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—— Trace Analysis ——

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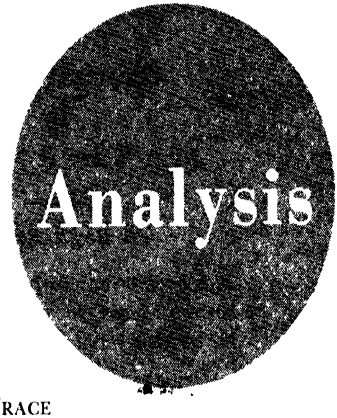
Trace Analysis



PAPERS PRESENTED
AT A SYMPOSIUM ON TRACE
ANALYSIS HELD AT THE NEW YORK
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Foreword

The Symposium on Trace Analysis, held at the New York Academy of Medicine on November 2, 3, and 4, 1955, came into being because of an unusual set of circumstances.

At a meeting of the Scientific Advisory Board of the Sloan-Kettering Institute, the subject of "trace elements" and the methods of analysis for them was brought before the board. It soon became apparent that the subject had not received the cooperative effort it deserved as a unified field of science.

It was thereupon decided that the Rockefeller Foundation and the Sloan-Kettering Institute would jointly finance a symposium. The purpose of the symposium was to be as follows:

1. To bring together for comparison and discussion authorities in the various scientific disciplines which are related to the analysis of trace constituents in industrial, agricultural, biological, and medical fields.

2. To consider which of the various techniques are directly applicable to the various fields of investigation.

3. To discuss the separation and concentration of millimicrogram quantities of metals and contamination hazards.

Accordingly, a chairman and a coordinator were selected and given the responsibility of organizing a symposium. It was decided to emphasize the three major areas in the field trace analysis: namely, (1) methodology, (2) instrumentation, and (3) separation, concentration, and contamination hazards. Speakers and their discussion leaders were selected and invited to participate. Presiding officers were chosen for the seven 3-hour sessions; they served as moderators. Ample time for discussion was provided for each paper. The audience was made up of invited guests and the participants, numbering about 150. In order to make the papers and discussions available to a much larger group, it was agreed that they would be published as a book on TRACE ANALYSIS. In so doing it was hoped that all who have an interest in the minor constituents of matter would benefit from the experience of many of those who have formed the vanguard in the study of the analytical techniques in this comparatively new, fascinating, and important field.

There is always in such a cooperative undertaking a large group of individuals whose unrelated activities are necessary for the proper functioning of the whole. A listing of all such people is seldom complete. Such must be the case here. The authors, the moderators, the discussors, the guests, all must be thanked for their unstinted efforts in behalf of the symposium and this volume. Doctor C. P. Rhoads, director of the Sloan-Kettering Institute, and Doctor Warren Weaver of the Rockefeller Foundation met the not inconsiderable expenses of the Symposium with foresight and generosity. Miss Diane Peckham, as secretary for the Symposium, rendered valuable service. To all of these we express our thanks and appreciation.

THE EDITORS

October 1957

Acknowledgment

In addition to those mentioned in the foreword, we wish to express our thanks to the several companies that supplied photographs for many of the figures in the text and to the scientific and technical publishers for permission to use copyright material.

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Introduction

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NEW YORK

The importance of the trace constituents of animate and inanimate matter has been increasingly demonstrated as methods for their detection and quantitation have been developed. Though the term *trace element* cannot be defined precisely, it may be considered to be a constituent of a biological, chemical, or structural system present in minute quantities in the system but having an important effect on the attributes of the whole system. The metal ion which acts as a specific activator of an enzyme system, the catalytic agent in a chemical process, and the germanium or tungsten content of a steel alloy are examples of such trace elements. Hundreds of other examples could be cited.

It is important to distinguish between quantity and concentration in considering the place of the trace element in biology. For example, iodine is present in the thyroid in a concentration of about one milligram per gram of tissue. The ratio of thyroid to body iodine concentration is about ten thousand to one. We may therefore consider iodine as a trace element since its quantity in the body is small; yet its con-

centration in the thyroid makes this element a principal constituent of this endocrine organ.

Of the 102 elements, at least 36 are known to be associated in one way or another with the fabric of life.[1] From these are derived all the compound chemical structures of the biological systems. These elements which have been found to be associated with animate matter may be grouped in several ways: chemically, as metals and nonmetals; physiologically, as essential and nonessential elements; and biologically, as plant and animal constituents. If the elements are classified according to the periodic table, a qualitative distinction may be made between variable and invariable elements, and a quantitative distinction can also be made among primary elements, secondary elements, and microconstituents.

The invariable primary constituents of animate matter are hydrogen, carbon, nitrogen, oxygen, and phosphorus. They are present in all forms of life and largely determine the physical structure of the organism. The invariable secondary elements are calcium, magnesium, sodium, potassium, iron, sulfur, and chlorine. They are as essential for life as the elements in the first group but are present in much smaller quantities.

Variable secondary elements are zinc, titanium, vanadium, and bromine. They reach a relatively high concentration in certain species where they are essential for life, but their occurrence and importance in other species are often doubtful. In other organisms they are completely absent.

Copper, molybdenum, manganese, iodine, boron, silicon, and fluorine are believed to be present in all form of life and, as such, represent the invariable microconstituents of animate matter. Whether some of these are present as contaminants rather than as essential constituents is still a matter of conjecture. The concentrations of these elements are very small, usually less than 0.005 per cent of tissue weight.

The variable microconstituents are lithium, rubidium, cesium, silver, beryllium, strontium, cadmium, germanium, tin, lead, arsenic, chromium, cobalt, and nickel. In some species they are essential; in others they may actually be toxic.

Finally, there is a group that has been reported as occurring in isolated instances in particular species or subspecies. It is composed of argon, barium, mercury, thallium, selenium, and gold. There appears to be no reason to regard them as other than fortuitous con-

taminants which may, in some species, produce a pathological reaction in the tissues of the host.

Although the fields of organic and inorganic chemistry have provided much information regarding the function of the primary constituents of biological materials, the trace constituents have largely remained an enigma for lack of proper methods of analysis. With the development of the new techniques during the past few years, the biologist has attained a position where he is now able to probe the functions of the elements which form a very minute part of the organic matrix, and to do so with a high degree of precision and accuracy. The determination of the definitive function of a particular element is, of course, in the hands of the enzyme chemist or physiologist. However, the clue to the possible function of an element remains with the analytical chemist who demonstrates its invariable presence and concentration range in a particular organ of a given species. Until this has been established, the enzyme chemist or physiologist cannot proceed with his functional studies.

It is the purpose of this book to acquaint the biologist, the physiologist, and the chemist, interested in the trace constituents of matter, with the new as well as the established techniques which are available for their analysis. Moreover, it is hoped that this collection of papers will serve as a useful and stimulating reference work in the rapidly growing field of trace analysis.

REFERENCE

1. Fearon, W. R., "A Classification of the Biological Elements," *Sci. Proc. Roy. Dublin Soc., 20,* 531 (1933).

Part I

METHODOLOGY

— 1 —

Chromatography and Electrochromatography

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ABSTRACT Chromatography, electrochromatography and related techniques are effective for the separation of mixtures and for the detection and recovery of the trace components. These methods are useful with all kinds of soluble and volatile substances.

The various modifications of chromatographic and electrochromatographic methods are described, and the conditions requisite for the application of these methods to the separation of trace substances are examined. The formation of zones of the minor constituents that are discernible or detectable and the formation of zones that are not detectable are considered. The distribution of molecules and ions in the migration zones are examined, because these distribution phenomena often determine the detectability and the recoverability of the separated substances. Emphasis is placed upon critical phenomena which are illustrated by selected examples.

This report summarizes those aspects and modifications of chromatography and electrochromatography of major significance to trace analysis. It emphasizes the methodological approaches to these subjects including the classification of the various experimental conditions. Limitations of space have prevented summarization of the his-

torical evolution [1,8,9] and have precluded the compilation of numerous applications and literature citations, most of which are now available in review articles,[6–12] in recent monographs,[1–5] in special issues,[13] and in *Chemical Abstracts.*

Definitions of Chromatographic Methods

Chromatographic and electrochromatographic methods are readily defined in terms that are descriptive of the chromatographic arrangements and procedure. These definitions apply primarily to the principal modifications of the techniques. They must be modified when they are employed to indicate particular details or, for example, the kind of sorbent, the principal reaction between the solutes and the sorbent, or the kind of solutes being examined.

Chromatography is a differential-migration method of analysis in which flow of solvent or gas causes the components of a mixture to migrate differentially from a narrow initial zone in a porous, sorptive medium. Some of the principal modifications of chromatography are *adsorption chromatography, partition chromatography, ion-exchange chromatography, columnar chromatography, inorganic chromatography, paper chromatography.*

Electrochromatography is a differential-migration method of analysis in which d-c electric potential causes the components of mixtures to migrate differentially from a narrow initial zone in a stabilized, permeable, background electrolytic solution. A number of names have been proposed for electrochromatographic methods and their modifications. The more common are *ionography, zone electrophoresis, electropherography, electrochromatophoresis,* and *paper electrophoresis.*

Continuous electrochromatography is a new differential-migration method of analysis in which flow of background electrolytic solution through a wide porous medium and transverse d-c electric potential cause the components of mixtures to migrate differentially from a narrow continuous stream flowing contiguously with the background electrolytic solution.

Characteristics of Trace Analysis

In the broadest sense, trace analysis is the detection and estimation of the minor, barely detectable constituents of mixtures. Dependent upon their properties, the trace components vary enormously in de-

tectability and in determinability, both of which vary in turn with the composition of the mixture. In practice, the amounts regarded as traces may vary from a few micrograms to a few atoms per gram of mixture. These trace components may be the free chemical elements, ions of the chemical elements, or all kinds of chemical compounds, inorganic and organic.

The precision of trace analysis varies with the quantities of the minor constituents of mixtures. When the quantities to be determined are near the limits of detectability, the analysis is usually qualitative. With quantities above the limits of detectability, the analysis is more quantitative. The limits of detectability vary not only with the trace materials to be estimated but also with the nature of the other substances comprising the mixture, with the nature of the method of estimation, and with the methods for resolution of the mixture and for the removal of interfering substances.

Requirements for Trace Analysis

The primary requisite for trace analysis is a sensitive detection method. This method may be specific for the minor constituents, or it may be nonspecific. Most nonspecific detection methods must be supplemented by various special classification or identification procedures.

Perhaps the second most significant requirement for trace analysis is a quantitative method of estimation. This method may also be specific or nonspecific.

For the detection and estimation of trace constituents when interfering substances are present, effective methods for resolution of the mixtures are required. Similarly, the determination of several similar trace substances, such as minor carotenoid or chlorophyll pigments, may require an effective preliminary resolution of the trace substances themselves.

Roles of Chromatography and Electrochromatography in Trace Analysis

Chromatography and electrochromatography have become indispensable tools in many applications of trace analysis. These techniques and their modifications have facilitated many analyses that could not have been accomplished in any other way.

In spite of their usefulness in trace analysis, chromatographic and

electrochromatographic methods do not serve directly for the detection of substances. Neither do they suffice for the estimation of substances. Their principal service is the resolution of complex mixtures.

The resolution of mixtures by chromatography and by electrochromatography depends upon the differential migration of the components through a porous medium. This differential migration is carried out so that each component, including each trace constituent, separates as a discrete zone. The separated substances may then be detected and estimated either in the medium or in extracts of the medium.

Chromatography, electrochromatography (also called zone electrophoresis, paper electrophoresis, electropherography, and ionography),[1,3] and several analogous differential-migration techniques [10-12] serve remarkably well for the resolution of mixtures of all kinds of soluble and volatile substances. Unlike chemical methods of analysis, they do not alter the materials during the separation, and they permit the recovery and isolation of all the separated substances including the trace substances. All the components of the mixture may be resolved in a single operation, even though many of them may be unknown and their presence unsuspected. Under ideal conditions, discussed below, virtually all of each trace component can be separated and isolated.

For their maximum effectiveness, the chromatographic methods must be employed in conjunction with various sensitive detection and estimation techniques. Because the chromatographic methods are themselves highly specific, the detection techniques employed with them often need not be specific.

Even though chromatographic methods are effective with all kinds of soluble, dispersible, and volatile substances, there are some significant limitations to their use in trace analysis. These limitations are the presence of contaminants in the migration systems so that the separating trace substances become contaminated; the relatively small quantities of mixtures that may be separated in migration systems of convenient size; imperfections of some migration systems that cause poor separation and cross-contamination of the zones of the constituents; and the natural distribution effects of the substances being separated so that, occasionally, ill-defined, diffuse, incompletely separated zones are formed. Some of these limitations are more pronounced with certain chromatographic systems than with others. Consequently, an understanding of these limitations must be based

on knowledge of the various systems of porous sorbents and solvents and sorbents and gases that have been employed for chromatography and on knowledge of the various porous stabilizing media and background electrolytic solutions that have been employed for electrochromatography.

CHROMATOGRAPHY

Features of Chromatography

All chromatographic methods have many features in common. These methods depend upon the differential migration of the components of mixtures through a porous sorptive medium, such as paper or a tube filled with a powdered sorbent. For effective separations, a narrow initial zone of the mixture is formed in the medium, and this zone is caused to migrate by washing with fresh solvent or with a gas. These sorbent–solvent and sorbent–gas combinations or systems must be selected so carefully that the components of the mixture are sorbed selectively and reversibly. The differential migration of each constituent of the mixture is inversely proportional to the degree of sorption. This differential migration, the formation of the chromatogram, resolves the mixture into a series of bands or zones, the chromatogram, each zone containing a single component of the mixture. These separated constituents may be carried into the effluent and collected separately, or they may be recovered from mechanically separated portions of the system by elution with suitable solvents.

In chromatographic separations, the presence of particular trace constituents need not be known to the operator. But there must be some method, either specific or nonspecific, for the detection and location of the separated trace substances. The migration of the constituents of the mixture relative to the migration of the wash liquid, the R value, serves for their description and identification, provided the migration conditions are carefully standardized. With unstandardized conditions, the migration of the constituents relative to the migration of particular reference substances, the sorption sequence, also provides a basis for the description and identification.

Chromatographic systems are almost infinitely variable in detail, in size, in shape, in sorbent, and in solvent. This great variability permits the adaptation of chromatographic methods to use with all kinds of chemical substances, but it also presents numerous practical problems in the selection of suitable migration conditions. It like-

wise presents many difficulties in the classification of the various methods and their modifications.

Most chromatographic methods are discontinuous or batch processes. They do not readily lend themselves to continuous operation, and they must be operated on a large scale to provide large quantities of the trace substances.[35]

Classification of Chromatographic Methods

Chromatographic methods are commonly classified with respect to one or more variable conditions. Those conditions of major interest are the dimensions of the initial zone of the mixture, the arrangement or geometry of the migration system, the nature of the migration system, and the principal kind of reaction occurring between the sorbent and the sorbed constituents of mixtures. Many of the conditions are interdependent; hence the classifications are often involved, and the terminology is frequently ambiguous. Moreover, with minor changes of conditions, the principal kind of reaction occurring in the sorption system may vary enormously. This variation depends also upon the substances that are sorbed as well as upon the sorption conditions. With chloroplast pigments dissolved in petroleum ether, for example, there is sorption upon the cellulose in dry paper; there is sorption at the petroleum ether–solvent interface in paper moistened with aqueous polar solvents; there is solution (partition) in polar solvents when the paper is moistened with these liquids; and there is solution in the nonpolar phase when the paper is saturated with paraffin, and the sorbed pigments are washed with aqueous methanol as in reversed-phase partition chromatography.[1,2,4,5,32]

The complexity of the classification is apparent from Tables I to IV, which have been simplified to illustrate some of the most impor-

TABLE I

CLASSIFICATION OF CHROMATOGRAPHIC METHODS BASED ON SIZE OF INITIAL ZONE

Zone	Use	Terms
Flow from a reservoir	Determination of migration rates	Break-through analysis
	No separation	Frontal analysis
Wide zone	Determination of migration rates	Trailing analysis
	No separation	Frontal analysis
Narrow zone	Determination of migration rates	Chromatography
	Complete separation	

tant conditions. Table I illustrates the importance of the size of the initial zone of the mixture. Only with a narrow initial zone is the mixture extensively resolved.[11] Table II indicates some of the many

TABLE II

CLASSIFICATION OF CHROMATOGRAPHIC METHODS BASED ON ARRANGEMENT OF THE MIGRATION MEDIUM

Arrangement	Sorbent	Terms
Columns	Various powdered sorbents, resins, gels, etc.	Columnar chromatography
Porous solids	Chalk	Chromatobar
	Pads of paper	Chromatopile
Fibrous strips, sheets, and pads	Paper	Paper chromatography
	Chamois	

arrangements of the migration media, and Table III shows some of the variations of solvent flow that are effective in modifications of chromatographic methods.[2]

TABLE III

CLASSIFICATION OF CHROMATOGRAPHIC METHODS BASED ON FLOW OF SOLVENT, METHOD FOR COLLECTION OF EFFLUENT, AND GEOMETRIC PATTERN OF SOLVENT FLOW

Flow	Arrangement	Terms
Downward	Columns	Column chromatography
Downward (solutes in effluent)	Columns	Flowing chromatogram
Downward	Paper	Descending paper chromatography
Upward	Paper	Ascending paper chromatography
One-way	Paper (strip or sheet)	One-way paper chromatography
Two-way (transversely)	Paper (sheet)	Two-way paper chromatography
Radial	Paper (segment of circular sheet)	Radial or circular paper chromatography
Spherical	Paper (solid mass)	Spherical paper chromatography

Tables IV and V show that several kinds of sorption effects, such as surface adsorption, ion exchange, and partition, may be made the basis of chromatographic methods. Surface adsorption is effective

TABLE IV

CLASSIFICATION OF CHROMATOGRAPHIC METHODS BASED ON PRINCIPAL REACTION IN THE SORBENT

Reaction	Sorbent	Terms
Adsorption Solution–solid Solution–liquid Liquid–interface [32]	Various substances as sugar, starch, calcium carbonate, alumina, magnesia, etc.	Adsorption chomatography
Gas–solid [2]		Gas-adsorption chromatography
Ion exchange Solution–resin Solution–zeolite	Cation-exchange resins Anion-exchange resins Amphoteric substances as alumina and zeolites	Ion-exchange chromatography
Partition (with a fixed liquid) Solution–liquid	Hydrated gels [2, 5] Moist paper [1, 4]	Partition chromatography Paper partition chromatography
Phases reversed [1, 2, 32]		Reversed-phase partition chromatography
Gas–liquid [24]		Gas–liquid partition chromatography
Complex formation (Various others)		

TABLE V

KINDS OF SORBENTS AND SOLVENTS EMPLOYED FOR CHROMATOGRAPHY OF VARIOUS SOLUTES

Sorbents	Solvents	Solutes
Adsorbents, Surface-active solids, droplets of liquids [32]	Nonpolar hydrocarbons to polar acids and bases Gases	All soluble or volatile organic substances Many soluble or volatile inorganic substances
Ion-exchange substances Zeolites, resins	Water, aqueous solutions of complexing solutes	All ionized organic and inorganic substances Non-ionized substances that form ionized complexes
Partition Fixed liquids (usually polar liquids)	Nonpolar to weakly polar solvents	All weakly polar to moderately polar soluble or volatile organic substances
	Gases	Many gaseous or volatile inorganic and organic substances

with all kinds of substances. Partition is useful with most weakly polar and polar organic substances, and ion exchange is effective with ionized substances and with non-ionized substances that can be converted into ionized complexes. For the examination of all kinds of substances, surface adsorption is therefore more widely applicable than ion exchange or partition. For many ionized solutes, however, ion-exchange sorbents, particularly the synthetic exchange resins, are the most effective.

Selection of Chromatographic Conditions

As may be inferred from Table V, the chromatographic systems and numerous variable conditions must be carefully selected and controlled for the effective resolution of mixtures. Thus far, these selections have been based primarily on empirical methods and on the use of analogy. Even after many conditions have been examined, substances may often be separated efficiently by a variety of chromatographic methods. The final selection may depend, therefore, upon the experience and prejudices of the worker as well as upon careful consideration of the variable conditions themselves.

For the empirical selection of sorbent and solvent, solutions of the mixture are added to the sorptive system until a small initial zone is formed. This is then washed with the fresh solvent to form the chromatogram. If the sorbed substances do not migrate or if they migrate as fast as the solvent, sorbent and solvent must be varied until suitable reversible or dynamic sorption is obtained.

For the systematic empirical selection of sorbent and solvent organic substances are commonly dissolved in the least polar solvent in which they are soluble. Then the sorbability on a series of surface-active sorbents of increasing activity is determined. Examples of solids with increasing sorption capacity are sucrose, starch, calcium carbonate, magnesium carbonate, calcium phosphate, activated alumina, and activated magnesia. If the sorbability is too great, even on the weakest sorbents, more and more polar solvents are employed. In partition chromatography with fixed liquids, a similar procedure is employed, care being taken that the selected solvents are immiscible and saturated with the fixed liquid. Ionized substances are usually dissolved in water and sorbed on an ion-exchange resin. To promote the formation of the chromatogram, the sorbed ions are usually brought into dynamic equilibrium with the solvent by the use of complex-forming solutes and by variation of the *p*H.

Detection of Separated Substances

The location of trace substances separated from mixtures by chromatography depends upon the use of sensitive detection methods. These detection methods depend largely upon the properties of the materials that are separated. They are nearly as variable as the materials to be detected. None is universally applicable. Selected examples of the kinds of properties basic to the tests are presented in Table VI.

TABLE VI

PROPERTIES UTILIZED FOR THE DETECTION OF VARIOUS KINDS OF SUBSTANCES SEPARATED BY CHROMATOGRAPHY

Properties	Kinds of Substances (Selected Examples)
Nuclear	Radioactive substances (tracers) Substances activated with neutrons after separation (activation analysis)
Physical	Colored and fluorescent substances Substances detectable with flame photometer Isotopic substances (stable isotope dilution) Substances (nonvolatile) that can be weighed Gases (thermal conductivity)
Chemical	Substances convertible to colored or fluorescent products (either before or after separation)
Biological	Substances that produce biological effects, as antibacterial products, growth hormones, enzymes

Separated substances may be detected within the intact chromatographic systems, in sections of the chromatographic systems, or in the effluent from the systems. The trace substances may also be collected from several migration systems, combined, and re-examined. Regardless of the technique employed, the detection tests must be sufficiently sensitive to reveal the trace substances provided by the quantity of the mixture that is sorbed.[23]

Estimation of Separated Substances

Trace substances separated by chromatography may be estimated by modifications of many of the detection methods, some of which are classified in Table VI. Radioactivity, for example, not only serves for detection, but the half-life serves for identification, and the intensity of the radiation serves for estimation. For some elements radio-

activity is by far the most sensitive detection method, but for others, owing to the nature and intensity of the radiation and to an unsuitable half-life, chemical and physical methods of estimation are the more sensitive.

For estimation as well as for detection, a sufficient quantity of the mixture must be separated in order to provide estimable quantities of the trace substances. With mixtures of unknown composition, much empirical experimentation may be required for the selection of suitable conditions.

Distribution Phenomena in Chromatographic Zones

For the effective recovery of trace substances from chromatographic systems, the distribution of the trace substance within its zone should be considered. For ideal systems with rapid sorption and desorption,

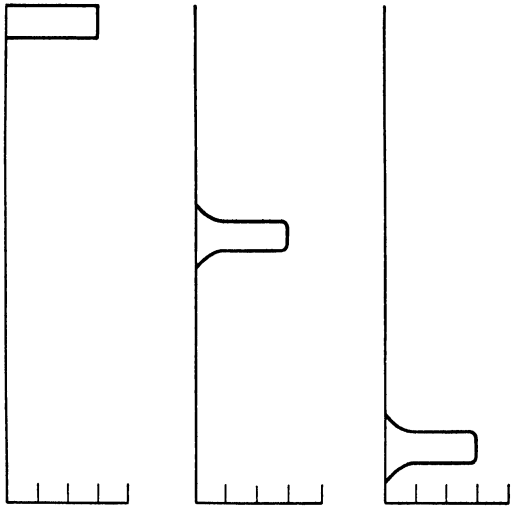

FIG. 1. Schematic representation of successive stages in the migration of a narrow zone of solute through a sorption column when equilibrium of solute between sorbent and solvent is instantaneous, when there is uniform flow of solvent, and when the sorption isotherm is linear (when the fraction sorbed is independent of concentration). Vertical position indicates distance of migration in the column. Horizontal distance indicates the amount of solute at a particular point in the zone. Atypical of most useful systems.[8]

there should be uniform concentration of each component throughout the zone, and the boundaries should be sharp, as indicated schematically in Fig. 1. But with many chromatographic systems, particularly

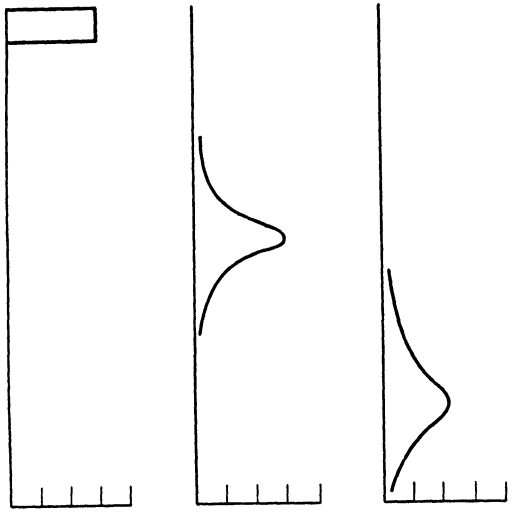

FIG. 2. Schematic representation of successive stages in the migration of a narrow zone of solute through a sorption column when the flow of solvent may be uneven, when the equilibrium of solute between sorbent and solvent may be incomplete, and when the sorption isotherm is linear. Typical of ion-exchange and partition systems.[1, 2, 8, 12]

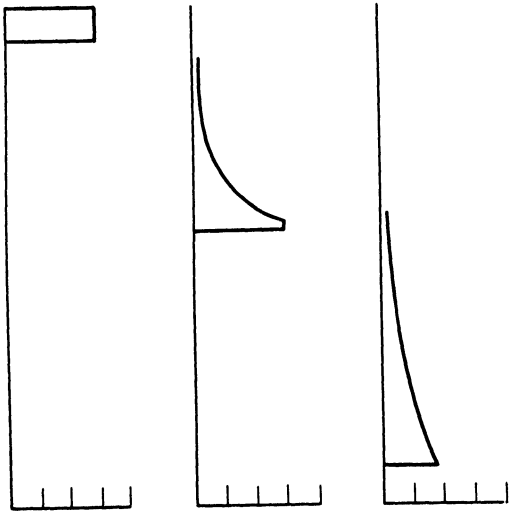

FIG. 3. Schematic representation of successive stages in the migration of a narrow zone of solute through a sorption column when the flow of solvent may be even or uneven, when the equilibrium of solute between sorbent and solvent may or may not be completed rapidly, but when the sorption isotherm is not linear (particularly when the fraction sorbed increases with decreasing concentration). Typical of surface-active sorption systems.[1, 2, 8, 12]

with the ion-exchange resins and with the fixed partition liquids, the concentration of each component is at a maximum near the center of the migration zone and decreases gradually at both leading and trailing boundaries, as shown in Fig. 2. With most surface-active sorbents, by contrast, the concentration of each component is greatest near the leading boundary of the zone and decreases very slowly in the trailing region as reported in Fig. 3.[1,2,8]

The two different effects shown in Figs. 2 and 3 may be attributed to variation of the sorption with concentration and to imperfections in the migration system. In partition systems and in ion-exchange systems, the migration of the zone relative to the flow of the solvent (R value) varies little with the concentration of the sorbed substances until the sorbent is saturated. As the zone becomes diffuse through various imperfections such as uneven flow and nonequilibrium exchange, all portions of the zone continue to migrate at the same rate thereby maintaining the symmetry of the migrating zone. In surface-active sorption systems by contrast, the migration of the zone usually decreases with the concentration. As the zone becomes diffuse through various imperfections of the system, the more dilute region of the zone migrates more slowly than the more concentrated region. At the leading boundary, the diffuse region is continually overrun by the more concentrated region so that the frontal region remains sharp and the trailing region becomes very diffuse.[8]

In most sorption systems, the imperfections in the solid phase are greater than those in the liquid phase. As a result, the concentration of solute in the sorption zones for partition and ion-exchange systems is frequently distorted, approaching that shown for the surface-active adsorption systems in Fig. 3.

These distribution effects may result in the cross-contamination of one zone by another. As a rule, each zone is contaminated by traces of the constituents of the zones that precede it. Each zone is less likely to be contaminated by the constituents of the zones that follow it.

At extremely low concentration, the atoms or molecules of a trace substance may be distributed irregularly through the region normally occupied by the sorption zone. As the number of particles increases, however, the distribution should rapidly approach that characteristic of the system, as already indicated schematically in Figs. 2 and 3. So far as has been determined, the separation of substances into discrete zones in the columns occurs at concentrations as low as those detectable by the most sensitive methods as with carrier-free, radioactive ions.

For the quantitative isolation of separated substances, it is desirable that the zones should not overlap. With many chromatographic systems, this overlapping is difficult to avoid. Occasionally it may be reduced by the addition of small quantities of polar solvents to the mixtures being separated or by the use of systems with sorption gradients.[10] More often the contaminated trace substance must be isolated from a wide section of the sorption system. This recovered mixture is then resolved more extensively by a second chromatographic analysis.

Rate of Separations

The rate at which the zones of different substances separate in a chromatographic system depends principally upon the speed of sorp-

TABLE VII

APPLICATIONS OF CHROMATOGRAPHIC METHODS TO THE SEPARATION AND ISOLATION OF VARIOUS TRACE SUBSTANCES

Trace Substances	Sorbent	Solvent	Detection and Estimation
Ag and Zn from Cu	Paper (one-way)	*n*-butanol + acetone + 3N HCl (pts/vol 25:25:3)	Radioactivity [18]
Elements 99, 100, 101	Cation-exchange resin	Aqueous complexing agents	Radioactivity [14]
Ra–Ba	Paper	Ethanol + water (4:1)	Radioactivity [2]
Cu (traces in milk)	Cation-exchange resin	Neutralized perchloric acid extract	Polarography [15]
SO_4	Anion-exchange resin	Water	— [2]
Y (traces in urine)	Cation-exchange resin	Urine	Radioactivity [30]
Rare earths	Cation-exchange resin	Solution of citrates	Radioactivity [2]
Volatile fatty acids	Celite plus silicone and stearic acid	Nitrogen	Titration [19]
Nucleic acid and nucleotides	Anion-exchange resin	Aqueous acids	Spectral absorption in ultraviolet [36]
Amino acids	Paper (two-way)	Phenol collidine	Ninhydrin reagent [16]
Chlorophyll *b* in red algae	Powdered sugar	Petroleum ether + propanol	Visual, spectral absorption [23]
ζ-carotene in carrots	Activated magnesia	Petroleum ether	Visual, spectral absorption [33]

tion and desorption, upon the sorbability of the components of the mixture, and upon the flow rate of the wash liquid. The speed of sorption and desorption is a function of particle size, and it is also a function of the permeability of the sorptive particles.

For most permeable ion-exchange and partition sorbents the sorption and desorption of the migrating particles occurs more slowly than in impermeable surface-active sorbents. As a rule, therefore, flow of solvent through the ion-exchange and partition sorbents must be much slower than with the surface-active sorbents. As examples, the separation of several ions in a column of permeable, cation-exchange resin may require some 8 to 24 hours whereas the separation of about a dozen chloroplast pigments in a column of impermeable, powdered sugar may be completed in 20 minutes.[23]

Applications

Virtually all the modifications of chromatography have been employed for the separation of various trace elements and numerous trace substances from mixtures. Some of these applications are summarized in Table VII. They range from the isolation of new chemical elements to the isolation of labile pigments and various biologically important substances. Numerous additional examples may be found in the books by Lederer and Lederer [2] and by Pollard and McOmie.[4]

ELECTROCHROMATOGRAPHY

Features of Electrochromatography

Various electrochromatographic methods have many critical features in common. All these techniques depend upon the differential electrical migration of the ionized or electrically charged components of mixtures through a background electrolytic solution that is fixed or stabilized in a porous medium. Stabilization media may be columns or troughs of powdered substances, strips or sheets of fibrous substances such as paper or chamois, or tubes of gels such as agar, gelatin, resins, and silicic acid. These stabilization media may be sorptive or nonsorptive. The d-c electric field, usually obtained with electronic rectifiers, may vary from about 2 to 20 or more volts per centimeter. The separation of the migrating substances depends primarily upon their differential electrical migration, upon their selective sorption by the medium, or upon both these effects acting simul-

taneously. With a narrow initial zone of the mixture, substances separated by electrochromatography yield a series of zones, an electrochromatogram, analogous to the chromatogram. The substances separated by the differential electrical migration may be detected, estimated, and recovered by any of the appropriate techniques that have been perfected for chromatography (see Table VI).[1,4]

Electrochromatographic systems are extremely variable in detail: in the stabilization medium, in the background electrolytic solution, in the arrangement and dimensions of the apparatus, and in the electric potential that may be employed. All these variable conditions must be carefully controlled if effective reproducible separations are to be obtained.[1,3]

In most electrochromatographic systems, the electrical migration of the charged particles is accompanied by migration or electro-osmosis of the background electrolytic solution through the stabilizing medium. This electro-osmosis, including its direction and magnitude, varies with the stabilizing medium and with the background electrolytic solution.[3] It also varies with the arrangement of the electrodes. It is small with the electrodes in contact with the migration system but is large with the electrodes in reservoirs of the background electrolytic solution that bathes the ends of the migration system.[17,37] For precise values of the electrical migration, the total migration of the particles must be corrected for the electro-osmotic transport which is usually determined by the addition of spots or zones of easily detectable, non-ionized substances, such as hydrogen peroxide, to the migration medium.[17,37]

At a given potential, the rate of migration, like the chromatographic R values, serves for the description and identification of the components of mixtures. For this kind of identification, however, the migrations must always be carried out in the same medium under the same conditions. Owing to the variability of the systems, particularly the variability of the stabilizing media with respect to sorption and electro-osmosis, the rate of migration of various substances is not easily reproduced. It is usually less in the moist porous media than in the unstabilized, background solutions themselves.[21]

Electrochromatographic methods are effective with virtually all kinds of soluble, ionized substances, inorganic and organic. They are also effective with un-ionized substances that can be converted into ionized complexes in solution as, for example, the complex borates of the sugars. They are especially useful with charged colloidal par-

ticles such as proteins that are stable during electrical migration but that are denatured by sorption in chromatographic systems.

Nomenclature and Classification of Electrochromatographic Methods

As indicated already, electrochromatography is known by a variety of names. Some of these names are more widely used in certain fields than in others, but there is no widespread agreement regarding usage. Electrochromatography, the oldest term, is frequently employed in a generic sense. Attempts to restrict this term to systems in which sorption plays an important role have not been popular because sorption not only plays a key role in some systems but it probably also plays some role in all systems. Zone electrophoresis and paper electrophoresis are widely employed terms in biological fields. Electropherography, electrochromatophoresis, and ionography have not been widely adopted, although the latter is the title of McDonald's comprehensive monograph.[1,4]

Various forms of electrochromatographic apparatus have been employed with different kinds of stabilizing media, as shown in Table VIII. The selection of apparatus and media depends upon a variety of conditions, perhaps the principal one being the amount of material

TABLE VIII

CLASSIFICATION OF ELECTROCHROMATOGRAPHIC METHODS BASED ON FORM OF APPARATUS AND KIND OF STABILIZATION MEDIA

Form of Apparatus	Stabilization Media
Columns	Inert powders [3,4] Sorptive powders [1,3,4] Ion-exchange resins [1,3,4]
Strips	Paper (one-way) [1] Chamois (one-way)
Sheets	Paper (one-way) [1] (two-way) [1,3,31]
Pads	Paper [11,27]
Troughs	Starch [3]
Tubes	Gels [3,4] Agar Gelatin Silicic acid

that is to be separated. Usually more material is separable in large columns, tubes, or pads of stabilized solution than in moist strips or sheets of paper. Where sorption of ions is desired, ion-exchange resins may be employed. When mild sorption effects are permissible, paper may be utilized. When sorption must be reduced to a minimum, troughs of moist starch or gels of agar or gelatin are usually employed.

The background electrolytic solution, particularly, must meet rigid requirements. It must not be too conducting. For strong electrolytes, such as salts, the concentration is usually about 0.01 to 0.05 *M*. For weak electrolytes, such as lactic acid, the solutions are about 0.1 to 0.2 *M*, and for very weak electrolytes, such as aqueous ammonia, the concentration may be 1 to 4 *M*. Various acids, bases, buffers, and complex-forming reagents have been added to the electrolytic solutions to facilitate the separation of different constituents of the mixture. Some of these reagents cause certain ions to migrate in opposite directions so that they facilitate complete or absolute separations.[27] Thus far no simple rules for the selection of the background electrolytic solutions have been formulated, and no convenient methods for the classification of these solvents have been devised.[3,11,29]

Solutions of mixtures to be resolved by electrochromatography must not be much more concentrated than the background electrolytic solution. If they are much more concentrated, the migrating zones become elongated with diffuse leading regions.[27] The lower limit for the concentration of the mixture depends upon the detectability of the separated substances.[27] The size of the initial zones should be kept small, usually not more than a centimeter in diameter.

Paper Electrochromatography

Of the several electrochromatographic methods that have been devised (Table VIII), paper electrochromatography has received by far the most extensive use, and it, in turn, has been studied in greatest detail.[1,3,4]

Various kinds of filter paper have been employed for paper electrochromatography. These papers have varied in thickness, in density, and in the isoelectric point.[1,3,17,37] The paper has been used as strips and pads for one-way migrations and as sheets for two-way migrations.[3] The paper has been supported in various ways, as horizontal strips on glass points, as in a catenary shape between supports, as an inverted V over a glass bar, and as horizontal strips and sheets on glass

plates, on water-cooled glass plates (Thermopane window),[29] and on an insulated, shallow, covered, water-cooled steel pan. The paper has also been placed in an inert atmosphere, submerged in nonconducting organic liquids, and wrapped in polyethylene plastic sheets.[1,3,17,27]

Electrodes are commonly used in separate reservoirs. But with weakly dissociated, background electrolytic solutions, the electrodes have also been applied directly to the migration medium. This latter arrangement reduces the electro-osmosis, although it gradually produces acidic and basic zones about the anode and cathode, respectively.[27]

One-way electrical migrations are usually carried out in paper strips, sheets, or pads.[27] For the description, identification, and comparison of substances by their relative migration rates or electrochromatographic sequences, the paper strips may be made wide enough for several parallel migrations.

Two-way electrical migrations are carried out in paper sheets, the second migration at right angles to the first.[1,3,4] In this technique, the second migration must be performed in a solvent different from that used for the first migration.[31] Chromatography by flow of the electrolytic solution in one direction may be substituted for the electrical migration in that direction. With this arrangement, it is not necessary to vary the solvent, because, in one direction, flow of solvent is a unique driving force, and, in the other direction, electric potential is a second unique driving force.[11] Moreover, the flow of solvent and the electrical migration may be carried out simultaneously.

Although frequently regarded as a nonsorptive or weakly sorptive stabilization medium, paper has significant sorptive affinity for many ions. This effect has been demonstrated with carrier-free radioactive lead and bismuth ions. Moreover, the proportion of the ions sorbed by the paper appears to increase with decreasing concentration, because the migration rate decreases with concentration.[8,29]

The distribution of solutes in migrating zones is remarkably uniform when the solutes are not sorbed by the medium. Sorption, however, may produce distribution effects like those shown in Fig. 3.[20,29] Precipitation usually produces small, dense zones with long, attenuated, leading regions.

Applications of Paper Electrochromatography

One-way paper chromatography has found extensive use for the examination of many kinds of substances. Typical examples are pre-

sented in Table IX. Like all chromatographic methods it too is effective with trace quantities of various elements and with organic and inorganic compounds.

TABLE IX

PAIRS OF TRACE SUBSTANCES SEPARATED BY PAPER ELECTROCHROMATOGRAPHY

Substances	Detection	Electrolytic Solution
Nd–Pm	Radioactivity	Lactic acid [26]
Ce–Pr	Radioactivity	Lactic acid [26]
Ba–La	Radioactivity	Lactic acid [26]
Sr–Y	Radioactivity	Lactic acid [26]
Ca–PO_4	Radioactivity	Lactic acid [27]
Co–Ni	Chemical	Thiocyanate [22]
Ba–Ra	Radioactivity	Lactic acid [29]
Pb–Bi	Radioactivity	Lactic acid [29]

Thus far two-way electrochromatography has found limited use.[1,3,31] It does, however, facilitate some separations not readily achieved by other methods, and it can be employed with minute quantities of radioactive tracers.

CONTINUOUS ELECTROCHROMATOGRAPHY

Principles and Applications

The procedure for the continuous resolution of mixtures utilizes flow of the background electrolytic solution transverse to an electric field. For continuous operation, the solution of the mixture is added as a narrow stream. The ions in this stream are exposed simultaneously to a chromatographic migration and to an electrical migration. They follow separate paths through the migration medium and emerge with the effluent at separate points at the bottom of the apparatus. The migration medium is usually a bed of powdered glass or a sheet or pad of soft filter paper. When the zones do not overlap or traverse the same regions, there is believed to be no cross-contamination such as may occur in one-way chromatography and in one-way electrochromatography.[1,3,4]

Many modifications of the apparatus for continuous electrochromatography have been devised.[1,3,4,25,28,34] These systems for continuous electrochromatography have been utilized for the separation of a great variety of carrier-free radioactive cations and anions. The separations are as effective at the lowest concentration of tracer detectable by radioactivity as at higher concentrations detectable by chemical

methods.[28,34] This continuous electrochromatography has also been employed for the isolation of a variety of biological products at very low concentration.[1,3]

DISCUSSION

Discussion Leader
W. J. Frierson
Agnes Scott College

In the preparation of a chromatogram reference has been made by Dr. Strain to the selection of the solvent and to detection methods. This brief discussion will be limited to some remarks concerning the selection of the solvent mixture and to describing several separations in which radioactive isotopes are used and serve as a means for the detection and identification of trace amounts. These remarks will be based on the separation of inorganic ions on paper but in many instances would apply as well to column work.

The selection of the solvent mixture is primarily based on empirical methods and on the experience of the individual worker, owing to the number of interrelated variables involved. With this number of variables it would be difficult to make a systematic study of a solvent mixture, that is, vary one factor while the rest are kept constant. The following factors are involved.

Organic Solvent. The organic solvent must be miscible or partially miscible with water. Acetone, butanol, pyridine, and methanol are among those that have been frequently used. In some cases two solvents may be used in the same mixture.

Water Ratio. If the organic solvent is partially miscible with water, for example, butanol, it is often used saturated with water. If a water-miscible solvent, such as acetone, is used, the most satisfactory water content must be determined. For some separations it should be low and for other separations it should be high for the best results.

Acidity. The acid content of the solvent may affect the results by giving the most favorable *p*H for the formation of complexes or by preventing the hydrolysis of some salts which would otherwise not be moved on paper. The kind of acid used to adjust the acidity is also a factor and must be determined.

Complexing Agent. Many separations are achieved by including a complexing agent in the solvent. Such agents may be selected on the

basis of their known action toward the ions involved in the separation, but it is often necessary to try several before finding a satisfactory one.

Other Ions. The zone position of an ion may be affected by the presence of other cations or anions. This would involve the ions present in the salts used or the addition of other ions to aid in the separation.

Detection Methods

Dr. Strain has mentioned the following four properties utilized for the detection of separated substances: nuclear, chemical, physical, and biological. The method used, of course, depends upon the types of substances being separated. Although the separations themselves are not affected by the method of detection, it is evident that a separation is useful only if we have some means of detecting the separated substances.

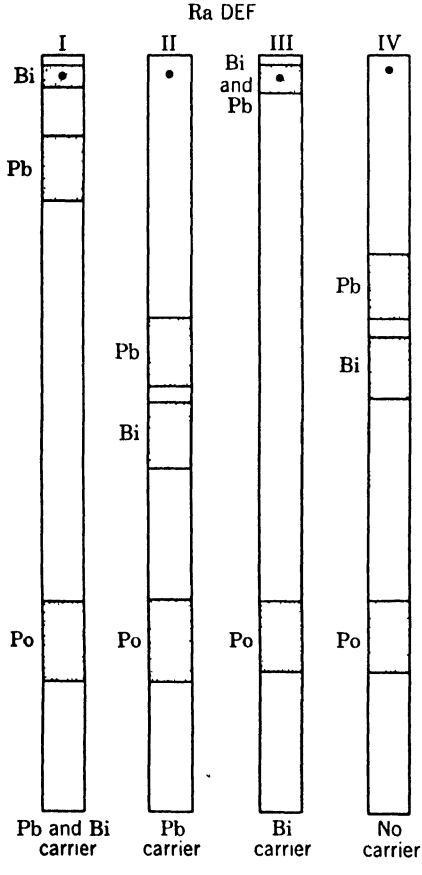

FIG. D1. Butanol, pyridine, acetic acid solvent.

Reagents are known for many inorganic ions which will convert them to colored or fluorescent substances and often the test is sufficiently sensitive to detect microgram amounts. For many of the ions, however, sensitive color or fluorescent tests are not known, and so there is a real need for such tests to be developed.

The most sensitive method for the detection of ions is based on nuclear properties. A paper-strip chromatogram containing radioactive isotopes may be scanned by the arrangement described by Frierson and Jones [38] or small sections may be cut from the strip for individual counting.

The separation of Pb^{210}, Bi^{210}, and Po^{210} from a RaDEF mixture (Fig. D1) illustrates the effectiveness of paper in separating small

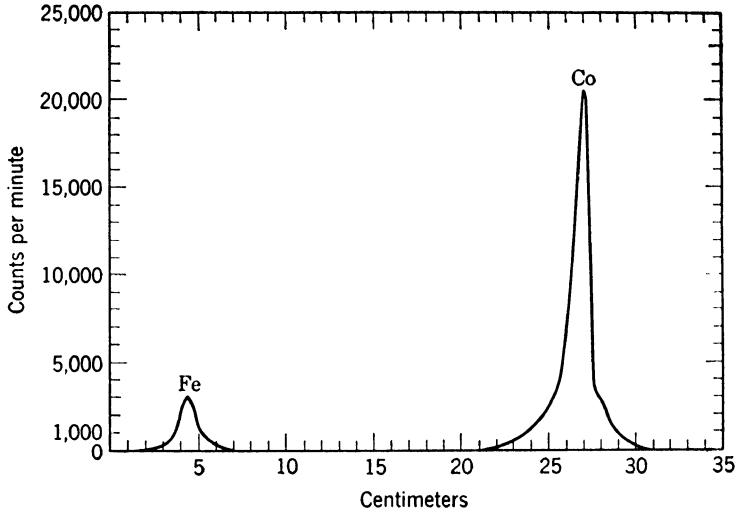

FIG. D2. Iron (old), in butanol pyridine solvent. No carrier added.

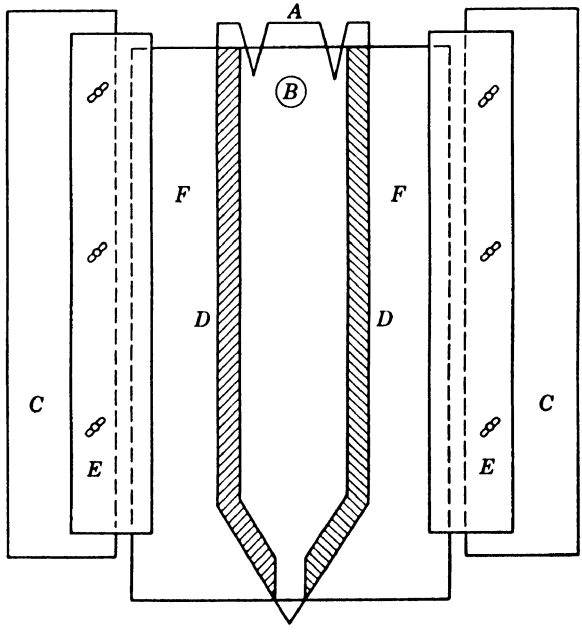

FIG. D3. Assembly for elution chromatography with thick paper. (*A*) Solvent added. (*B*) Solution spotted. (*C*) Plywood (*D*) Edges of paper covered with platinum foil. (*E*) Two stainless steel strips, back one screwed to plywood strip. Front one tightened on glass with wing nuts. (*F*) Glass plates with strip sandwiched between.

quantities and the use of nuclear properties for the location and identification of the ions. The nuclide Pb^{210} has a long half-life and emits a beta particle too weak to detect with thin-window Geiger tubes. Since the bismuth activity grows into the lead, the position of the lead may be determined by a growth curve. The Bi^{210}, with a 5-day half-life, emits a strong beta particle and so may be identified by a decay curve. The Po^{210} may be identified as an alpha emitter. Strip IV with no carrier shows a satisfactory separation with submicrogram quantities, the amounts present being 10^{-5} μg of bismuth, 10^{-4} μg of polonium, and 10^{-2} μg of lead.

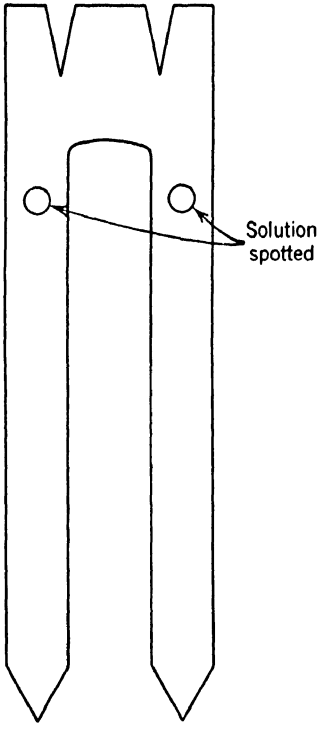

FIG. D4. Strip for simultaneous elution of a standard and sample.

A radioactive sample of iron was chromatographed on paper (Fig. D2), and two peaks of activity found. The iron did not migrate, and so its activity was located at the point of application. The other peak of activity was at the liquid front and was identified as Co^{60} from the absorption characteristics in aluminum, chemical separation of cobalt from iron, and the position on the chromatogram.

Thick paper elution [39] has been applied to this separation for a rapid and accurate determination of trace amounts of cobalt in radioactive samples of iron (Fig. D3). A 2×8-inch strip of thick (0.1-inch) filter paper is cut and mounted as shown. The sample is placed at *B* and about 50 ml of the solvent are allowed to flow onto the paper at *A*. The cobalt is eluted quantitatively from the end of the strip and determined by some standard method. A control analysis may be made by using a strip with a center section cut out (Fig. D4). A sample containing a known amount of cobalt is eluted under identical conditions with the unknown. For each analysis this procedure permits a rapid check with a standard.

In conclusion one application to metabolism studies might be mentioned. Many radioactive elements have been used in such studies, and it is very important that no trace impurities be present in the

sample. Copper (Cu^{64}) with a half-life of 12.9 hours has been used by a number of workers for biological studies. One worker reported an anomaly in the activity recovered in the urine of the injected rats. Although the animal was injected with the copper sample with a half-life of 12.9 hours, the activity collected in the urine was about 28 hours.

A paper chromatogram of the copper sample showed two activities separated from the copper.[40] From a study of their radiation characteristics they were identified as Ag^{110} and Zn^{65} with half-lives of 270 days and 250 days, respectively. These trace amounts present with large amounts of copper did not cause a detectable change in the original half-life; however, when they were concentrated in the kidney with small amounts of copper the half-life of 12.9 hours was increased to about 28 hours. This would suggest the importance of making a chromatographic analysis of radioactive samples to be used for metabolism studies.

QUESTIONS AND ANSWERS

SIDNEY UDENFRIEND: The data that both Dr. Strain and Dr. Frierson presented, showing the large differences in movement with concentration or amount of material placed on the chromatogram, are, I think, of great importance, especially since chromatography is now being used for standardization of and proof of purity of radioactively labeled material. If such large differences in migration occur with concentration, then trace impurities might not be detected. We have had experience with derivatives of amino acids where one could change the order of the compounds on the chromatogram by varying the concentrations over a wide enough range. In Calvin's work on photosynthesis, where one material might appear in traces and another in milligram quantities, R_f values could certainly be misleading. In other words, one should not use as a guide 5 gammas or 10 gammas of a compound when dealing with a thousandth of a gamma of radioactive material in an experiment.

I wonder if you have any comment?

W. J. FRIERSON: Dr. Strain has recently studied the effect of concentration on migration. Do you have any comment on that?

HAROLD H. STRAIN: Separability will depend on the relative proportions, and the separability will also depend on the sequence of the two substances. If the major component is the more absorbed, the more you increase the concentration the poorer the separation; but if the minor constituent happens to be the more absorbed, variation of concentration does not make too much difference.

Your comment brings up another important point; the use of R values. These values cannot be standardized very easily, and they will vary. One has to take R values with a grain of salt.

The selection of solvent that you put on the board seems to me to be an

over-all simplification. The primary factor is the mixture. You have to have a solvent that dissolves that mixture, and until you get the mixture into solution you cannot do anything with it.

You can proceed on an empirical basis. The tendency should be to dissolve the mixture in the least polar solvent or solution. Then you can increase the polarity of the solvent, using a given sorbent, and you can vary the sorbent. You have solubility, polarity of the solvent, sorbability or capacity of the sorbent system, and you have an equilibrium among solvent, solute, and sorbent as important variable conditions.

W. J. FRIFRSON: That would apply to the selection of the organic solvent.

HAROLD H. STRAIN: This determines nothing about selectivity. I know of no way of predicting selectivity. I would like to raise the question. Could someone here provide an answer? What determines selectivity in these sorptive systems, and can you select two immiscible solvents so that partition becomes as selective as sorption?

ELWOOD O. TITUS: May I comment on that. I would say that probably depends on the nature of the solvent you are using. It has been our experience that, when one uses a partition system in which there is a marked difference in polarity between the two solvents, there is quite marked selectivity, and closely related materials separate rather well. If one is forced to resort to solvents that are not greatly different in polarity such as butanol–water mixtures, then it is more difficult to find solvent pairs which exhibit much selectivity.

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Ion Exchange

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ABSTRACT Ion exchange is promising to become an important tool for research and analysis involving trace materials. As a first approximation, ion-exchange phenomena, particularly with high-capacity exchangers, are independent of the concentration of the trace materials, and their behavior is principally determined by the solution medium. Control of the medium, in turn, allows control over the degree of adsorption of the solute of interest. By choice of suitable conditions, ion exchange permits concentration of trace constituents, sometimes to an enormous extent. Ion exchangers can be used to separate materials which often have similar properties or to identify new compounds not separable by more conventional techniques. Ion-exchange column techniques can be used to remove interfering materials and to prepare the materials of interest for analysis by conventional means. The great simplicity of the technique makes ion exchange a very attractive and inexpensive tool. With modern ion exchangers the bed characteristics remain sufficiently constant over long periods of time to permit the use of elution characteristics for the identification of compounds. Ion-exchange reactions can be used for the elucidation of the structure of trace materials, for identification of functional groups, charge on the ions, and quantitative information regarding equilibria involving these trace constituents.

INTRODUCTION

The field of analytical applications of ion exchange is now generally and rightly considered a branch of chromatography. Approximately 100 years elapsed between the discovery of ion exchange by Thompson and Way (1850) and its broad application to chromatographic analysis. As pointed out by many (see, for example, Lederer and Lederer [80]), modern chromatography began with Tswett in 1903. However, it did not become of general use until its rediscovery by Kuhn and Lederer in 1931. The presently popular method of elution analysis apparently began in 1938 (Reichstein), and the first application of chromatography to inorganic chemistry began about that time.[110] Publication of the wartime work of the Manhattan Project [54] is credited by Lederer and Lederer with being an important milestone in the application of ion exchange to inorganic chemistry. Although ion exchange is historically and, to some extent, logically an inorganic technique, it is surprising that its development as a chromatographic method in this field has been so long delayed.

As recently as 1948 ion exchange still appeared to be the stepchild of chromatography: In a symposium of the New York Academy of Sciences [17] in 1948 a paper on ion exchange was added after the meeting, at the suggestion of one of the editors. A year later in a similar symposium at the Faraday Society [18] 10 out of 38 papers contained "Ion Exchange" in the title, although many more papers either dealt with it or were written by people actively engaged in the field. Today ion exchange appears to have become the dominant field of chromatography. A review here of the enormous ion-exchange literature seems both impractical and unnecessary, since the field is being reviewed frequently (see, for example, the yearly reviews by Kunin [76,77] and the excellent coverage in the *Annual Review of Physical Chemistry* [5,10,58,108,119]). A guide to the large number of excellent textbooks and review articles is given in the bibliography (references with prefix R).

We may wonder what caused this sudden and striking increase in the popularity of ion exchange. Unquestionably one of the main reasons is the availability of commercial organic ion-exchange materials of high capacity, reasonably reproducible properties, and high chemical stability. In the middle 1930's Adams and Holmes obtained their basic patents, which became the starting point for the preparation of modern organic ion-exchange resins. The improvement of

the materials in the following years sparked broad exploitation in practically all fields of chemistry—inorganic, organic, biochemistry and analytical chemistry, as well as hydrometallurgy and other large-scale engineering processes.

Increased understanding of the principles of ion exchange no doubt also contributes to the present popularity of the method. A great deal of work has been carried out with the synthetic exchangers, and predictions regarding the effect on adsorbability of a number of variables, such as ionic strength, cross-linking, solution composition, may be made with considerable confidence.

Present great interest in ion exchange probably also results from increasing awareness that many adsorption phenomena, with materials not usually characterized as ion exchangers, can effectively be described by ion-exchange terminology. As a result, many fields of chromatography are being incorporated in the field of ion exchange by virtue of the method of interpretation used and the nomenclature applied. It is believed that this rewording of "adsorption" phenomena in terms of ion-exchange terminology will continue. Although such rewording is only a semantic device, it may be expected to lead to the discovery of new ion-exchange materials and should permit their more rapid exploitation, because available ion-exchange knowledge may be directly transposed and adapted to the new materials. This is especially true when it can be shown that a given material behaves essentially like an "ideal" ion exchanger, permitting immediately deductions regarding the effect of many variables on the degree of adsorption.

Ion exchange cannot be considered a complete analytical method by itself, because in general it does not permit direct detection or quantitation. For most applications, ion exchange is an adjunct to standard analytical techniques. Specifically, it is principally a preparative method which, through concentration, separation, and isolation, allows preparation of materials in a form suitable for examination by standard analytical techniques, be they volumetric, colorimetric, potentiometric, radiometric, or otherwise. However, the elution rates (or adsorbabilities) under a given set of conditions may be used for identification of materials, for example, of radiotracers, and in some cases, particularly certain biochemical applications, this may be the only technique available for identification.

Application of ion exchange to the analysis of traces presupposes a definition of the term "trace" which at present seems to be lacking.

The term is commonly used in connection with materials at extremely low concentration, often presumed to be so low that accepted analytical techniques fail. From this point of view the definition will thus vary with time and with development of new sensitive detection methods. There seems to be no lower limit to the concentration range at which ion exchange could be used, since most ion-exchange materials, and in particular the modern organic-synthetic ion-exchange resins, though useful at macroconcentrations, work even more effectively and more predictably at extremely low concentrations. Most striking in this connection is the use of ion exchange for the discovery of the synthetic heaviest elements at concentrations of a few atoms per cubic centimeter [117,120,121] and for their isolation at macroconcentrations under essentially the same conditions.

Aside from the sensitivity of available reagents, the concentration range at which trace problems arise may vary enormously with the presence of interfering materials. For such problems ion exchange is an almost ideal tool, since it allows ready separation even of extremely similar materials and often permits simultaneous separation, concentration, isolation, and identification.

In a less usual connotation the term "trace" is applied to materials available in minute amounts, though not necessarily at low concentration. Since ion exchange may be scaled down to very small columns (or sheets), it can effectively be applied to these problems as well.

Sometimes materials may be present at reasonably high concentrations and in relatively large amounts, but their presence may be masked by other materials, which either have similar properties or interfere with available analytical techniques. Though the term "trace" is usually not applied to such systems, they appear to fall logically within the same framework. For many of these, chromatographic (ion-exchange) techniques may be the only ones available, leading to the discovery of the materials and their separation and characterization. One of the most powerful features of ion exchange is its adaptability to any of these "trace" problems with almost equal ease, whether they involve low concentrations, small amounts, or difficult-to-identify materials.

The use of ion exchange in trace analysis does not differ significantly from its application at macroconcentrations. Therefore, essentially all papers on ion exchange are of interest to the trace analyst. Usually information obtained at macroconcentrations is directly applicable, necessitating only minor extrapolations and interpolations of conditions. Conversely, quite frequently analytical methods are de-

veloped with radioactive tracers in the absence of "carriers" and then applied at macroconcentrations. Although such extrapolation must be done with caution because of possible polymerization reactions in the solutions and loading effects, the reverse extrapolation from high to low concentrations is usually simpler and more reliable because, if such difficulties exist, they generally have become apparent at the higher concentrations.

In summary, the principal advantages of ion exchange as an analytical tool are as follows:

Simplicity of Experimental Techniques. Equipment for ion-exchange chromatography is cheap and readily available. Commercial ion-exchange materials have relatively reproducible properties and are stable toward most common reagents. They have considerable intrinsic stability and show little change with time. Columns made from them are generally re-usable, and their regeneration is simple and in some instances may be achieved with water. Most ion-exchange separations can be accomplished with small columns and in a fraction of the time required by other techniques.

Once an ion-exchange method is developed, it is usually adaptable to routine work and with only minor supervision may be entrusted to technicians. Exceptions might be those cases where elements to be separated have extremely similar elution behavior, and judgment (or analysis) is necessary to decide which fractions must be collected for quantitative recovery of each constituent.

Quantitative Separations. Though most commercial ion-exchange materials do not have large specificity for ions of similar structure, excellent separations may still be achieved, since even small columns are equivalent to a large number of equilibrations (theoretical plates), and with modern small-mesh resins the effective height of a theoretical plate is considerably less than a millimeter at quite satisfactory flow rates. Further, a column height suitable for almost any desired degree of separation can usually readily be selected.

Broad Applicability. Essentially any material that can form ions in solution is the proper target for ion-exchange analysis. This includes not only well-recognized ions, i.e., those commonly found in aqueous solutions, but also applies to the less common ions formed by complexing and to non-ionic materials which can be made ionic by various devices such as acid–base or complexing reactions.

Versatility. With essentially the same equipment one may obtain concentration, separation, isolation, and identification of the mate-

rials, and often, with not more than minor modifications, many properties of the solutes may be studied as well.

Insensitivity to Concentration. Adsorption of most materials on ion-exchange resins is insensitive to the concentration of the material in the solutions. The amounts that can be handled safely are, of course, limited by the size of the columns and the capacity of the exchangers. Since the latter is high for most commercial exchangers, this is rarely a problem in analytical work and, in general, if these factors are kept in mind there is generally no upper limit to the concentration that can be handled.

There is no lower limit to the concentration at which ion exchangers can be used. It is not necessary to add "carriers" in the sense that this word is used in radiochemistry, as one might do in precipitation reactions. Actually, from some points of view ion-exchange materials may be considered universal carriers which remain insoluble under practically all conditions but still permit easy isolation of the materials of interest.

Sensitivity to Solution Conditions. Adsorbabilities of ions as well as relative adsorbabilities of ions (i.e., their separability) can be controlled over wide limits by the composition of the solutions. Some of the more important variables are ionic strength (supporting electrolyte concentration), acidity, and presence of complexing agents. If information is available regarding the acid constants of acids (or bases) which are to be adsorbed, or if the complex constants are known, the effect of a change in medium on adsorbabilities and separations can be predicted. Conversely, ion exchange is a tool for the elucidation of the solution properties of the solutes.

Ease of Combination with Other Techniques. Ion exchange lends itself readily to combination with other rapid, modern analytical techniques such as spectrophotometry, polarography, and spot testing. Most powerful, at least in the inorganic field, is the combination of ion exchange with radiometric techniques.

Theoretical Simplicity. Although it is impossible at present, except perhaps in the simplest cases, to predict how strongly an ion is adsorbed by a given exchanger under a given set of conditions, variations in adsorbability with conditions can often be estimated with a high degree of certainty. For trace materials the necessary theoretical considerations are usually simple, involving basically nothing more than a combination of mass-action equilibria.

Ion-Exchange Materials

Ion exchangers might be defined as materials that contain a network or matrix to which are fixed either positive or negative charges. In order to preserve electrical neutrality, these solids must contain either mobile or displaceable ions of the opposite charge (counter ions). If the network carries a negative charge, the displaceable ions will be positively charged (see Fig. 1), and the materials are cation

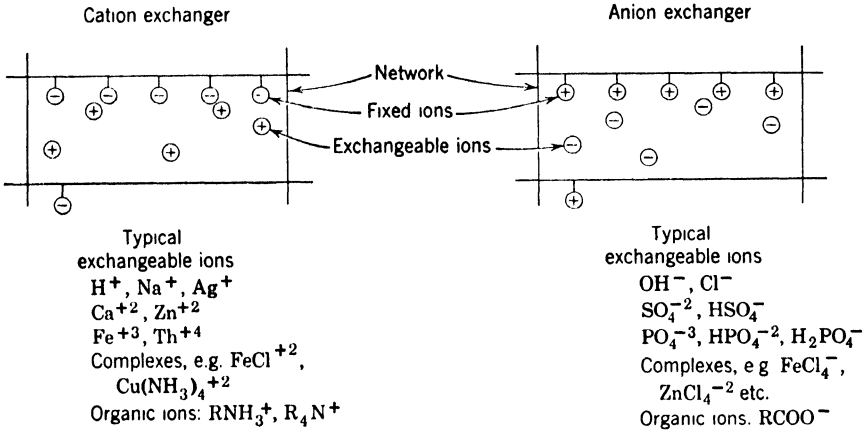

FIG. 1. Diagram of ion exchangers.

exchangers (sometimes, particularly in the older literature, also called base exchangers). Conversely, for a positively charged network the displaceable ions are negatively charged, and the materials are anion exchangers. A very large number of compounds, of course, fall within the framework of such a definition, and even the surfaces of certain salts composed of ionic lattices could be included. A successful ion exchanger, however, must meet additional requirements. Principally, the exchanger must be so constructed that the displaceable ions are accessible: i.e., that they can diffuse out at reasonable rates so that (practical) exchange equilibrium is reached within a relatively short time. For those exchangers that carry their exchangeable ions on the surface of larger particles, e.g., various inorganic solids and to some extent the clays, the solutions in which they are immersed might be considered the diffusing medium. In the synthetic-organic exchangers it is presently believed that the solvent, e.g., water, penetrates throughout the particles or beads more or less uniformly. The

dry exchangers swell on immersion in water, and the swelling water is considered the medium for diffusion. For these, exchange rates will depend not only on particle size, but also on the tightness of the network (degree of cross-linking), which in turn controls the ability of the exchangers to swell.

Unquestionably the most common ion-exchange materials are the clays and similar minerals (zeolites). These exchangers are of fundamental importance in the chemistry of soils. Conversely, study of soils, and particularly of the question why they tend to retain highly soluble salts without the latter being washed away by rains, gave rise

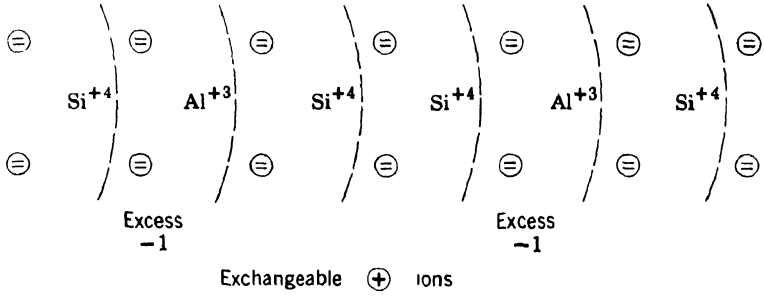

FIG. 2. Diagram of cation-exchange property of clay.

to discovery of ion exchange. For a rather detailed history of the early ion-exchange work see, for example, Kelley.[60] In addition to the clay minerals, the soils contain also other ion-exchange active materials, many of which are organic in nature, such as the humic acids, lignin, lignoproteins.

The clay minerals contain aluminosilicate networks, often sheets, into which negative charges are built by substitution in the lattice of some positive ion by another of lower charge. This leaves an excess of negative charge in this region of the network. The clay will then contain a concordant amount of replaceable positive ions; it is a cation exchanger. This mechanism is illustrated highly schematically in Fig. 2, which confines itself to the substitution Al^{+3} for Si^{+4}. In most clays the substitution of Mg^{+2} for Al^{+3} is at least as important, though it is not so easily described schematically. The actual structure of clays is, of course, very much more complicated, and detailed discussions may be found in various books, such as that by Grim.[44] In addition to the mechanism shown in Fig. 2, further reasons have also been proposed for the cation-exchange properties of clays, and, as mentioned by Grim, clays are also believed to have anion-exchange

properties. However, the mechanism for anion-exchange action is apparently not clearly established and is believed by some to be due to decomposition products of the clays: e.g., oxides.

The natural zeolites, including glauconite (greensands) and the corresponding synthetic aluminosilicates (synthetic zeolites, permutites), comprised the bulk of the commercially available ion-exchange materials until quite recently. Though of great commercial importance, for example in water softening, their lack of stability in acids and bases is the main deterrent to their widespread use in chromatography. Nevertheless, there is a resurgence of interest in the cation-exchange properties of clays, aside from the long-range interest of soil chemists, sparked by the atomic energy waste-disposal problems.[32, 35, 36]

In 1935 Adams and Holmes [1] showed that ion exchangers can also be prepared from organic networks to which certain functional groups are attached. Although they used condensation reactions such as of phenolsulfonic acid with formaldehyde (cation exchanger) or of phenylenediamine with formaldehyde (anion exchanger), various polymerization reactions can also be used. Many such synthetic, organic exchangers are now available. They are produced by a number of companies, such as the Dow Chemical Company (Dowex resins marketed under exclusive license by the National Aluminate Corporation under the name Nalcite resins); the Chemical Process Company (Duolite resins); the Permutit Company, and Rohm and Haas (Amberlite resins). In some cases different companies make exchangers of the same type, whose differences are smaller, at least for an analytical chemist, than the advertisements of the companies would lead one to believe. Extensive lists of exchangers may be found in various books and review articles,[14, 103] as well as in the trade literature of the various manufacturers. Resins may be prepared with aliphatic and aromatic networks. Most widely used at present are the aromatic resins, particularly those based on the copolymerization of styrene and divinylbenzene (DVB), because of their excellent stability. Sulfonation of these copolymers leads to cation exchangers which include such current favorites as Dowex-50, Nalcite HCR, Amberlite IR 120, and Permutit Q. Through chloromethylation and amination, anion-exchange resins may be prepared. With quaternary amines as the functional groups, the hydroxide form of the exchanger behaves as a strong base, and the exchangers are commonly called strongly basic (typical examples, Dowex-1 and -2, Nalcite SBR and SAR, Amberlite IRA 400 and 410, Permutit S). Conversely, resins containing lower amines (primary to tertiary) are classed as weakly basic anion exchangers

(typical examples, Dowex-3, Amberlite IR 45 and IR-4B, Permutit W, Duolite A-2 and A-3). Following the same nomenclature, the sulfonic exchangers in their hydrogen form act like strong acids, and hence are called strongly acidic exchangers, while resins containing more weakly acidic groups such as carboxyls are called weakly acidic exchangers (typical examples: Amberlite IRC-50, Permutit H-70).

One of the main differences between weak and strong exchangers is the unusual position the hydrogen or hydroxide ions occupy in the selectivity series. The weakly acidic exchangers are easily regenerated from an ionic to the hydrogen form, and, conversely, they are generally not satisfactory exchangers in strongly acidic solutions. Similarly, the weakly basic exchangers are most effective in strongly acidic solutions, where the amine group carries a proton and hence a charge. They can be regenerated readily by base to the free-amine form. These weakly acidic and basic resins offer some advantages when regeneration or elution of an ion is difficult. In addition, they also have some unusual selectivities.

The resins so far mentioned are those in widest use. However, many other resins have been prepared, some of which are of interest in specialized applications. Some resins contain phenolic hydroxyl groups (cation exchangers). The older resins, particularly, were often polyfunctional and contained the phenolic hydroxyls in addition to sulfonic acid groups. Also might be mentioned phosphonic ($R-PO(OH)_2$) and phosphonous ($R-P(OH)_2$) acid exchangers and resins containing special chelating groups which are introduced to impart unusual selectivities. A resin that contains heavily nitrated aromatic groups reminiscent of picric acid and that shows high selectivity for potassium over sodium was prepared by Skogseid.[111] Most ingenious is the use of ion exchangers to which antigens are linked for the isolation of specific antibodies from blood serum.[57]

Most of the organic exchangers were originally produced for certain industrial applications, principally water purification, and from the manufacturers' point of view analytical applications are presumably only a minor sideline. Nevertheless, many manufacturers are agreeable to producing and marketing materials more suitable for research work and analytical applications. Thus, many of the resins are available in a variety of mesh sizes, smaller than used in commercial products, and in a wide variety of cross-linking (% DVB).

Although the synthetic organic resins are at present more widely used in analytical work than any other exchangers, it should be pointed out that the list by no means ends with them. Rather, an

enormous number of materials show interesting ion-exchange properties, and with further research some of these may become popular favorites. For example, there are cation exchangers based on sulfonated coal, sulfonated butadiene rubber, and unbleached sulfite pulp. Because of the presence of carboxyl groups, cellulose, as well as oxidized cellulose, has interesting cation-exchange properties. Cellulose pulp, possibly because of cation-exchange properties, has been shown to be an effective chromatographic agent, particularly by British workers.[127] Although filter paper is not normally considered an ion exchanger, it does contain ion-exchange activity,[113] and some of the successes of paper chromatography may be due to this activity. Of considerable interest is the observation of Guthrie, Hoffpauir, and co-workers [45,49,50,59] that cotton fibers may be converted to ion exchangers of considerable capacity. These authors used phosphorylation to produce cation exchangers and amination to prepare anion exchangers. Relatively little use has been made of these materials for analytical purposes, though one might expect them to have many successful applications. Recently Kember and Wells [61] showed that modified cellulose of this type may be made into papers and used for paper (ion-exchange) chromatography.

In the inorganic field many materials other than the clays presumably have ion-exchange activity. Thus, Schwab [110] suggested that aluminum oxide, if washed with base, would behave like a cation exchanger, and, if washed with acid, as an anion exchanger. A similar conclusion may be drawn from many adsorption studies, e.g., those of Kolthoff and Overholser on adsorption of dyes on ferric hydroxide.[65] Presumably at some intermediate acidity anion- and cation-exchange properties would overlap, yielding materials that can adsorb both positive and negative ions of a salt. Anion-exchange properties of hydroxyapatite and calcium phosphate presumably are responsible for the adsorption of fluoride ions [2,81] and of other materials.[122] A systematic investigation of the ion-exchange properties of various amorphous or microcrystalline inorganic materials has been initiated at the Oak Ridge National Laboratory. It has been found that many oxides such as those of Zr(IV), Ti(IV), Sn(IV), and Th(IV) have anion-exchange properties in acidic solutions and that the materials prepared by precipitation and subsequent drying have reasonably high capacities and satisfactory ion-exchange rates.[69] Similar to the earlier observations for aluminum oxide, most of these materials were found to develop cation-exchange properties in basic solutions. In a search for new cation-exchange materials, certain precipitates of zir-

conium with polyvalent anions such as phosphate, tungstate, molybdate, and arsenate have been investigated. Some of these, too, show reasonably high capacities, interesting selectivities, and satisfactory exchange rates. Zirconium phosphate [70] and zirconium tungstate,[71] for example, have been used for the separation of the alkali metals with results that compare favorably with the organic-synthetic exchangers, as illustrated in Fig. 3. In view of these results, it is my

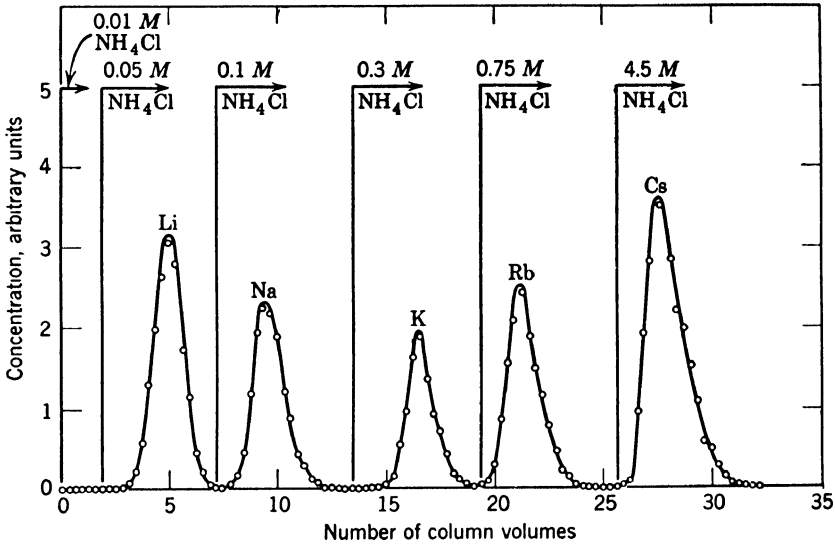

FIG. 3. Separation of the alkali metals. Zirconium tungstate: 12.3 cm × 0.13 cm^2 column. Flow rate: ca. 0.75 cm per min.

belief that the future holds considerable promise for the development of new inorganic exchangers which at times may not only be competitive with the synthetic organic exchangers but in some applications may even supplant them. These materials, however, are mainly a promise of the future, and hence the present paper will not consider them further. The remaining pages will be devoted entirely to the organic ion-exchange resins: particularly their most widely used representatives, the strongly acidic cation exchangers and the strongly basic anion exchangers.

SOME PROPERTIES OF THE SYNTHETIC-ORGANIC EXCHANGERS

Since resins based on polystyrene–divinylbenzene copolymers are used mostly in analytical work, the following discussion will be ori-

ented toward them, though many of the considerations, of course, apply to the organic exchangers in general.

Swelling. When ion-exchange resins are immersed in aqueous media, a considerable amount of water is taken up, and as a result the resin usually swells. The amount of water pickup and swelling is strongly dependent on the degree of cross-linking of the exchangers. For the polystyrene–divinylbenzene resins, cross-linking is defined in terms of the per cent divinylbenzene added during polymerization. This is noted by some manufacturers on the package by a number preceded by X, e.g., Dowex-1 X 10 means Dowex-1 prepared with 10% DVB in the copolymerization step.

While swelling is relatively small at high cross-linking, it may become enormous at low cross-linking. Thus, Calmon [15] showed that a sulfonated polystyrene exchanger containing 0.25% DVB had a 50-times larger volume when immersed in water than when dry. At 0% DVB, no cross-linking, swelling would become infinite: One then deals with the soluble polyelectrolyte on which the exchanger is based. At a given degree of cross-linking, swelling of the resins is sensitive to the composition of the solutions. In general, the higher the salt concentration of the solutions, the smaller the swelling of the immersed resin.[4] Swelling is also influenced by the mobile ions in the exchanger, and at least in some cases, as pointed out particularly by Gregor and co-workers,[42] the more strongly adsorbed ions permit the least swelling. Differences in the degree of swelling of the same resin containing different ions can, especially for the low cross-linked resins, be utilized for analysis. For example, Calmon [15] took advantage of the fact that the calcium form of the exchanger is very much less swollen than the sodium form for detecting calcium breakthrough in water-softening processes. He also suggested the utilization of these volume characteristics for the determination of *p*H and dissociation constants of acids.[16] In the usual analytical work these changes in volume with resin composition and aqueous electrolyte concentration are a considerable disadvantage, because they tend to destroy good packing of the columns, and with excessive volume increases may even shatter them.

Rates. Ion-exchange rates, at least with the sulfonic acid exchangers, seem to be principally diffusion-controlled.[9,11,73,98,112] We shall ignore here the case where the rate-controlling step is film diffusion (through the "stationary" solution film surrounding the beads) and confine ourselves to particle diffusion. Then, the rates are clearly dependent on the degree of cross-linking of the resin, presumably

because of differences in the amount of swelling. This is also borne out by the observation that the carboxylic exchangers in the hydrogen form, which show little swelling, show very slow exchange rates compared with the sulfonic acid exchangers or compared with carboxylic exchangers in ionic forms in which they are swollen. At very low cross-linking, the diffusion rates within the exchanger appear to approach those in aqueous solutions, at least for ions of low charge. At high cross-linking, diffusion coefficients may be several orders of magnitude smaller than at low cross-linking. Diffusion coefficients, at a given degree of cross-linking, are strongly dependent on the charge of the ions for cation exchangers and less so for anion exchangers.

Since most studies of diffusion rates in ion exchangers are carried out at low ionic strength where the resins show essentially maximal swelling, the reported diffusion coefficients are probably the largest that can be observed with given ions and a given exchanger. At high ionic strength where the resins are less swollen, decreased diffusion rates are expected, with the result that under these conditions equilibration may become extremely slow.

Screening Effect. If one visualizes an ion-exchange resin immersed in an aqueous medium to consist of a three-dimensional organic network distended by the imbibed swelling water, one can see that it has an effective pore size that should impose limits on the size of ions that can readily penetrate into the exchanger. This pore size is dependent on the degree of cross-linking and the medium, and thus can to some extent be controlled. Conversely, it may also be taken advantage of as a screening effect for exclusion of large ions from the resin, decreasing their adsorptive capacity by confining the materials to the surface of the beads, while permitting penetration of smaller ions from which separation is desired.[74]

Selectivities. The selectivity of exchangers, i.e., the relative preference they show for one ion over another, is profoundly affected by the cross-linking. For simple ions, such as the alkali metals or alkaline earths, selectivities increase at moderate cross-linking from lithium to cesium and from beryllium to barium. Further, the selectivities tend to increase with cross-linking. However, at the same time selectivities become increasingly dependent on the fractional conversion X of the resin from one form to the other. In some cases [97] the effect may become so large as to amount to an inversion of selectivities. Although the reason for this behavior is not clearly understood, it is believed by some to result from uneven copolymerization, existence of highly knotted regions within the exchanger beads compared with the aver-

age knotting expected from the degree of cross-linking. Supporting this point of view is the work by Graydon and co-workers who have prepared, by carefully controlling the polymerization reactions, ion exchangers which show little change in selectivity with conversion from one form to the other.[116]

In the case of anion exchangers, selectivities for simple ions have not been studied as thoroughly, though extensive lists are given by Wheaton and Bauman,[128] Kunin and McGarvey,[78] Peterson,[94] and more recently by Gregor and co-workers.[43] For the halides, least adsorption is usually found for the fluorides and strongest adsorption for the iodides. Most striking is the enormous and quite unpredicted selectivity which the strongly basic anion exchangers have for a number of complex ions and which has become the basis of a large number of anion-exchange separations of metals. At present it is not known if this unusual selectivity results from a property of the organic network or from the quaternary amine groups of the resin, or both. However, it has been shown [47] that this selectivity is influenced by cross-linking (or swelling) in much the same way as the selectivities of cation exchangers are affected by cross-linking.

Current theory tends to interpret many ion-exchange phenomena, particularly observed selectivities within coherent groups of simple ions, in terms of a concentrated electrolyte analog, first apparently suggested by Bauman and Eichhorn.[4] In this model the swollen resin is considered as a system in which the functional groups and their counter ions are "dissolved" in the swelling water. If we compute the concentration of these ions in terms of moles per kilogram of swelling water, the figures become extremely high: 5 to 10 moles per kilogram of water for standard cross-linked resins (8–10% DVB) and considerably higher for special high cross-linked materials. Parallels have been drawn between the properties of exchangers and aqueous solutions of equivalent concentration. For example, properties of the sodium form of sulfonic acid exchangers may be correlated with concentrated sodium phenylsulfonate solutions. Not inconsistent with this picture is the model that ion-exchange resins are closely related to polyelectrolytes, though differing from them in that the exchangers are cross-linked into a three-dimensional network and hence made insoluble. This picture should continue to be fruitful not only for interpretation of resin phenomena, but also for the study of the nature of polyelectrolytes. For typical references, see 7, 8, 12, 13, 20, 29, 37, 40, 42, 53, 64, 93, 100.

Imbibed Electrolyte. The statement that ion exchangers imbibe water only is an idealization applying to solutions of negligible salt content. At finite salt concentrations the electrolyte of the medium also penetrates into the exchanger together with the solvent water.[4] This effect is relatively small at moderate ionic strength and has been taken advantage of in the process of ion exclusion [129,130] where neutral molecules are permitted to enter the exchanger in preference to the salt, with resultant separation of the two. The reason for the exclusion of the electrolytes from exchangers is usually described in terms of a Donnan membrane effect: The resin is compared with a membrane within which a high concentration of electrolyte exists, restricting further entry of the common ions. At high external electrolyte concentrations, however, this effect becomes essentially swamped, with the result that, for an exchanger immersed in concentrated electrolyte solutions, the "inside" electrolyte concentration (per kilogram of swelling water) may not only equal the "outside" concentration, but may actually exceed it.[67]

Conductivity—Membranes. The strongly acidic and strongly basic ion-exchange resins have considerable conductivity. The same appears to be true for the weakly acidic exchangers in their salt form, though not in their acid form. This conductivity appears to be qualitatively consistent with the "concentrated electrolyte analog" currently used as a resin model.[58,115] In recent years considerable effort has been expended in the study of membranes made from these exchangers. Membranes made from cation exchangers permit transport of cations principally, and those made from anion exchangers permit principally transport of anions. These properties have considerable implication both for electrochemical applications and water purification. Little advantage seems so far to have been taken for analytical uses. Detailed discussion of the properties of such membranes may be found in a recent symposium.[19]

Equivalent and Nonequivalent Exchange. It is usually assumed that during exchange for each equivalent of ions entering a resin an exact equivalent must leave it. Thus, if a cation exchanger in the sodium form is permitted to exchange with potassium ions, one mole of sodium ions is assumed to leave for each mole of potassium ions entering the exchanger. This assumption is a direct consequence of electroneutrality considerations, provided the exchanger contains no other mobile ions and particularly no mobile ions of the opposite charge. This assumption of equivalent exchange is no doubt very good in dilute electrolyte solutions. Nevertheless, it is only an idealiza-

tion and equivalent exchange does not necessarily occur in concentrated electrolyte solutions. Sometimes, even at moderate electrolyte concentrations, noticeable deviations might occur.[3] At high electrolyte concentrations, deviations from equivalence may become very large because the amount of imbibed electrolyte depends on the form in which the exchanger exists. For example, in concentrated HCl a large amount of hydrochloric acid enters an anion exchanger in the chloride form. However, if the resin has adsorbed a considerable quantity of complex ions, e.g., $FeCl_4^-$, the HCl content even in concentrated HCl solutions may be quite small. Hence, stoichiometrically, for each $FeCl_4^-$ ion entering, more than one chloride ion has left, taking along of course (electroneutrality) a sufficient number of hydrogen ions. Many other examples may also be cited where equivalent exchange is not expected to occur. The situation will always arise if the exchanger contains in one way or another a significant amount of the ions of opposite charge than implied by the type of the exchanger.

Capacity and Loading. The capacity of exchangers C may be defined in terms of the number of equivalents of exchange sites per unit weight or unit volume of exchanger. This quantity is needed for estimation of the amount of a material that can be adsorbed on a given amount of resin. Organic exchangers in general have rather high capacities, usually of the order of 3 to 6 equivalents per kilogram of exchanger (or milliequivalents, meq, per gram). Since the density of the exchanger beds is considerably less than unity, capacities on a volume basis are substantially lower, usually one to two equivalents per liter (meq per ml) and of course strongly dependent on swelling characteristics. For trace-analytical purposes the limited capacities of the exchangers are rarely a problem. Capacity of the exchangers, however, enters analytical problems, also indirectly, through the effect of fractional conversion of the exchanger, or loading, on adsorbabilities of a given ion. In this sense we shall define loading, L, by the relationship

$$L = a(A^{\pm a})_r/C$$

where $\pm a$ is the charge on the ion A and $(\,)_r$ indicates concentration in the resin phase. As pointed out earlier, selectivities and adsorbabilities are strongly affected by loading, particularly for highly cross-linked resins.

Adsorption of Non-Ionic Materials. Non-ionic materials can be adsorbed by the organic ion-exchange resins. Typical early work on

this adsorption is the rather extensive study of Gregor and co-workers.[41] Such adsorption is of considerable interest in connection with ion exclusion which was mentioned on page 49. Taking advantage of differences in rates (screening effect) and differences in adsorbabilities, it is now possible to use the organic ion exchangers for the separation of various non-ionic materials [130] in typical partition chromatographic manner. Though the future looks bright for such applications of ion exchangers, so far it does not seem to have been exploited widely for analyses.

EXPERIMENTAL TECHNIQUES

As implied earlier, the use of ion-exchange resins in analytical work is exceedingly simple. In the present section an attempt is made to elaborate on this theme, and to discuss some of the techniques. Resins have been used in such a large number of different methods, often designed for special applications, that a review of these techniques is not feasible here. Again, reference will have to be made for further details to the books (particularly the excellent monograph of Samuelson [103]), review articles, and individual papers. Instead, this discussion is written principally from the author's point of view, whose experience in the main has been confined to anion-exchange separations of metals, which were preceded by a search for conditions where the ions to be separated have large differences in adsorbability. For these the techniques need usually not be so elaborate as those employed for separation of materials with small separation factors: e.g., the rare earths. The techniques to be described are thus considered the simplest available, and for more difficult problems, as well as routine analytical use, some elaboration may become necessary.

Preparation of Resin. For many purposes ion-exchange resins may be used essentially as received from the manufacturer, and often it is only necessary to wash out the "fines" and to convert them from the form shipped to the form desired. The latter is usually desirable even if the resin is to be used in the form in which it is nominally shipped. Conversion may be achieved either by repeated contacting of the exchanger with a moderately concentrated solution of the appropriate electrolyte or, preferably, by placing the exchanger in a relatively large-diameter tube and passing the solution through the column. To avoid excessive volumes (minimum volume is determined by the amount of exchanger, its capacity, and the solution concentration), conversion should be carried out at high electrolyte concentration, but concentrated electrolyte solutions are undesirable

because, with these, exchange rates may become so slow as to necessitate extremely slow flow rates. For exchangers in the range of 8 to 10% DVB we have found 0.5 to 2 *M* electrolyte solutions satisfactory. After conversion (or regeneration) the exchangers may be washed free of interstitial and imbibed electrolyte. Particularly in trace analysis, extremely pure water is needed, or water specifically purified from the ions whose analysis is desired, to avoid recontamination. The same problem arises, of course, with the reagents used for conversion or regeneration. Sometimes "prepurification" of the reagents and water may be achieved by passing them through a small exchanger column which precedes the main column.

Many exchangers as received from the manufacturer contain considerable organic and inorganic impurities, such as the monomers or low-molecular-weight polymers from which the resins were prepared, or impurities picked up from catalysts, corrosion products, etc. This is particularly serious for cation exchangers from some of which ions typical for the composition of stainless steels may be eluted before purification. Removal of these foreign materials can usually be achieved by washing the exchangers (e.g., in a column) with relatively concentrated and dilute acids. Sometimes it is also advantageous to wash with strong bases. Organic impurities are best removed by washing with acetone, alcohol (methyl or ethyl alcohol), or ethers, followed by washing with water. At present a number of suppliers sell a so-called analytical grade of ion exchanger, usually at a considerable premium in price. It is doubtful if some purification of such resins would not still be needed for trace-analytical applications.

Preparation of Columns. For most purposes ion-exchange columns for analytical use can be surprisingly small. Thus, most of the work carried out in our laboratory involves columns less than 5 cm long and usually less than 0.2 cm^2 in cross-sectional area (A). Since the time necessary for a given separation is directly proportional to the height of the column, unnecessary length should be avoided. From preliminary experiments, particularly regarding the number of theoretical plates per unit length and the relative adsorbabilities of the materials to be separated, optimum column sizes may readily be estimated, as discussed later under Column Theory.

While the length of columns is principally determined by the ratio of the adsorbabilities of ions to be separated, their diameter mainly reflects the volume of solution to be processed or the quantity of material (number of equivalents of ions) to be adsorbed. Thus, if the charge of the ion is known, the number of grams (or cubic centi-

meters) of resin necessary for complete adsorption can be calculated and a length–diameter ratio chosen for the column concordant with the desired separation. Since only the unloaded portion of the exchanger is effective for separations, the amount of resin used is larger than is implied by this minimum calculation. Sometimes a factor of two above this minimum is sufficient, though this factor may become as large as twenty for difficultly separable materials.

For analytical purposes, where flow rates are only a minor consideration, it is always advantageous to use resins of small mesh size, e.g., in the range 100 to 200 mesh, and in some instances it may be essential. Rates of equilibration increase with decreasing particle size, and hence at a given flow rate much sharper elution bands may be obtained with small particles than with larger ones. Use of small mesh particles also has the additional advantage that (in aqueous solutions) no additional devices are needed to prevent small columns from running dry, capillary forces being sufficiently great to prevent drainage of the solution below the top layer of the columns.

A narrow range of mesh size is desirable because uniform particles give more satisfactory flow properties. It is usually worth while to screen the resin even though this is a tedious operation, particularly with spherical "beads" which tend to clog the screens. Wet screening is greatly preferred by us over dry screening, since in the latter method the beads tend to break and large amounts of "fines" are produced which later must be washed out again.

Typical columns as used in our laboratory are made from small-diameter glass tubing, pulled out at the bottom, or, more usefully, they can be made directly from serological pipets (or burets), cut off to a convenient size if necessary. A wad of glass wool is placed in the conical section to retain the resin. With a small length of rubber or plastic tubing at the bottom of the columns and a pinch clamp, flow rates can be controlled or column flow stopped. The tubes may easily be filled by first pulling into them water through the bottom and then adding a slurry of the resin through the top. The resin is permitted to settle, and the columns are usually ready for use within a few minutes, the density difference between resin and water being sufficient for rapid settling. In some cases, e.g., if concentrated electrolytes are to be added in which the resins tend to float or if the top layer of the exchanger bed is disturbed during reagent additions, it is advisable to top the column with a layer of glass wool or of small-mesh glass beads.

Essentially the same technique for preparing columns may be used for solutions that attack glass by substituting plastic tubings. For these, retaining plugs can be made of plastic (e.g., Lucite) shavings, or similar inert materials.

The simple columns described can be prepared in a few minutes if properly treated resins are available and thus are particularly attractive for research use. For routine operations or in special cases, more complicated columns may be advantageous, e.g., with glass stopcocks, enlarged upper sections for greater volumes of solution, side arms for addition and change of reagents, etc.[103]

Column Operation. Techniques of operating columns depend largely on the purpose of the experiments. Thus, for large-volume reduction, as in the concentration of a trace constituent, the solution can be placed in a large separatory funnel or bottle which is connected to the ion-exchange column. Flow is permitted until break-through. For such purposes, devices for maintaining constant flow are not necessary.

For separations, an aliquot of the solution may simply be placed into the void volume above the column. The solution is permitted to seep under gravity into the column. Wash liquors and eluting solutions are added when necessary. If effluent fractions of known volume are desired, e.g., to plot an elution curve, it is often simplest to add the eluent in discrete fractions from a micropipet, adding a new aliquot only after the previous one has drained to the top of the bed. If the column was prepared from a serological pipet (or from a buret), the top section can be reserved for the eluent and the volumes read directly from the graduations. For larger volumes the column can be connected to a buret. More elaborate devices are usually needed for separations involving extremely difficultly separable materials where constancy of flow rates is important. For these, automatic fraction collectors are also desirable. However, they should not be necessary in many, if not most, analytical applications where, after a search, adsorbing and eluting conditions may often be found for completion of separations with only a few column volumes of solution.

For most small-mesh modern organic exchangers, equilibration rates are sufficiently fast for operation at flow rates of the order of 1 cm per minute (1 cm^3 per cm^2 per minute). Much slower flow rates should be necessary only in difficult separations, such as of the rare earths, whereas in some cases considerably higher flow rates may be satisfactory. To be sure, large flow rates may necessitate overdesign-

ing the columns to some extent, but the advantage gained from the higher flow rates could easily compensate for this.

Development of Separation Methods. Although prescriptions for the search for new analytical methods are very dangerous, since the most effective methods of the future will probably not be foreseen, a few guiding principles may be listed.

In general, it is most desirable to find adsorption conditions under which the trace constituent is adsorbed and the macroconstituent not. This allows use of minimum-sized columns and in addition often yields a volume reduction. In adsorption of the macroconstituent, the capacity of the exchanger will impose design limitations, and the trace constituent usually becomes diluted.

For the adsorption of a trace constituent, conditions for highest possible adsorbability should be sought to permit maximum concentration. To take advantage of this high adsorbability for large-volume reductions, another set of conditions should be available where the trace constituent shows little adsorbability and hence can be eluted in a small volume.

Although it appears trivial to state that for adsorption of positive ions a cation exchanger should be selected and for adsorption of negative ions an anion exchanger, it must be remembered that the sign of the charge of the ion to be adsorbed can often be controlled, as well as the magnitude of this charge. Thus, normally positively charged metals can be complexed to yield negative ions and then adsorbed on an anion exchanger. The reverse should also be possible, though this as yet has not been exploited. Similarly, neutral molecules can be complexed to yield charged materials. If solution equilibria are rapid compared with the ion-exchange equilibrium, the various complexes of a given element or compound are not separated from each other, and the degree of complexing controls the adsorbability of the material as a whole. When solution equilibria are slow, as is the case, for example, with Cr(III) complexes or the polyphosphates, separation of the complexes from each other occurs.

It is apparent that development of an analytical technique implies a rather extensive search for optimum conditions which, in turn, implies a large amount of preliminary experimentation. A number of the available methods will be discussed in the next section.

Determination of Adsorbability. Adsorbability of an ion may be expressed in terms of the number of column volumes necessary for its elution. If a thin band of an element is placed on a column and elution carried out with a given medium, the effluent concentration

will trace out the characteristic bell-shaped curves (Fig. 4), approximately Gaussian, if the column is operated near equilibrium and if the adsorption isotherm is linear: i.e., if the concentration in the

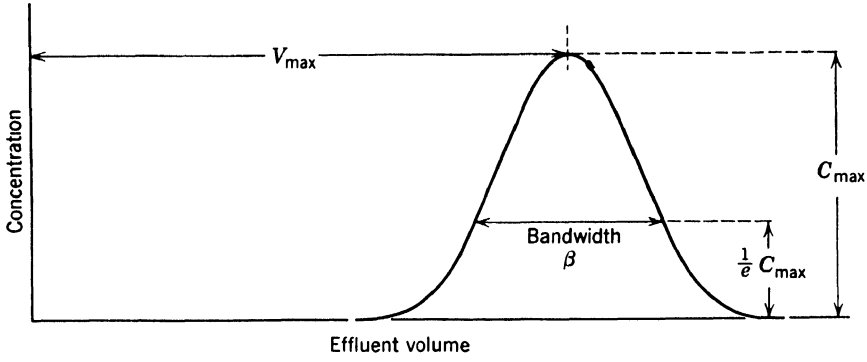

FIG. 4. Typical elution curve.

resin is proportional to the concentration in solution. Actually, as illustrated in Fig. 5, ion-exchange reactions cannot follow linear adsorption isotherms except at low loading, where the resin remains

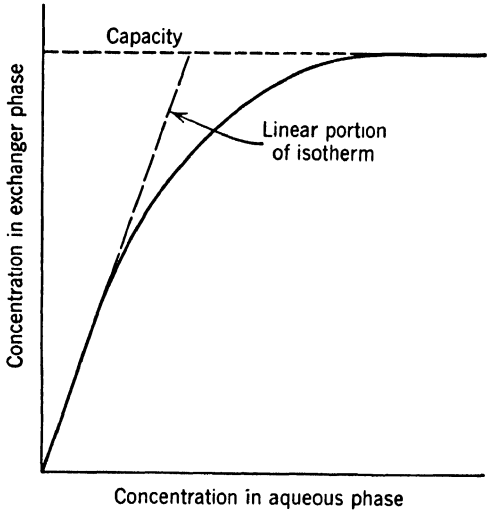

FIG. 5. Adsorption isotherm of an ion exchanger.

essentially in one form and, for practical purposes, independent of the amount of a second ion adsorbed.

The volume at which the effluent in an elution experiment like Fig. has maximum concentration is characteristic for the adsorbability

of the ion. We shall designate it by the symbol V_{max} if expressed in terms of column volumes, where the column volume is here defined by the geometry of the column: cross-sectional area A (cm^2) times length l (cm). The elution method, in addition to characterizing adsorbabilities, also gives a check on ideality of column behavior (symmetry of elution band) and permits estimation of the number of theoretical plates in the column, and hence is an aid for computation of the optimum column lengths for separation (see later under Column Theory). Asymmetrically shaped elution bands may result

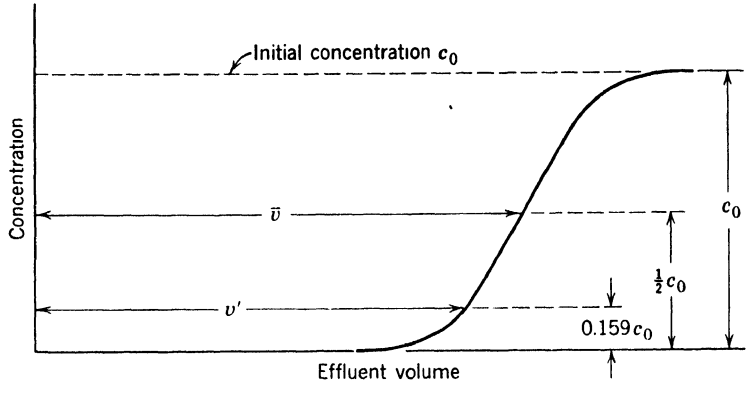

FIG. 6. Typical break-through curve.

either from too high a flow rate (nonequilibrium behavior) or from nonlinearity of the adsorption isotherms, which always occurs if column loading is too high.

Adsorbabilities may also be determined in break-through experiments. A solution containing the ion to be adsorbed is continuously passed through the column, and the effluent concentration traces out a characteristic S-shaped curve (see Fig. 6). The volume $\bar{v}$ at the inflection point is characteristic of the adsorbability and should be equal to V_{max} if the measurements are carried out at comparable loading and expressed in the same units.

Effluent concentrations may be obtained by almost any analytical technique, either continuously or with successive aliquots. If only determination of V_{max} is the objective, semiquantitative tests are satisfactory. In particular, we have found a series of spot tests to be of advantage and location by eye of the volume at which the test is maximal. In this case the column is best designed to have a volume small enough so that a single drop constitutes an appreciable fraction of a column volume. Then, all drops can be collected in the succes-

sive depressions of a spot plate, and aliquoting for analysis becomes unnecessary.

In spite of the many advantages of the effluent-column method, it has several disadvantages: A large number of analyses is necessary for completion of a single elution curve which, though no serious drawback with a rapid analytical technique such as spot testing or radiotracer analysis, may become a considerable deterrent with slower techniques. Further, if adsorption is at all large, the method is very time-consuming since many column volumes are necessary to reach V_{max}.

Measurement of the rate of migration of a band along an adsorption column may be substituted, though often with a loss of accuracy, for the elution method. In this case, one can measure the elution constant E, defined by the equation

$$E = dA/v \tag{1}$$

where d is the distance the adsorption band travels on addition of v cm^3 of eluent in a column of cross-sectional area A cm^2. Since for complete elution d becomes equal to 1, it is easily seen that

$$E = 1/V_{max} \tag{2}$$

For this method d may be determined essentially with a meter stick for colored ions and for radiotracers by scanning the column.

Adsorbability of an ion may also be determined by the technique standard for any two-phase equilibrium: equilibration of an aliquot of the solution with a weighed amount of resin. From determination of the relative concentrations in the resin and solution phases, often simplest (though not most accurately) achieved by measuring solution concentration before and after equilibration, the distribution coefficient D, defined by the equation

$$D = m_{(r)}/m \tag{3}$$

may be determined, where $m_{(r)}$ and m are the concentrations in the resin and solution phases, respectively. These concentrations will be expressed in the units amount per kilogram dry resin divided by amount per liter of solution. Though many other units could also be chosen, we shall additionally define only the volume distribution coefficient D_v in terms of amount per liter of exchanger bed divided by amount per liter of solution. Distribution coefficients expressed

in various units are related by conversion factors, and the relationship between D and D_v is given by

$$D_v = D\rho \tag{4}$$

The bed density ρ (kilograms dry exchanger per liter of bed) is determined in separate experiments: e.g., by measurement of the volume of a column filled with a known weight of exchanger. It is strongly dependent on cross-linking and to some extent on the packing of the column. For the exchanger that we have used most frequently (Dowex-1, ca. 10% DVB), ρ is ca. 0.45.

Distribution coefficients determined in this manner may readily be related to column behavior by the relationships

$$E = 1/(i + D_v) \quad \text{and} \quad 1/E = V_{\max} = i + D_v = i + D\rho \tag{5}$$

where i is the fractional interstitial space of the column, usually ca. 0.4.

Unfortunately, i is difficult to determine accurately. Presumably, it is given by the elution constant or band velocity for $D = 0$. However, because of penetration of electrolyte into the resin, D is rarely zero, even for nonadsorbable ions. The uncertainty in i is usually not large, however, and is not of great import when D is large.

The equilibration technique is specially adapted to measurement of fairly large distribution coefficients; i.e., it is ideal in the search for conditions where optimum adsorption should be carried out or for establishment of conditions where maximum degree of concentration may be achieved.

In some cases it may be desirable to determine distribution coefficients considerably larger than 1000, e.g., in extremely difficult concentration problems or if the results are necessary for theoretical purposes such as determination of characteristic properties of the solutes. It is then often more efficient to take recourse to another type of column method where the material is first adsorbed on an exchanger, preferably under conditions where the rates of adsorption are fast, even if the distribution coefficients are low. The resulting uniformly loaded resin is placed into a column. The eluting solution in passing through the column will rapidly come to equilibrium with the exchanger, and analysis of the concentration of the effluent combined with the known concentration in the exchanger permits computation of distribution coefficients. The principal advantage of this method results from the fact that equilibrium is rapidly reached at high values of D since only a small amount of material needs to leave the

exchanger for equilibration and since the time necessary for equilibration varies with the square of the distance of penetration necessary.

One additional method may be mentioned, though it is principally designed to measure adsorbabilities at high loading (adsorption isotherms from zero to 100% conversion). In this method [35] the exchanger is first equilibrated with a given solution, and then an essentially identical solution which contains tracers of these same elements is passed through it: Isotopic exchange will occur. From the integral under the elution curve the necessary quantities may be computed. If two tracers are simultaneously passed through the bed, the method even permits an unambiguous evaluation of the interstitial volume of the column.

Capacity, Dry Weights, Water Content, Interstitial Volume. Capacity of an exchanger is usually defined in terms of the number of fixed charged (or ionogenic) groups in a given amount of exchanger. It may, with reasonable precision, be determined for the strong-acid and strong-base exchangers by displacement of the replaceable ions present with other ions. For example, the chloride content of a strong-base anion exchanger may be determined by treatment with an excess of sulfate or perchlorate solution, followed by chloride analysis of the total effluent. To avoid difficulties from imbibed electrolyte, the resin should previously be washed, though in moderation, because otherwise some "decomposition" or "hydrolysis" may occur: e.g., displacement of chloride ions by carbonate or hydroxide ions of the wash water.

It has become customary to refer capacities, distribution coefficients, etc., to the dry weight of the exchanger. Usually this means an exchanger that had been dried near 100° C in an oven to constant weight. Actually, however, such constant weights are rarely achievable because of decomposition at elevated temperatures. Drying at lower temperature (40° to 60° C) in a desiccator over a good dehydrating agent (e.g., Anhydrone) appears preferable and has been used extensively in our laboratory. Even this method, however, has some disadvantages if highest precision is desired, since decomposition may still occur and since resin weights tend to change, particularly when handled in a humid atmosphere. Since the reference state is arbitrary, it appears more suitable to dry the resins at a constant and clearly defined humidity, e.g., 50%, where handling problems become less serious, as well as decomposition. Experimentally such weights can be obtained by equilibrating the resin in a desiccator containing a saturated salt solution of definite humidity. For most practical purposes it is

sufficient to air-dry the resins, keep them in a well-stoppered bottle, and only occasionally check for moisture content by drying either over Anhydrone or at a given humidity. Because of danger of irreversible changes, dry weights should be determined only on aliquots of the resin which are not needed for any other experiments.

Water content of the exchangers is difficult to measure accurately, since it implies a weight determination at zero and 100% humidity. At 100% humidity the resins will cake, and a considerable amount of "interstitial" water is adsorbed. At 0% humidity the resins tend to decompose, and, at any rate, a definite weight can only be obtained ideally. The water content, which is the hypothetical difference between resin at 100% and at 0% humidity, thus can only be obtained by extrapolation. Various methods have been proposed, and for detailed discussion reference should be made to the original literature.[92,67]

Estimation of the interstitial volume of a column is difficult, and its definition is obscure. Ideally it implies the volume, e.g., of water, between the resin particles in a bed. Various experimental procedures have been used to approximate this volume. They are principally based on measurement of the volume at which a nonadsorbable small ion or large dye molecule appears in the effluent. Though satisfactory for approximate or practical evaluation of interstitial volumes, they cannot be considered precise. Unambiguous determination seems feasible by the double-tracer technique proposed by Gaines and Thomas.[35]

Determination of Properties of Solutes. Investigation of the properties of solutes by ion-exchange techniques usually involves determination of adsorbability as a function of a number of variables. The method has been used by a large number of investigators and particularly by Schubert and co-workers.[104,105,107,109] Any of the methods listed earlier for determination of adsorbability are applicable and some of the pertinent theoretical considerations will be discussed under the headings Ideal Exchange and Effect of Solution Equilibria on Separations.

COLUMN THEORY

The chief considerations on the behavior of ion-exchange columns are given by chromatographic theory. As first proposed by Martin and Synge,[84] chromatographic columns may be compared with distillation columns, and hence the pertinent column parameter is expressed in terms of the effective height of a theoretical plate (EHTP)

which, together with column length, gives the number N of effective plates. Many valuable contributions have been made to this theory, such as the work of Mayer and Tompkins [86] and more recently of Glueckauf.[38,39] Exact kinetic equations (not involving a stepwise process) are discussed by Thomas,[118] Hiester and Vermeulen,[48] and others, and tables for predicting column performance computed by Opler and Hiester.[91] Since Glueckauf treats in considerable detail the question of purity to be expected in a chromatographic separation of two substances and especially stresses ion exchangers, we shall use his papers as a basis for present discussion.

Column behavior is determined by many factors, such as magnitude of the distribution coefficients, their variation with loading (isotherm), flow rates, particle size, diffusion coefficients in the solution and the resin, and temperature. The shape of the elution curves depends on whether particle diffusion or film diffusion are the rate-controlling steps.

Most theoretical considerations have been confined to the cases where the adsorption isotherm is linear, i.e., where the concentration in the resin phase is proportional to the concentration in the aqueous phase, or where D is constant. We shall restrict our considerations also to this limiting case.

Glueckauf gives two principal methods for the determination of the number N of theoretical plates in a column and hence of the effective height of a theoretical plate EHTP. He shows that in a break-through elution curve (see Fig. 6) N is given by

$$N = \frac{\bar{v}v'}{(\bar{v} - v')^2} \tag{6}$$

Here $\bar{v}$ is the volume of effluent at the center of the S-shaped break-through curve where the concentration c is one-half of the initial concentration c_0 and v' is the volume at which the effluent has the concentration $0.1587c_0$.

In the elution of a thin adsorption band where the effluent concentration traces the characteristic bell-shaped curve (Fig. 4), N may be determined from the volume V_{max} at which the concentration is at a maximum (c_{max}) and from the band width β measured at the concentration $(1/e)c_{max}$, by the equation

$$N \approx 8(V_{max}/\beta)^2 \tag{7}$$

The number of effective theoretical plates in a given column is not a constant. Not only does it vary with flow rates and diffusion prop-

erties of the solutes, but in particle diffusion EHTP varies almost linearly with $1/D$, and hence, according to Glueckauf, "It cannot be considered constant even within the same experimental run for different ionic species."

The values of EHTP for fine-mesh ion-exchange resins may become surprisingly small, and hence it is feasible to carry out separations with short columns, even when the separation factor S (defined as the ratio of the distribution coefficients of the ions to be separated) is quite small. Glueckauf states that in the excellent rare-earth separations of Ketelle and Boyd [62] EHTP was ca. 0.1 cm and that this value was still a factor of 10 too large, presumably because of channeling effects. Actually, under optimum conditions one might expect EHTP to be of the order of the diameter of the particles (i.e., 0.14 mm for 100 and 0.07 mm for 200 mesh). Rarely is such an ideal obtained in practice because of packing irregularities, channeling, and need for excessively slow flow rates.

Glueckauf discusses further the importance of film diffusion versus particle diffusion in determining EHTP, and the points to be considered for optimum flow rate and optimum distribution coefficients.

The effectiveness of a separation can be measured in terms of the overlap between the bell-shaped elution curves of the materials to be separated. Optimum separation is achieved if the cut is made at the volume given by the geometric mean of the values of V_{max}. The overlap is determined by the ratio of the distribution coefficients and the number of theoretical plates in the column. At a given required degree of purity, N increases extremely rapidly as the distribution coefficient ratio (separation factor S) decreases toward unity. Thus, for an overlap of 0.01%, according to Glueckauf, one needs N = ca. 20 for $S = 5$; $N = 100$ for $S = 2$; $N = 1000$ for $S = 1.2$; and $N = 10^5$ for $S = 1.02$. Combining these values of N with the conservative value 0.1 cm of EHTP shows that for $S \geq 2$ a column need rarely be larger than 10 cm and presumably, if packed carefully, could be considerably shorter. Thus, in the development of analytical procedures care should be taken to find conditions where the ratio of the distribution coefficients S is at least 2. Preferably this ratio should be larger, because then greater liberties may be taken regarding adherence to optimum conditions. For example, one could then operate at higher flow rates or decrease the distribution coefficient at which elution of the faster component is carried out. This is of considerable importance because, if elution can be carried

out under conditions where $D \leq 1$, extremely sharp elution bands may be obtained, and tailing, in general, becomes less serious.

ION EXCHANGE AS A TWO-PHASE EQUILIBRIUM

Many methods have been used for describing ion-exchange equilibria. Thus, Freundlich and Langmuir adsorption isotherms have been applied. Kinetic equations such as those of Gapon and Jenning have been proposed. However, it can be shown that many of these reduce to standard mass-action expressions which today are most favored in the ion-exchange field.

It is common practice to describe ion-exchange equilibria by equations of the type

$$B^+ + A^+_{(r)} \rightleftharpoons B^+_{(r)} + A^+ \tag{8}$$

with the equilibrium expression

$$K_{AB} = \frac{(B^+)_r(A^+)}{(B^+)(A^+)_r} \frac{g_{B(r)}g_A}{g_B g_{A(r)}} = K^{(\,)}_{AB} G_{AB} \tag{9}$$

where parentheses indicate concentration of species and g their activity coefficients. Subscript r refers to the resin phase; no subscript to the solution phase. K_{AB} is the mass-action constant, $K^{(\,)}_{AB}$ is the concentration quotient of the species, and G_{AB} the activity coefficient quotient. The concentration quotient is also often called the selectivity coefficient, though we shall refer to it as the *practical* selectivity coefficient to avoid confusion, since the literature also sometimes uses the term, selectivity coefficient, for the product of concentration quotient times the activity coefficient ratio for the ions in the aqueous phase. Though equations 8 and 9 are written for the cation exchange of univalent ions, similar equations, of course, apply to anion exchange. The more general case will be discussed later.

At first glance, it appears that these equations only apply to equivalent exchange: i.e., when for each equivalent of ions entering the exchanger one equivalent leaves it. As mentioned on page 49, this is not generally so, and particularly not at high electrolyte concentration where presently a great deal of work is being carried out. Such restriction would thus impose serious limitations on the applicability of the equations. Equivalent exchange, however, is actually not implied by these equations, and to illustrate their meaning and use they will be derived by consideration of the distribution of compo-

nents in a two-phase equilibrium without specific reference to ion exchange.

Consider the distribution of two ("Gibbs") components, I and J, between the exchanger and solution phases. At equilibrium the partial molal free energies of each component are the same in both phases: i.e.,

$$\bar{F}_I = \bar{F}_{I(r)} \quad \text{and} \quad \bar{F}_J = \bar{F}_{J(r)} \tag{10}$$

Hence

$$\bar{F}^\circ_I - \bar{F}^\circ_{I(r)} = RT \ln \frac{a_{I(r)}}{a_I} \tag{11a}$$

and

$$\bar{F}^\circ_J - \bar{F}^\circ_{J(r)} = RT \ln \frac{a_{J(r)}}{a_J} \tag{11b}$$

where a is the activity of the components and $\bar{F}^\circ$ the standard partial molal free energy. For convenience we may refer activities in the exchanger phase to the same standard state that is used for the component in the aqueous phase. In this case, $\bar{F}^\circ_I - \bar{F}^\circ_{I(r)} = 0$; $\bar{F}^\circ_J - \bar{F}^\circ_{J(r)} = 0$; and equations 11 become

$$a_I = a_{I(r)} \tag{12a}$$

and

$$a_J = a_{J(r)} \tag{12b}$$

Though not generally realized, equations 12 could be used for description of ion-exchange equilibria and for evaluation of the activity coefficients of the pertinent components in the resin phase. To illustrate, we shall consider the distribution of HCl between a hydrochloric acid solution and an anion-exchange resin in the chloride form and the adsorption of (tracer) bromide ions from such a medium.

If we set $I \equiv$ HCl and $J \equiv$ HBr and expand equations 12, we obtain

$$\frac{m_{H(r)} m_{Cl(r)}}{m_H m_{Cl}} = \frac{\gamma^2_{\pm HCl}}{\gamma^2_{\pm HCl(r)}} \tag{13a}$$

and

$$\frac{m_{H(r)} m_{Br(r)}}{m_H m_{Br}} = \frac{\gamma^2_{\pm HBr}}{\gamma^2_{\pm HBr(r)}} = D_{Br} \frac{m_{H(r)}}{m_H} \tag{13b}$$

According to equation 13*a*, measurement of the hydrogen-ion and chloride-ion concentrations in the resin phase, together with the known concentration of HCl and the known activity coefficients $\gamma_{\pm HCl}$ for the aqueous phase, permits computation of the stoichiometric activity coefficient $\gamma_{\pm HCl(r)}$ of HCl in the resin phase. The same applies to any

other component that distributes between the two phases; i.e., it also applies to HBr. In equation 13*b*, the additional substitution $D_{Br} = m_{Br(r)}/m_{Br}$ was made as one would do in measuring the adsorption of bromide ions from HCl solutions. Though, in principle, evaluation of $\gamma_{\pm HBr(r)}$ could as easily be achieved as that of $\gamma_{\pm HCl(r)}$, in practice it it is usually more complicated because of insufficient knowledge of activity coefficients in the aqueous phase in such mixed electrolyte systems, here HCl–HBr.

The simplicity of equations 12 or 13 makes their use attractive whenever possible. Unfortunately they need determination of the concentration of, e.g., hydrogen ions in an anion-exchange resin which cannot be achieved with precision except at very high outside electrolyte concentration where the amount of imbibed electrolyte also becomes large. However, these difficult-to-measure quantities may be canceled out if equations of the type of 12 are divided by each other after being raised to an appropriate power. In the present example, adsorption of bromide ions from HCl solutions, division of equation 12*b* by equation 13*a* yields

$$\frac{m_{Br(r)}m_{Cl}}{m_{Br}m_{Cl(r)}}\frac{\gamma^2_{\pm HBr(r)}\gamma^2_{\pm HCl}}{\gamma^2_{\pm HBr}\gamma^2_{\pm HCl(r)}} = K^m_{Cl,Br}\Gamma_{Cl,Br} = 1 \qquad (14a)$$

and

$$D_{Br}\frac{m_{Cl}}{m_{Cl(r)}} = \frac{1}{\Gamma_{Cl,Br}} \qquad (14b)$$

the familiar ion-exchange equations expressed in stoichiometric quantities. (Note that Γ is the stoichiometric activity coefficient quotient and $K^m_{Cl,Br}$ the concentration quotient.) The analogous equations for the ions A+ and B+ are

$$\frac{m_{B(r)}m_A}{m_Bm_{A(r)}}\frac{\gamma^2_{\pm BX(r)}\gamma^2_{\pm AX}}{\gamma^2_{\pm BX}\gamma^2_{\pm AX(r)}} = K^m_{AB}\Gamma_{AB} = K^m_{AB}\frac{\gamma_{B(r)}\gamma_A}{\gamma_B\gamma_{A(r)}} \qquad (15a)$$

and

$$D_B\frac{m_A}{m_{A(r)}} = \frac{1}{\Gamma_{AB}} \qquad (15b)$$

Since these equations are obtained by consideration of independent distribution of components, equivalent exchange is not implied, and the equations are generally applicable, provided that stoichiometric quantities are inserted throughout.

Equations 9 and 15 are formally similar since K_{AB} in equation 9 can be set equal to unity by proper selection of the standard states.

However, they become identical only if the stoichiometric concentrations, m, equal the species concentrations, ( ), and if the stoichiometric activity coefficients, γ, are equal to the species activity coefficients, g, as is true for essentially completely dissociated electrolytes in both phases. Such cases are well recognized for the solution phase but remain a matter of definition for the resin phase where appropriate limiting laws are still lacking. The differences between stoichiometric concentrations and species concentrations in the aqueous phase, though of no import for HCl and HBr solutions, are the basis for the ion-exchange equations for complex and weak acid (or base) systems, as will be discussed in the following sections.

The various general equations for ions of charge $\pm a$ and $\pm b$ that are equivalent to equations 9, 15*a*, and 15*b* are

$$a\mathrm{B}^{\pm b} + b\mathrm{A}^{\pm a}{}_{(r)} \rightleftharpoons a\mathrm{B}^{\pm b}{}_{(r)} + b\mathrm{A}^{\pm a} \tag{16}$$

$$\frac{m^a{}_{\mathrm{B}(r)} m^b{}_{\mathrm{A}}}{m^a{}_{\mathrm{B}} m^b{}_{\mathrm{A}(r)}} \frac{\gamma^{a(b+1)}{}_{\pm \mathrm{BX}_b(r)} \gamma^{b(a+1)}{}_{\pm \mathrm{AX}_a}}{\gamma^{a(b+1)}{}_{\pm \mathrm{BX}_b} \gamma^{b(a+1)}{}_{\pm \mathrm{AX}_a(r)}} = K^m{}_{\mathrm{AB}} \Gamma_{\mathrm{AB}} = 1 \tag{17}$$

$$D^a{}_{\mathrm{B}} (m_{\mathrm{A}}/m_{\mathrm{A}(r)})^b = 1/\Gamma_{\mathrm{AB}} \tag{18}$$

if we restrict ourselves for convenience to singly charged counter ions. Analogous equations may be written in terms of concentration of species and their activity coefficients.

The ion-exchange equations developed here are identical in form with those derived by Bauman [4] on the basis of a Donnan membrane equilibrium. However, as shown here, such model is not a necessary prerequisite. Many authors do not select the same standard states for the components in the aqueous and resin phases. For those the equilibrium constant K generally will differ from unity and their activity coefficient quotients G' (or Γ') will differ from our activity coefficient quotient G (or Γ), though they are related by: $K/G' = 1/G$ and $K/\Gamma' = 1/\Gamma$. The practical selectivity coefficient (concentration quotient) is not affected by the choice of the standard states.

Some authors have used more complicated thermodynamic equations than developed here which include a pressure–volume term in addition to an activity coefficient quotient. These equations are used principally for interpretation of ion-exchange selectivities and for interpretation of volume changes but are unnecessary when only description of the phenomena is of concern, such as in most analytical uses.

While we have restricted ourselves to consideration of the distribution of the components AX and BX between the two phases, which

is satisfactory for description of many ion-exchange experiments, ion-exchange thermodynamics may also be developed in terms of the resin components AR and BR, where R stands for the resin. This point of view has been adopted by many investigators, such as Davidson and co-workers,[22] Ekedahl, Sillén and co-workers,[31] and Gaines and Thomas.[34]

IDEAL EXCHANGE

From the mass-action expressions discussed in the previous section the adsorption behavior of simple ions in a number of idealized cases may be deduced. In particular, we shall consider adsorption of an ion which occurs at low concentration from a solution that contains a large excess of another electrolyte—the case of most interest in trace analysis. We shall further assume that negligible electrolyte invasion of the resin occurs.

Consider a trace ion B^+ which is not complexed [i.e., $m_B = (B^+)$], that it is in a 1:1 electrolyte AX, assumed to be completely dissociated, that the exchanger is in the A form, and that the pertinent activity coefficient ratio $G_{AB} = \Gamma_{AB}$ is constant. Since, for complete dissociation, $D = m_{B(r)}/m_B = (B^+)_{(r)}/(B^+)$ and $m_A = (A^+)$, and since, for trace loading, $m_{A(r)} \approx \text{const} = C$, equation 15*b* may be written in the form

$$D_B m_A = \frac{1}{G_{AB}} m_{A(r)} = \text{const} \tag{19}$$

This equation implies that the distribution coefficient D_B of the trace ion under these idealized conditions is inversely proportional to the concentration of the eluting ion or that a plot of log D_B vs. log m_A should be a straight line with slope -1 (see Fig. 7).

In the more general case, i.e., for exchange of the ions A^{+a} and B^{+b}, the equation equivalent to 19 is

$$D^a{}_B m^b{}_A = \frac{1}{G_{AB}} m^b{}_{A(r)} = \text{const} \tag{20}$$

and a plot of log D vs. log m_A has a slope of $-b/a$, as shown by differentiation of equation 20:

$$\frac{d \log D_B}{d \log m_A} = -\frac{b}{a} \tag{21}$$

Thus, slopes of such plots permit evaluation of the charge b of the ion B if the charge a of the eluting ion A is known and if the assump-

tions apply. The equations, though derived for an idealized case and thus not accurately applicable to a real case, can still serve as satisfactory guides. They become increasingly applicable as the electrolyte concentration of the aqueous phase decreases.[68] Then, electrolyte invasion of the resin becomes negligible, and activity coefficients in the resin phase remain essentially constant. Also, the activity coeffi-

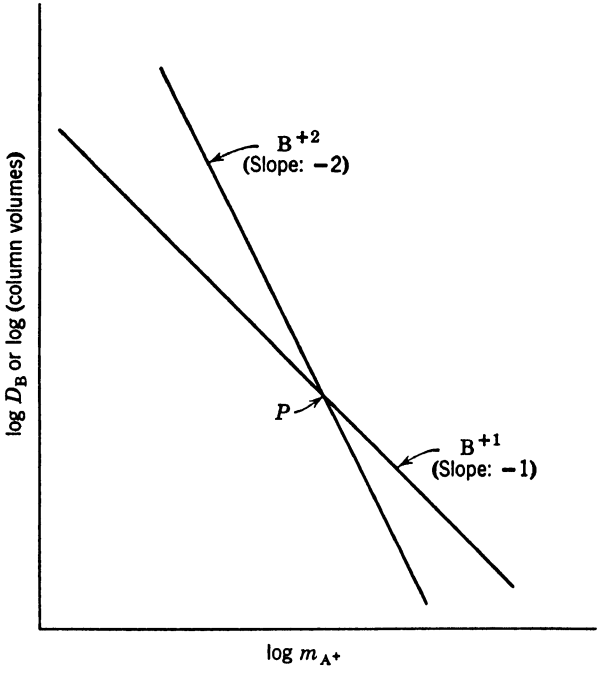

FIG. 7. Effect of charge on distribution function.

cient ratio for the ions in the aqueous phase approaches constancy at low ionic strength. For most practical purposes this happens below μ = ca. 0.5 if the ions have equal charge ($a = b$). For unequal charges of the ions, constancy of the activity coefficient ratio for the aqueous phase can only be expected at much lower concentration. At high electrolyte concentrations, e.g., above 1 *M*, the equations do not apply because then G_{AB} may vary rapidly with solution composition and m_A may deviate seriously from constancy.

From the mass-action equations the (ideal) effect of loading on observed distribution coefficients may also be estimated. We shall illustrate this for univalent ions. Equations for the more general case may readily be deduced from equation 18. For constant m_A,

constant G_{AB} and for negligible electrolyte invasion of the resin ($m_{A(r)} + m_{B(r)} = C$), equation 15*b* may be written as

$$\frac{D_B}{m_{A(r)}} = \frac{1}{G_{AB}} \frac{1}{m_A} = \text{const} \tag{22}$$

i.e., D_B is proportional to $m_{A(r)}$. Since in this instance loading L is given by $L = m_{B(r)}/C$, it is apparent that D_B will also be proportional to $(1 - L)$. For example, at 10% loading ($m_{B(r)}/C = 0.1$; $m_{A(r)}/C = 0.9$), D should only be 10% less than its maximum value at tracer loading, and, below 1% loading, D should remain practically constant. Only for $L > 0.5$ should D decrease seriously with L. Ideally, to avoid loading problems, one should thus work with enough resin to keep $L < 0.5$. Unfortunately, in practice loading effects become appreciable at much lower values of L, though it is still true that, below ca. 1% loading, D is usually constant.[51,72,123]

A number of deductions may also be made from these equations regarding separations. As mentioned earlier, separability S of two ions is determined by the ratio of their distribution coefficients. According to equation 21 values of D for ions of equal charge will vary in the same manner with supporting electrolyte concentration. The ratio is thus independent of electrolyte concentration, and separability cannot be improved by a change in supporting electrolyte concentration.

Separation of ions of equal charge is thus principally determined by the ratio of their selectivity coefficients, $K^{(\ )}$, with respect to the same eluting ion. From the success of many ion-exchange separations, e.g., of the rare earths or actinides, one might conclude that the organic exchangers have greatly different selectivities for ions of equal charge. However, this is not usually so. Rather, separation of ions of equal charge and small differences in selectivity is often achieved by taking advantage of the large number of equilibrations (theoretical plates) which even moderately sized columns afford. Still more effective is the use of complexing agents for increasing separability, and the spectacular successes of the rare-earth and actinide separations are largely due to this device.[56,62,114,121,123a] In this method, however, the effective charge of the ions is changed, and the severe problem of separating ions of equal charge is circumvented.

For ions of different charge, the distribution coefficients will vary differently with supporting electrolyte concentrations; the plots of log D vs. log m_A have different slopes. Hence the ratio of the distribution coefficients and the separability vary continuously with m_A. The

straight-line plots of log D vs. log m_A in general cross each other, as illustrated in Fig. 7, at a given supporting electrolyte concentration m_A (point P in Fig. 7). At this point the distribution coefficients of the two ions to be separated are equal, and in general no separation can be achieved by an equilibrium experiment, though advantage might still be taken of differences in rates of equilibration. At $m_A < P$, the distribution coefficient of the ion of higher charge will be greater than that of the ion of lower charge, and in column elutions the latter will come off first. For $m_A > P$, the reverse is true, and the ion of higher charge is less adsorbed and will elute first.

Though one cannot predict the concentration at which P is located, it appears to occur in many systems at reasonable values of m_A, so that one cannot generally state that an ion of higher charge is more strongly adsorbed than an ion of lower charge, as is often done. Instead, such statements should always be accompanied by information regarding the concentration at which the measurements are carried out. However, at very low electrolyte concentrations, ions of higher charge are almost always more strongly adsorbed than ions of lower charge.

The effect of supporting electrolyte concentration on separability of ions of different charge has often been utilized. The following examples are typical: In the anion-exchange separation of Zr(IV) and Nb(V) [66a] in HCl–HF mixtures, adsorbabilities of the two elements are approximately equal in 0.4 M HCl. At this point no separation is achieved. Zr(IV) under these conditions behaves like an ion of charge -2 and Nb(V) like an ion of charge -1. Hence at higher HCl concentrations Zr(IV) elutes more rapidly than Nb(V), and excellent separation can be achieved.

A similar problem arose in the separation of K^+ and Mg^{+2} by cation exchange.[6] A cross-over point apparently occurs in ca. 1 M HCl. For effective separation of the ions the HCl concentration was decreased to 0.7 M, where K^+ elutes first, well separated from Mg^{+2}.

NONIDEAL BEHAVIOR

Ideal ion-exchange behavior may be defined in terms of constancy of the practical selectivity coefficient $K_{AB}^{(\ )}$, which implies constancy of the activity coefficient quotient G_{AB} for the exchange equilibrium and negligible electrolyte invasion. In practice, G_{AB} is not constant under conditions where the exchanger imbibes electrolyte, and we shall, therefore, consider nonideality principally in terms of nonconstancy

of G_{AB}. The term G_{AB} may be written as the product of an activity coefficient ratio of the ions in the aqueous phase and a ratio for the resin phase, e.g., for the exchange of the ions A+ and B+, $G_{AB} = (g_{B(r)}/g_{A(r)})(g_A/g_B)$. At low ionic strength, g_A/g_B is not expected to vary appreciably if the ions have the same charge, since then the activity coefficients g_A and g_B are approximately equal and their ratio unity (the Debye–Hückel term vanishes). For ions of unequal charge, this ratio at low ionic strength μ may be approximated by the Debye–Hückel equation. Thus

$$\log (g_A/g_B) = -0.509\ (Z_A^2 - Z_B^2)\mu^{1/2}/(1 + A\mu^{1/2}) \tag{23}$$

and

$$\log (g_A{}^b/g_B{}^a) = -\ 0.509ab(a - b)\ \mu^{1/2}/(1 + A\mu^{1/2}) \tag{23a}$$

where Z (or a and b) is the charge of the ion and A a constant, which for many purposes may be set equal to 1.5.

At high ionic strength, g_B/g_A may vary rapidly with μ, and a linear variation of log (g_B/g_A) with μ is usually a good approximation; i.e.,

$$\frac{\partial \log (g_B/g_A)}{\partial \mu} = b\mu \tag{24}$$

where b is a constant.

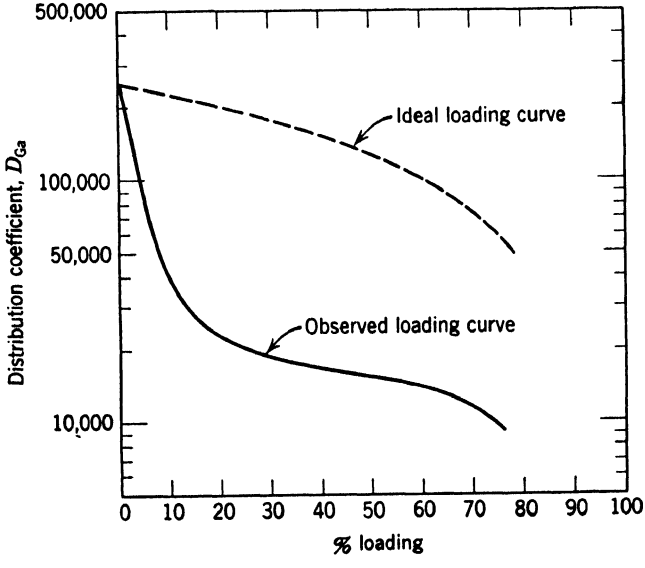

FIG. 8. Adsorption of Ga(III) as a function of loading (Dowex-1 X 10, 7 *M* HCl).

For the exchanger phase, two sources of nonideality may be distinguished: (*a*) effect of loading on $g_{B(r)}/g_{A(r)}$, and (*b*) effect of surrounding medium on $g_{B(r)}/g_{A(r)}$ and on electrolyte invasion.

At low ionic strength where electrolyte invasion is small, $g_{B(r)}/g_{A(r)}$ varies essentially only with loading. Deviations from constancy may

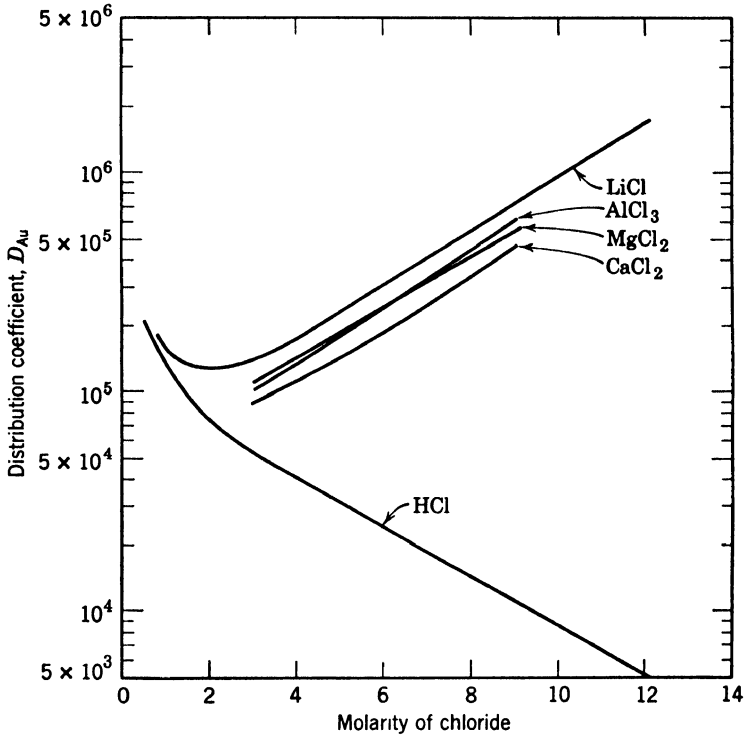

FIG. 9. Adsorbability of Au(III) from various media on a strong base anion exchanger (25% loading).

become serious for $L > 0.01$. In some instances loading effects may become so large that the selectivity of the exchanger inverts. For example, at high cross-linking an exchanger in the hydrogen form may preferentially adsorb sodium ions, and when principally in the sodium form it may preferentially adsorb hydrogen ions.[97] Loading effects are also very pronounced in the adsorption of many negatively charged complexes by anion exchangers. For these, distribution coefficients may decrease by an order of magnitude between 1 and 20% loading, as illustrated in Fig. 8 for adsorption of Ga(III) on Dowex-1 X 10 from HCl solutions.[72] For trace applications high loading is

usually not necessary, and it is thus advantageous to bypass loading problems whenever feasible.

Nonideality of exchangers resulting from the effect of the surrounding medium on resin properties has become of considerable interest in view of the effectiveness of anion-exchange methods for the separation of metals in concentrated electrolyte solutions. How serious this effect may become is demonstrated by the following example:

The distribution coefficient D for $AuCl_4^-$ decreases rapidly with increasing m_{HCl}. The same is true for $D_{AuCl_4} m_{Cl}/m_{Cl(r)}$. Ideally, this term should be independent of m_{HCl}. However, in other chloride solutions (low acidity), e.g., in LiCl, $CaCl_2$, $MgCl_2$, and $AlCl_3$ solutions, both D_{AuCl_4} and $D_{AuCl_4} m_{Cl}/m_{Cl(r)}$ increase rapidly with supporting electrolyte concentration with a resulting enormous divergence of adsorbabilities from HCl and from the essentially neutral chloride solutions (see Fig. 9). At present it is believed that this effect is principally a result of variations of the activity coefficient ratios in the resin phase. The reason for this effect is not clearly understood. It is very general, however, as shown by the study of a large number of negatively charged complexes.[68, 68a]

EFFECT OF SOLUTION EQUILIBRIA ON SEPARATIONS

The factors influencing adsorbability of materials may be classified as (*a*) factors operative in the resin phase which affect selectivities, (*b*) factors that take advantage of the properties of the mass-action expression for ion exchange, and (*c*) factors influencing the properties of the aqueous phase. Except for very special cases, it is usually inconvenient to attempt variation of the properties of the resin phase to effect improved separations, and at present most analytical applications have been restricted to commercially available exchangers whose selectivities for different ions are generally quite modest. The influence of the mass-action expression on separations was discussed in the previous section. It is often most convenient to take advantage of the fact that adsorption of a given material is greatly influenced by solution conditions, particularly acid–base and complexing reactions. The pertinent theoretical considerations will be discussed in this section. These are relatively simple, if we restrict ourselves to idealized cases, particularly those where variations in the activity coefficients of the species in the aqueous and resin phases can be ignored. The discussion, therefore, is highly restricted. The important case of ion exchange in concentrated electrolyte solutions will not be discussed in

detail. As mentioned (see also ref. 68), this problem is complicated by large variations of the activity coefficients of the ion exchange and complex equilibria. Nevertheless, some of the discussion here is sufficiently general to serve as a guide also for this case.

The effect of complex formation on ion-exchange equilibria could be derived thermodynamically without explicit reference to the species formed, as was done in part by Fronaeus.[33] Such derivation is essentially based on equations 12, which state that at equilibrium the stoichiometric activity of the component in the resin phase is proportional (or equal) to its activity in the aqueous phase. Complex formation in the aqueous phase is revealed by a decrease in the stoichiometric activity coefficients γ. Hence, with the assumption that the activity coefficients in the resin phase do not change significantly, the concentration of the component $m_{(r)}$ must decrease when at constant m in the solution phase γ, and hence $m\gamma = a$ decrease.

The equivalent arguments involving species have been discussed by a number of authors. Most prominent in this respect is the work of Schubert,[104,105,107,109] who extensively used the influence of solution equilibria on adsorbabilities for evaluation of complex constants. We shall discuss the equations, though only in general outline, because of their paramount importance to analytical problems. It is hoped that their extension to any specific problem will thus be facilitated. Derivation is based on essentially the following considerations: The adsorbability of an ion is determined by the specificity of the exchanger in a given medium and the concentration (activity) of the eluting ion. When complex equilibria or acid–base equilibria occur, the ratio, or "ion fraction," F, of the concentration of the ion to the total stoichiometric concentration of the component decreases below unity. Adsorbability decreases then by the same factor, provided that the complex ions formed (or the weak acid) show negligible adsorption compared with the original ion. When separation of two ions is desired and if the complex constants (acid constants) differ, the ion fractions F will also be different. This in turn affects the ratio of the distribution coefficients and hence the separability.

As an example, we shall consider the problem of separating two weak acids by anion exchange of the conjugate bases A^- and B^-. If the selectivity coefficients for these two ions are approximately the same, separation will be difficult in that pH range where the two acids are essentially completely dissociated. As discussed in the previous section, variation of the ionic strength (or eluting ion concentration) will also have little effect. However, if the two acids have dif-

ferent acid constants, control of the acidity might permit better separations, particularly if the more strongly adsorbed ion is also the conjugate base of the stronger acid. In the reverse case, separation might also become more difficult.

We shall derive the pertinent equations for the case where the eluting ion C^- has the same charge as the ions to be separated. The basic ion-exchange equations for the two species A^- and B^- will then be

$$K_{CA}^{(\,)} = \frac{(A^-)_r(C^-)}{(A^-)(C^-)_r} \tag{25a}$$

and

$$K_{CB}^{(\,)} = \frac{(B^-)_r(C^-)}{(B^-)(C^-)_r} \tag{25b}$$

Note that the selectivity coefficients $K_{CA}^{(\,)}$ and $K_{CB}^{(\,)}$ are generally not constants, but vary inversely with the activity coefficient quotients G_{CA} and G_{CB}.

The observed distribution coefficient is given in terms of the stoichiometric concentrations m_A, m_B, etc., which for weak acids would be the sum of the concentrations of the undissociated acids and its ions:

$$D_A = \frac{m_{A(r)}}{m_A} \tag{26a}$$

and

$$D_B = \frac{m_{B(r)}}{m_B} \tag{26b}$$

Combination of equations 25 and 26 yields

$$K_{CA}^{(\,)} = D_A \frac{m_A}{(A^-)} \frac{(C^-)}{(C^-)_r} = D_A \frac{1}{F_A} \frac{(C^-)}{(C^-)_r} \tag{27a}$$

and

$$K_{CB}^{(\,)} = D_B \frac{m_B}{(B^-)} \frac{(C^-)}{(C^-)_r} = D_B \frac{1}{F_B} \frac{(C^-)}{(C^-)_r} \tag{27b}$$

if we set $F_A = (A^-)/m_A$, etc., and if the stoichiometric concentrations of A and B in the resin phase are assumed equal to the concentration of the ions; i.e., $(A^-)_{(r)} = m_{A(r)}$ and $(B^-)_{(r)} = m_{B(r)}$. This implies that no undissociated acid is picked up by the exchanger or at least that its concentration in the resin is small compared with the concen-

tration of the ions. (In some instances, however, undissociated acids and other nonelectrolytes are specifically adsorbed, and so this assumption cannot be a universal one; see page 50 and refs. 94, 130.)

Solving equations 27 for D, one obtains

$$D_A = K_{CA}^{(\,)} F_A \frac{(C^-)_{(r)}}{(C^-)} \tag{28a}$$

and

$$D_B = K_{CB}^{(\,)} F_B \frac{(C^-)_{(r)}}{(C^-)} \tag{28b}$$

Hence, the distribution coefficients of A and B should be proportional to the ion fractions F if the selectivity coefficients $K^{(\,)}$ are constant as well as the concentrations (C^-) and $(C^-)_r$ of the eluting ion in the aqueous and resin phases. The separation factor S_{AB} of the two acids in a given medium is given by the ratio of the distribution coefficients:

$$S_{AB} = \frac{D_B}{D_A} = \frac{K_{CB}^{(\,)}}{K_{CA}^{(\,)}} \frac{F_B}{F_A} = R_K \frac{F_B}{F_A} = R_K R_F \tag{29}$$

i.e., it is equal to the product of the selectivity ratio $R_K = K_B^{(\,)}/K_A^{(\,)}$ and of the ratio R_F of the ion fractions (F_B/F_A) and is independent of (C^-) and $(C^-)_r$. The ion fractions F_A and F_B may be computed from the acidity (H^+) of the solutions and the concentration quotients ${k_A}^m$ and ${k_B}^m$ of the acid constants defined as

$${k_A}^m = \frac{(H^+)(A^-)}{(HA)}\,; \quad {k_B}^m = \frac{(H^+)(B^-)}{(HB)} \tag{30}$$

At sufficiently high acidity (low pH) where $(HA) \gg (A^-)$ and $(HB) \gg (B^-)$, the approximations

$$F_A = \frac{(A^-)}{m_A} \approx \frac{(A^-)}{(HA)} = \frac{{k_A}^m}{(H^+)} \tag{31a}$$

and

$$F_B = \frac{(B^-)}{m_B} \approx \frac{(B^-)}{(HB)} = \frac{{k_B}^m}{(H^+)} \tag{31b}$$

may be made. Insertion of equations 31 into equation 29 yields

$$S_{AB} \approx R_K \frac{{k_B}^m}{{k_A}^m} \quad \text{for} \quad \begin{matrix} (HA) \gg (A^-) \\ (HB) \gg (B^-) \end{matrix} \tag{32}$$

At high pH where undissociated acid formation is small and hence $F_A \approx F_B \approx 1$, one obtains from equations 28

$$S_{AB} \approx R_K \quad \text{for} \quad \begin{matrix}(HA) \ll (A^-) \\ (HB) \ll (B^-)\end{matrix} \tag{33}$$

Thus, though separability at high pH is only determined by the ratio of the selectivity coefficients in this idealized case, it also involves

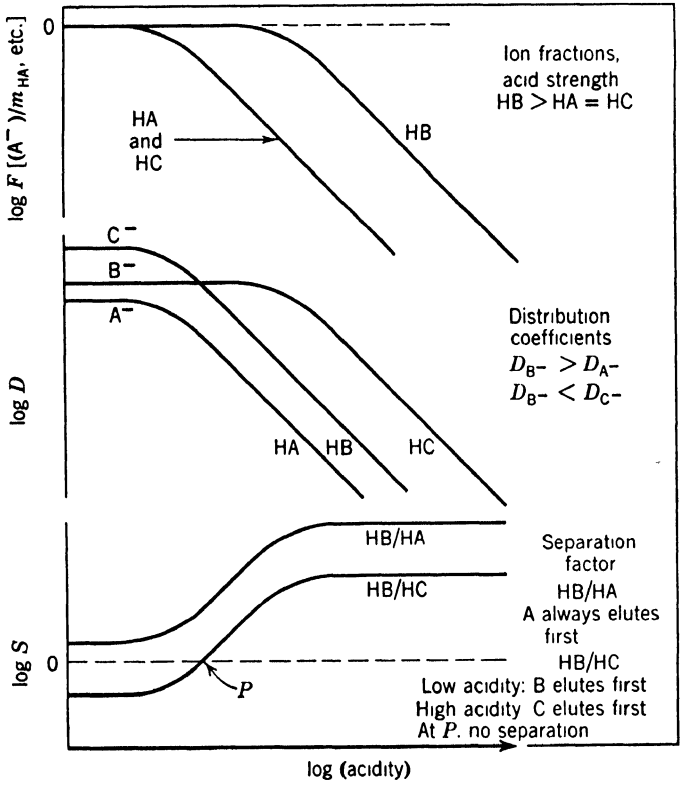

FIG. 10. Relationship among separation factor of weak acids, distribution coefficients, and ion fractions.

the ratio of the acid constants at sufficiently low pH. Operation at high acidities will thus cause improved separations if R_K and $k_B{}^m/k_A{}^m$ are both either larger or smaller than 1. If one term is larger and the other smaller than 1, better or poorer separations might result, depending on the relative magnitudes of the two terms. A diagram of the various relationships is given in Fig. 10.

Equation 29 which relates separation factor, selectivity ratio, and ion-fraction ratio, though derived for a simple acid–base equilibrium,

has actually much broader applicability. It holds if only one ion of each component is adsorbed by the resin and if these ions carry the same charge. If the adsorbed ions carry different charges but if still only one ion is adsorbed for each component, the right-hand side of equation 29 will have to be multiplied by the ratio of the eluting ion concentration in the resin to that in the solution raised to an appropriate power. If more than one ion is adsorbed, the equations are more complicated but may be derived by similar arguments.

Application of equations of the type of 29 to complex equilibria may be illustrated with cation separations of two ions in a complexing medium, and with anion-exchange separations of metal complexes.

Consider the cation-exchange separation of two divalent ions, e.g., Sr(II) and Ba(II), in a complexing medium, e.g., ammonium citrate. In the absence of the complexing agent (e.g., in NH_4Cl solutions) or at an acidity sufficiently high to prevent significant complexing, the distribution coefficients D_{Sr} and D_{Ba} will be determined by the selectivity coefficients $K_{Sr}^{(\,)}$ and $K_{Ba}^{(\,)}$, the concentration of the eluting ion (NH_4^+), and by loading, though the latter might be ignored in adsorption of traces. As complexing occurs, the ion fractions $F_{Sr} = (Sr^{+2})/m_{Sr}$ and $F_{Ba} = (Ba^{+2})/m_{Ba}$ will decrease below unity. At constant (NH_4^+), both D_{Sr} and D_{Ba} will decrease in direct proportion to the ion fractions, and the separation factor will be given by equation 29. When both metals are largely complexed, separability will remain constant and be determined (see equation 32) by the ratio of the selectivity coefficients and the ratio of the complex constants, provided the same type of complex is involved for both elements. As with the weak acids, the ratio of the selectivity coefficients and complex constants might both be larger than unity, whereupon improved separation always results by use of the complexing agent, or one of the terms may be larger and the other smaller than unity, which could cause either improved or poorer separations.

In anion-exchange adsorption of metal complexes, the development revolves around the fraction of the metal in the form of the adsorbable negatively charged complex (or complexes). With the restrictions given (equal charge of the adsorbed anions, only one anion adsorbed per component), equation 29 is applicable. Modifications of the equations may readily be made if these restrictions do not hold.

In spite of the inherent simplicity of these equations, however, it is usually not convenient to apply them to prediction of anion-exchange separations. Thus, identity of the (negatively charged) adsorbable complex is at present often obscure. At high ionic strength,

where much anion-exchange work is carried out, evaluation of $K^{()}$ is not readily achievable—the activity coefficient quotient for the ion-exchange equilibrium varying rapidly with ionic strength and in a manner not clearly understood. Equilibrium constants for the various complex equilibria are also at present not sufficiently well known, and in some cases completely unknown, making computation of the ion fractions virtually impossible. Actually, it is our hope to obtain information regarding the equilibria in the aqueous phase from anion-exchange studies, rather than the reverse. It is thus usually simplest and always more reliable to measure the distribution coefficients of the ions to be separated and from these evaluate S. Further discussion of anion exchange of metal complexes will be found on page 87.

Separability so far has only been discussed from the point of view of ratios of distribution coefficients. For the development of a good analytical procedure, however, a large ratio of adsorbabilities is not sufficient. In addition, it is necessary that the adsorbability of the least strongly adsorbed ion is also low enough to permit elution within a reasonable period of time. Such considerations might sometimes necessitate selection of conditions that are far from optimal from the point of view of separation coefficients. In other cases, it may be a simple matter of adjusting additional parameters such as ionic strength to achieve easy removal, together with optimum separability.

Although the present section concerned itself principally with determination of the separation factor, it is clear that the considerations used and the various equations may be applied to the determination of acid–base and complex equilibria in the aqueous phase from measurement of adsorbabilities. In trace analysis this may be of considerable importance, because the amount of material required is often much less than would have to be used for equally effective, more standard thermodynamic methods. Actually, from my point of view, the applicability of ion-exchange equilibrium measurements to elucidation of solution properties is one of the most attractive features of the ion-exchange method.

EFFECT OF TEMPERATURE

Except for a few papers, mostly fairly recent (see e.g., 30, 40, 43), the influence of temperature on ion-exchange equilibria has received little attention.

The effect of temperature on selectivity coefficients is believed to

be small. It might, however, be significant for the separation of two ions whose separation factor S is close to unity. Presumably $K^{()}$ can either rise or decrease with temperature, depending on the sign of ΔH for the ion-exchange equilibrium. A different dependence of $K^{()}$ on temperature could even happen for two ions of similar type and charge with approximately equal values of $K^{()}$ at room temperature. In this case, a sufficiently significant divergence of selectivity coefficients could result with change in temperature to cause considerable improvement in a previously difficult separation.

The effect of temperature on acid–base and complex equilibria and hence on the adsorbabilities they control may be large since ΔH for such reactions is often large. The effect on separability should be considerably smaller if the ions form similar complexes or undergo the same type of acid–base reaction. Many instances occur, however, where similar uncomplexed ions undergo different complexing reactions. Consider, for example, the anion-exchange separation of Ni(II) and Co(II) in concentrated HCl solutions. One ion [Ni(II)] does not form a negatively charged complex and is not adsorbed, while the other ion [Co(II)] forms one and is adsorbed.

The most important effect of temperature is its influence on rates. An increase in temperature causes more rapid equilibration with a resultant decrease in the effective height of a theoretical plate at a given flow rate. Thus, a rise in temperature usually permits more rapid separation of ions normally difficult to separate.[62,87,120]

APPLICATIONS

Ion exchange has been applied to a very large number of analytical problems whose detailed discussion here is not possible. Instead, we shall briefly review some of the major fields of application with special emphasis on the problem of trace analysis. A few typical illustrative references will be given.

Purification and Preparation of Reagents. The purity of the reagents used in trace analysis may often be the limiting feature in the procedures. This is particularly so in the analysis for the more common inorganic elements.

Of special importance is purification of water, the most commonly used solvent. Even distilled water often contains a considerable amount of ionic impurities which can be removed by successive (or, better, simultaneous mixed-bed) use of cation and anion exchangers. The cation exchanger in the hydrogen form will replace positively

charged ions by hydrogen ions, the anion exchanger in the hydroxide form will replace negative ions by hydroxide ions, with the result that total salt removal is achieved ("deionization"). By this method, water of very high specific resistance has been obtained. For analytical purposes it is usually best to use the ion-exchange step on distilled water, i.e., on water of initial high purity, rather than on tap water, since the ion-exchange step only removes ions and may leave in solution nonionic and colloidal impurities.

Relatively little work has been carried out on the purification of other solvents. Many such applications are probably difficult because of lack of resin swelling and correspondingly slow rates in organic solvents. In special cases, however, by employment of various ingenious devices, purification of organic reagents may be achieved, as illustrated by the purification of alcohols from aldehydes with the sulfite form of anion exchangers, as proposed by Samuelson (ref. 103, p. 90).

Application of ion exchange to purification of other reagents, e.g., acids, bases, and salt solutions, seems to be still in its infancy, as demonstrated by the briefness of the corresponding chapter in Samuelson's book.[103] As a typical example may be cited the removal of carbonate from moderately concentrated sodium hydroxide solutions with a strong-base exchanger in the hydroxide form.[23]

Purification of concentrated electrolyte solutions by cation exchange is inherently difficult because, in general, adsorbabilities become too low at high electrolyte concentrations. Fortunately, such limitations do not necessarily apply to anion exchangers, since many metallic impurities, presumably as negatively charged complexes, are strongly adsorbed by them even from very concentrated electrolyte solutions. For example, iron impurities [Fe(III)] can easily be removed, even from concentrated chloride solutions.[66] Originally only the removal of Fe(III) from HCl solutions was demonstrated, but it has since been shown that Fe(III) can be removed equally well, if not better, from other concentrated chloride solutions, e.g., LiCl, $MgCl_2$, $CaCl_2$, $AlCl_3$, etc., hence making purification of these salts feasible.[72] The procedure is extremely simple: It is only necessary to pass a concentrated salt solution through a strong-base anion exchanger in the chloride form. Since adsorption of Fe(III) is extremely strong, rapid flow rates are feasible. Regeneration of the resin may be achieved by washing with water or dilute HCl, since Fe(III) under these conditions is not adsorbed. Attack of the resin by the strong acids is apparently negligible and the same column may be used for many cycles. In the

same manner, a large number of other metallic impurities may be removed from HCl and other concentrated chloride solutions because they form strongly adsorbable negatively charged complexes, as will be discussed on page 87. This method has been used routinely for several years in our laboratory for the purification of chloride solutions.

At present, purification of reagents of this type apparently has been applied only to chloride solutions. However, the method should prove useful for many other salts as well: e.g., bromides, iodides, thiocyanates. In some instances it might prove profitable for the purification of a reagent from a specific impurity to add a small amount of a complexing agent and to adsorb the metal as the corresponding anionic complex from this medium. Since many metals are strongly adsorbed on anion exchangers in the presence of certain complexing agents, such as citrates, tartrates, and versenates, it should also be feasible to purify these complexing agents from inorganic impurities by anion exchange.

Ion exchangers have in some specific instances been used for the preparation of some reagents, particularly acids and bases. For example, if the hydroxide form of an anion exchanger is treated with a salt, e.g., sodium chloride, the effluent will be a sodium hydroxide solution. If the initial effluent is discarded, standard sodium hydroxide solutions may be prepared from standardized sodium chloride solutions. By the same device, acids may be prepared from the hydrogen form of the exchangers.

Sometimes it may be desirable to convert a certain salt from one form to another: e.g., the nitrate to the chloride. This may readily be achieved by passage of the salt solution through an anion exchanger in the appropriate form. The method is particularly useful if the ion to be removed is more strongly adsorbed by the exchanger than the ion to be substituted. When the reverse is true, the effective capacity of a given bed may be too small for successful utilization.

Sample Preparation. The use of ion exchange in the preparation of samples for analysis is presumably the main application, and most of the topics to be discussed fall logically under such a heading. We shall, however, restrict this section to a few items and discuss further applications in the following sections.

Ion exchange may effectively be applied to the analysis of ions for which no simple techniques exist, by using it for quantitative substitution of an easily analyzable or titratable ion for a more difficult one.[101] For example, an alkali-metal solution may be passed through

the hydrogen form of a cation exchanger. Hydrogen ions will be substituted for alkali-metal ions in the solution, and analysis is achieved by titration of the effluent plus necessary washings. By similar methods other substitutions can of course also be achieved. For detailed discussion of this type of analysis, see Samuelson's book (ref. 103, p. 117). In salt mixtures the method permits determination of the total salt content. Since many organic acids are adsorbed by ion exchangers, some caution must be exercised if the corresponding anions are present. Although anion exchangers in the hydroxide form could be used in an analogous manner, their applicability is less general, principally because of possible (hydrolytic) precipitation reactions. Less restricted appears to be the use of anion exchangers for other substitutions.

Closely related is the removal of interfering ions of the opposite charge with ion exchangers. A typical example is the removal of sulfate or phosphate from alkali metals.[103] This may be achieved with the hydrogen form of a cation exchanger (corresponding acids in effluent) or the chloride form of an anion exchanger (corresponding halides in effluent).

Dissolution of various insoluble materials may be achieved with ion exchangers. Thus, Samuelson [102] dissolved acid-soluble phosphates by adding the hydrogen form of a cation exchanger. Even highly insoluble materials may be opened up in this manner. Most striking is the dissolution of barium sulfate with either cation or anion exchangers.[52,90] This method is essentially based on the continuous removal of barium or sulfate ions from solution by adsorption on the resins. No doubt dissolution of many other insoluble salts could be aided by such a technique.

Ion-exchange techniques may at times permit analysis of solids without need for dissolving them first. This is true in the analysis of other ion-exchange materials for their replaceable ions. Wiklander, for example, contacted the hydrogen form of a sulfonic acid exchanger in water with soils to be analyzed. The soils are thus converted to the hydrogen form, the releasable cations are picked up by the organic exchanger, and analysis may be completed after the organic resin is washed free of soils.

Concentration. One of the most important uses of ion exchangers in analysis, particularly in trace analysis, is for concentration of materials. Concentration may always be achieved if adsorption can be carried out at a higher distribution coefficient than elution. Maximum concentration is hence obtained if adsorption is carried out at as

high a value of D as possible, and elution at the lowest practical value. In fact, for elution it is desirable to find a set of conditions where D is less than unity, because then the bulk of the material may be contained in not much more than one column volume of effluent. For effective concentration the various devices discussed on pages 70 and 74 may be used. For example, if a trace cation is to be concentrated with a cation exchanger, adsorption should be carried out at the lowest possible electrolyte concentration, and elution at the highest practical value (mass-action effect). The same of course applies to the adsorption of simple negative ions on an anion exchanger.

For cation adsorption on a cation exchanger, highest values of D usually demand absence of complexing agents. Conversely, addition of complexing agents for elution usually decreases D considerably beyond the value achievable by an increase in supporting electrolyte concentration. This is of particular importance because there is a practical upper limit to the electrolyte concentration of the eluent. As pointed out earlier, equilibration rates tend to become sluggish at very high electrolyte concentrations, with a resulting tailing of bands, which of course is detrimental to both quantitative recovery and volume reduction. Further, it appears that at high electrolyte concentration adsorbabilities may go through a minimum,[25,26] setting a limit to the maximum concentration of the eluent quite apart from rate considerations.

In the concentration of metals by anion exchange the relevant considerations, though in principle the same, differ considerably in detail because of the wide divergence of the possible adsorption functions as discussed under Anion Exchange of Metal Complexes.

Separations. Separations may be subdivided into two major parts: (1) separation of positive ions from interfering negative ions and vice versa, and (2) separations of ions of the same charge type: e.g., positive ions from each other. The former subject was discussed under Sample Preparations and is reviewed in considerable detail by Samuelson.[108]

The second part of this topic was discussed to some extent in earlier sections from a simplified theoretical point of view. In general, cations and anions that carry the same charge are more difficult to separate than those that carry different charges when advantage may be taken of the mass-action law. In addition, ions carrying different charges (different oxidation states) usually differ more widely in the stability of their complexes (and hence are more easily separated) than ions carrying the same charge. However, even "similar" ions of the same charge, such as the alkali metals and the halide ions, have been

successfully separated by ion exchange. In view of the limited selectivity of organic exchangers, these methods unfortunately are often tedious, though almost always more efficient and less time-consuming than competitive non-ion-exchange methods. Most powerful is the combination of complexing and acid–base reactions with ion exchange, which has led to the spectacular separations of the rare earths, the discovery of the "missing" rare earth promethium, and the discovery and isolation of the new heavy synthetic elements, not to speak of the excellent separations of materials of biochemical interest.[21] Success of these methods is largely based on a change in the average charge of the components of interest.

This change in the average charge is carried to an extreme in the anion-exchange method for separating metals, since here even the sign of the charge may be inverted. Some separations of metals may become as simple as the separation of ions of opposite charge. Further, complexing metals to their negatively charged complexes often accentuates differences between so-called similar elements, and it appears that the anion-exchange method often permits exploitation of the maximum differences between elements.

Recognition and Identification. The rate at which an adsorbed material moves down an ion-exchange column under a given set of eluting conditions is a characteristic property that may be used for the recognition of the material and its identification. This type of analysis has at present been exploited to a relatively small extent in spite of its great success in many biochemical problems, as well as in the discovery and characterization of the transplutonic elements. The method is extremely simple if a rapid analytical device is also available, because no more is involved than determination of the volume V_{max} at which the material appears in the effluent in maximum concentration.

In our laboratory the technique has been used almost routinely for the identification of tracers, usually coupled with an anion-exchange method which permits selection of one or more conditions under which the ion of interest shows essentially unique behavior.

Use of Ion Exchangers as Reagents and Catalysts. Ion exchangers have some unusual advantages as reagents, since they permit addition of an ion without simultaneous introduction of a (soluble) oppositely charged counter ion. Thus, the hydrogen form of a cation exchanger may be used as an acid, the hydroxide form of an anion exchanger as a base. Resins in the S^{-2} form may be substituted for H_2S in sulfide precipitations, in the CN^- form for soluble cyanides, etc. Since

the reacting ions are within the exchanger, their release can often be better controlled than if they were added as a solution. If reagents are introduced in this manner, they often are considerably purer than in more conventional techniques.

Closely related is the use of ion-exchange resins as catalysts (in contrast to their use as catalytic substrates in gas reactions). Exploitation for these purposes is a relatively recent development, though the catalytic activity of the hydrogen form of cation exchangers for sugar inversion has long been known. If ion exchangers are used in this way in solutions, removal of the catalysts may be achieved by simple phase separation at the end of the reaction. In addition, there is promise of considerable specificity and repression of undesirable side reactions. A typical example for materials of biochemical interest has been given by Khym and Cohn.[63] So far the principal uses have been restricted to the hydrogen form of cation exchangers as acid catalysts and the hydroxide form of anion exchangers as base catalysts. Condensation reactions and hydrolysis reactions have been studied most. The field was recently reviewed by Helfferich.[46]

Anion Exchange of Metal Complexes. In more recent years it has become apparent that concentration, separation, and isolation of metals can often be more efficiently achieved by anion exchange than by cation exchange. The method is based on the adsorption of negatively charged complexes of the normally positively charged metals. For some of these, commercial anion-exchange resins, e.g., the quaternary-amine resins of the Dowex-1 type, show extremely high selectivities. This permits concentrations and separations, even in concentrated electrolyte solutions where cation exchange often fails. As mentioned, differences in metals are often amplified, and elements that are difficult to separate by cation exchange may often be separated in a few minutes by anion exchange. The method appears to be the logical tool for those elements of the fourth and fifth groups, which must be complexed to be kept in solution. In applying the method to metals, one might first think of the extremely strong complexes characteristic of the transition elements, e.g., $AuCl_4^-$, $PtCl_6^{-2}$, etc. While these complexes can be adsorbed effectively on anion exchangers, the method is much broader and turns out to be most effective for the more labile complexes.

Conditions have been found for adsorption of practically all metals on anion exchangers, the only exceptions being the heavy alkali metals. Some of the results have been summarized with special reference to the fission products.[68] Many types of complexes have been

investigated, including citrates, versenates, oxalates, fluorides, bromides, thiocyanates, and the anions of the common mineral acids, hydrochloric, sulfuric and nitric. Most complete is the study of the adsorbabilities of the metallic elements in HCl solutions. These data were summarized in reference 68 for essentially the whole periodic table as a series of plots of log D_v vs. M HCl, and the corresponding figure is reproduced here as Fig. 11. Inspection of this figure clearly demonstrates the enormous divergence of the adsorbabilities of the various elements and how easily many separations may be achieved. One should realize that ion-exchange column separations can readily be carried out if the distribution coefficients differ by a factor of 2, and, as shown in Fig. 11, it is rare indeed to find two elements that throughout their usable HCl concentration range show ratios of D as small as 2, if the elements are adsorbed at all. To be sure, the applicability of the method is somewhat more restricted because, in addition to a favorable ratio of the distribution coefficients, elution conditions with low values of D must also be available. Otherwise the time necessary for separations and the effluent volumes become inconveniently large. This restriction is felt severely in anion exchange from HCl solutions of many of the heavier transition elements which, though showing decreasing adsorption with increasing molarity of HCl, never reach very low values of D.

In HCl solutions one may recognize several types of adsorption behavior:

A number of elements [alkali metals, alkaline earths, group IIIB, and surprisingly Al(III), Ni(II), Th(IV)] show no adsorption at any HCl concentration. Other elements show increasing adsorbability with M HCl. Others show shallow adsorbability maxima, others pronounced maxima, and still others show only decreasing adsorbability with M HCl in the range studied (0.1 to 12 M). Qualitatively one may state that, when elements show increasing adsorbability with M HCl, the negatively charged complexes in solution are not the principal species (the ion fractions F are less, often much less, than unity), whereas, when they decrease with M HCl in moderately concentrated HCl solutions, the metals are principally in the form of negatively charged complexes (i.e., the ion fractions F are approximately unity).

It was pointed out earlier that ideally adsorbability of an ion should decrease with ionic strength because of a mass-action effect; the selectivity coefficients, however, ideally remain constant. The decrease in adsorbability that is observed in strong HCl solutions under conditions where F is postulated to be unity, however, does not arise from

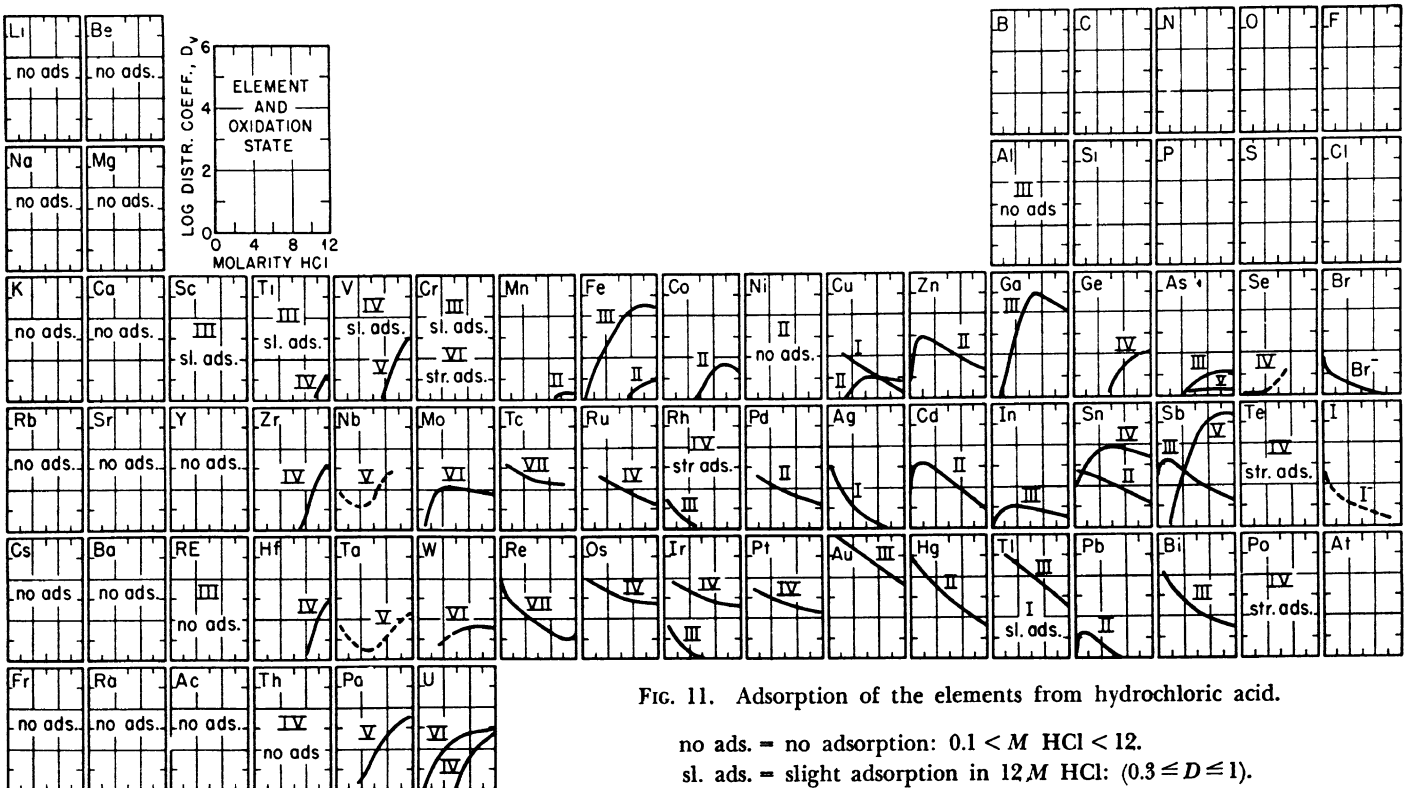

FIG. 11. Adsorption of the elements from hydrochloric acid.

no ads. = no adsorption: $0.1 < M$ HCl < 12.
sl. ads. = slight adsorption in $12M$ HCl: $(0.3 \leq D \leq 1)$.
str. ads. = strong adsorption: $D_V \gg 1$.
RE = rare earths.

this mass-action effect. Rather, it results from variations in the pertinent activity coefficient quotients. The selectivity coefficients decrease almost as rapidly, since, at high HCl concentration, $m_{HCl(r)} \approx m_{HCl}$. In lithium chloride and other low-acid chloride solutions, these decreases generally do not occur, and instead the values of D increase with M HCl (see also Fig. 9).

Data of the type of Fig. 11 may be used directly to design a multitude of separations. In view of the variability of commercial exchangers, even of the same type, it is best to check the procedures for each new batch of resin. Presumably variations of adsorbabilities between different resins of the same type (here, polystyrene–10% DVB–quaternary amine) will be only minor. Use of resins of higher or lower cross-linking will cause corresponding changes in adsorbability.

The anion-exchange method in HCl (or other chloride) solutions, though of broad applicability, has some obvious restrictions. The elements that are strongly adsorbed at all HCl concentrations can only effectively be removed by employment of special techniques: e.g., use of lower cross-linked resins, displacement chromatography with another strongly adsorbed complex, etc. Some elements tend to hydrolyze (and polymerize) before the HCl concentration can become low enough for elution—for these (particularly elements of the fourth and fifth groups), addition of another complexing agent is often desirable. In many cases HCl–HF mixtures have been found to be satisfactory. Then, of course, there are the elements that cannot be adsorbed from chloride solutions. For these, other complexing agents must be used. Satisfactory results have been achieved with citrates and versenates, though sometimes nitrates and sulfates [as for instance Th(IV)] are quite effective. Considering the multitude of complexing agents available, it is probably not too optimistic to state that essentially all metallic elements may be separated from each other, often uniquely, by the technique of anion exchange of metal complexes.

GLOSSARY AND SYMBOLS

Ion-exchange literature is plagued with a multitude of terms and symbols which are often used interchangeably. At other times the same symbol or term may have more than one definition. Comparison of papers by different authors (and often by the same authors) must thus be made with considerable caution. Some of the terms and symbols used in this chapter are defined below.

Absorption See adsorption.

Adsorbability Tendency of an ion to be adsorbed by an adsorbent or exchanger; it is quantitatively expressed in terms of the distribution coefficient.

Adsorption This term is used here whenever ions or other solutes are taken up by an exchanger. It does not imply any specific type of forces responsible for this uptake. While this term is widely used in ion-exchange literature, it may be considered interchangeable with absorption or sorption, terms used by some authors.

Adsorption function A plot of log D vs. a solution concentration variable.

Adsorption isotherm A plot of concentration in the resin vs. concentration in the solution. Adsorption isotherms are called linear if these concentrations are proportional to each other.

Break-through The term is usually used to signify appearance of a solute in the effluent in significant concentrations if a solution containing this ion is continuously passed into a column. It is generally used in a qualitative sense. A quantitative expression is given when the effluent concentration is a definite percentage of the inflowing concentration c_0; e.g., at 50% break-through c in the effluent is equal to $\frac{1}{2}c_0$.

Capacity, C In this paper capacity is defined in terms of the number of available ion-exchange sites in a given amount of exchanger. It is a constant. In the literature, particularly in industrial applications, it is often used as a practical term signifying the amount of material taken up by an exchanger under break-through conditions in a given experiment. When used in this sense, capacity (better called effective or apparent bed capacity) is a variable and depends on conditions.

Chromatography A method of separation or analysis involving the flow of a fluid over a stationary phase.

Column volume, V This term is used here to signify the geometric volume occupied by the bed; i.e., $A \times l$. The term in the literature is also often applied to the interstitial column volume, i.e., $i \times A \times l$. The latter definition appears less desirable because it involves the fractional interstitial volume i, a quantity that is difficult to obtain with precision.

Distribution coefficient, D A measure of adsorbability defined as the ratio of concentrations in the adsorber and solution phases.

Effluent Solution leaving a column.

Electrolyte invasion See Imbibed electrolyte.

Eluent Inflowing solution used for removal of material adsorbed on a column.

Equivalent exchange The term is used when the number of equivalents of ions entering an exchanger is equal to the number of equivalents leaving it. See page 49.

Ideal exchange The term is used when the practical selectivity coefficient (or concentration quotient) $K( )$ can be assumed constant. It is also sometimes used when the activity coefficient ratio for the resin phase remains constant.

Imbibed electrolyte Soluble electrolyte in the exchanger phase. See also page 49.

Interstitial volume Usually, *fractional* interstitial volume *i;* fraction of volume of column bed not occupied by exchanger.

Ion exclusion Electrolyte exclusion from an exchanger phase based presumably on a Donnan membrane effect and used as a separations tool. See also page 49.

Isotherm See Adsorption isotherm.

Loading, L Equivalent fraction of capacity converted to ionic form of interest; in the literature often also called fractional conversion with symbol *X*.

Practical selectivity coefficient, K^0 See Selectivity coefficient.

Regeneration Treatment of an exchanger with reagents to return them to the ionic form used in the beginning of an experiment or cycle.

Screening effect See page 47.

Selectivity The term is used here to describe the relative adsorbabilities of two ions and is expressed quantitatively in terms of K^0.

Selectivity coefficient Quantitative expression of selectivity. Here usually expressed in terms of the concentration quotient K^0 and then called the practical selectivity coefficient. In the literature, the selectivity coefficient is also often defined as the product of K^0 and the ratio of the activity coefficients of the ions in the solution phase raised to the appropriate power. K^0 is independent of the concentration units selected if the exchanging ions carry equal charges but is dependent on concentration units when they carry different charges.

Separability See Separation factor.

Separation factor or separation coefficient, S Ratio of distribution coefficients of ions to be separated measured under identical conditions.

Sorption See Adsorption.

Swelling See page 46.

Swelling water Same as imbibed water, water in the exchanger phase. See also page 46.

a	Activity; component indicated as subscript.
A	Cross-sectional area of column (square centimeters).
$A^{\pm a}$	General symbol for an ionic species; should be read A^{+a} for a cation and A^{-a} for an anion.
$B^{\pm b}$	General symbol for an ionic species; see $A^{\pm a}$.
c	Concentration (moles per liter).
c_0	Initial concentration of inflowing solution in a break-through experiment.
C	Capacity. Usually expressed in equivalents of sites per kilogram exchanger, though other units may also be used.
d	Distance (centimeters) an adsorption band travels on a column.
D	Distribution coefficient (amount per kilogram dry adsorber/amount per liter of solution).
D_v	Volume distribution coefficient (amount per liter of bed/amount per liter of solution).

E	Elution constant ($E = dA/v$).
EHTP	Effective height of a theoretical plate.
F	Ion fraction.
$\bar{F}$	Partial molal free energy.
$\bar{F}^\circ$	Standard partial molal free energy.
g	Activity coefficient of species.
G_{AB}	Species activity coefficient quotient for ion-exchange equilibrium; subscripts refer to ions in the resin phase: first (A) subscript for denominator, second (B) for numerator.
ι	Fractional interstitial volume.
k	Equilibrium constant for homogeneous reactions.
k^m	Concentration quotient for homogeneous equilibrium.
K	Ion-exchange equilibrium constant or mass-action constant determined by difference in standard free energies; $K = 1$, usually taken here.
$K^{()}$	Practical selectivity coefficient. $K^{(\ )}_{AB} = \dfrac{(B^{\pm b})^a_{(r)}(A^{\pm a})^b}{(B^{\pm b})^a(A^{\pm a})^b_{(r)}}$.
K^m	Concentration quotient for ion-exchange equilibrium expressed in stoichiometric quantities.
l	Column length.
L	Loading; $L = a(A^{\pm a})/C$.
m	Concentration; usually used here in arbitrary units.
N	Effective number of theoretical plates in a column.
P	Cross-over point where adsorbability of two ions of different charge is equal.
(r)	Used as subscript to denote resin phase.
R	Ratio: i.e., R_K ratio of selectivity coefficients; R_F ratio of ion fractions.
S	Separation factor; $S_{AB} = D_B/D_A$.
v	Volume (cubic centimeters) of effluent or of eluting solution.
V	Column volume; $V = l \times A$.
V_{max}	Number of column volumes at which elution band has maximum concentration.
γ	Stoichiometric activity coefficient.
Γ	Stoichiometric activity coefficient quotient.
μ	Ionic strength.
ρ	Bed density (kilograms dry exchanger per liter of bed).
( )	Concentration of species.

DISCUSSION

Discussion Leader
Robert Kunin
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I would first like to compliment Dr. Kraus on his excellent presentation on ion exchange and the use of ion exchange in trace-analysis work. In the few comments that I would like to make, I will confine

myself to those aspects of ion exchange in trace-analysis work that do not involve chromatographic procedures.

From a routine point of view, many of the chromatographic procedures involving ion-exchange resins or materials do not lend themselves to either field work or routine analytical laboratory work. However, there are many other applications of ion-exchange resins in trace-analysis work that do not involve chromatographic procedures.

One of the most important properties of ion-exchange resins in trace-analysis work is to concentrate a very dilute solution of a metal or other ionic substance in order that one may apply a conventional and simple analytical procedure. Since many of the resins used are commercial products involving trace impurities, one has to be extremely cautious in dealing with these resins in trace-analysis work for such common trace impurities as copper, lead, aluminum, nickel, zinc, tin, and mercury. If one is therefore interested in using resins for concentrating dilute solutions of these metals, one may have considerable difficulties. I think many of the difficulties analysts have had with the use of ion-exchange resins in trace-analysis work are due to the small traces that are present in these resins. These impurities are usually present regardless of whether they are made in the laboratory or on a large industrial scale, because, in order to achieve the properties one desires, huge quantities of water and catalysts are required.

Fortunately, many of the ion-exchange resins that are available can stand drastic treatments, and so they can be put into a suitable form free of these trace elements. This can be done with molar concentrations of acids and alkalies. In some instances one may use both acid and alkali alternately to advantage. Through such a process of conditioning the resins, one can lower the trace impurities to a point where they will not interfere during the subsequent analysis. Sometimes the resins after conditioning have to be stored in plastic containers.

One of the more recent applications of ion-exchange resins in trace analysis is in geochemical field work or in the analysis of rivers and lakes. In such work, one has to analyze streams and lakes that are fed from water passing through various geological formations. Unfortunately, the metal or constituent to be analyzed for exists in concentrations of one or two parts per billion. Many samples must be collected and sent back to the laboratory for analysis. Since it is quite difficult to send back 50 gal of solution to the laboratory for analysis, cartridges of these well-cleaned-up resins are prepared in a plastic

container. They are then taken to the field, and large measured volumes of the river, stream, or lake are passed through the resin. The resin cartridge is then sealed and sent back to the laboratory and analyzed spectrographically, or it is eluted with suitable reagents and suitable colorimetric procedures are applied.

Another application is the purification of reagents and the preparation of certain reagents which one cannot obtain in a pure state or in a state that is free of a particular element for which one wishes to analyze.

An example I would like to cite is the analysis of silica in the range of a hundredth part per million to perhaps a tenth part per million. This analysis is quite important industrially, particularly in the power field where high-pressure boilers are operated and the amount of silica entering the boiler or turbine must be low: a hundredth part per million to a tenth of a part per million.

The caustic required for dissolving the colloidal silica that may be present in a water sample usually contains more silica than is present in the sample for analysis. The key to this analytical problem is a grade of caustic that is sufficiently low in silica to permit an analysis of less than a tenth part per million of silica. This has been accomplished by means of ion-exchange resins. Caustic generated by passing a salt solution over a column of the hydroxyl form of a quaternary-ammonium anion-exchange resin is lower in silica than any reagent grade of caustic. By passing a 1.0 *M* solution of K_2SO_4 over a well-regenerated column of Amberlite IRA-400, a 0.9 *M* solution of KOH is produced that is sufficiently low in silica so that it contributes but a negligible blank in the analysis of a water containing but 0.01 ppm SiO_2.

There is one other interesting application of ion exchange that touches on the selectivity of ion-exchange resins. I am very grateful to Dr. Kraus for his statement that resins are not very selective, and I myself am beginning to feel that the terms selectivity and selectivity coefficient are misleading and are creating much confusion. The uninitiated are expecting the resins to be more effective than they are; however, there are occasions when resins are more selective than is supposed. The carboxylic cation-exchange resins are an excellent example.

The sulfonic acid resin will not function at all in removing and concentrating dilute traces of zinc or copper present in blood. The zinc and copper are well complexed and pass right through a sulfonic

acid column. On the other hand, a carboxylic acid resin column will remove almost quantitatively all the zinc and copper present in the blood. Another problem is the analysis of traces of copper in calcium chloride solutions, where perhaps a trace of copper is present in a very concentrated solution of calcium chloride. In this case the sulfonics are again worthless, and the carboxylic acid resins are quite effective. The carboxylic resins in calcium form will remove quantitatively all the copper from a 1 *M* calcium chloride solution. The advantage of the carboxylic acid is that, once the material is adsorbed and concentrated on the resin, it may be eluted as a concentrated solution with almost a stoichiometric amount of acid.

Resin selectivity is an important factor, and the choice of the resin does become quite important with certain elements in the field of trace analysis.

Before opening the general discussion from the floor, I would like to make one or two concluding remarks about the field in general. As many of you know, ion exchange is a most promising technique for trace-element analysis and for many other analytical purposes. However, successful use has been limited by a lack of understanding of the principles of ion exchange and the nature of the characteristics of the various resins available. Too many analysts are not familiar with the characteristics of ion-exchange resins, and too many do not fully understand the principles of ion exchange. Perhaps very little has been done in developing a working hypothesis for the various analysts. I believe a proper understanding of the analytical principles is important in analytical chemistry; but ion exchange has been somewhat unsuccessful because a proper understanding has not been available, for most routine analysts have not been aware of the fundamental principles involved. I would like to indicate that this is rapidly changing, and that many of the theories have been simplified to a point where they can be used in a routine way by the analyst applying ion-exchange resins. Dr. William Rieman's work in simplifying the theory of column operation as applied to ion-exchange chromatography has been very successful in this direction.

QUESTIONS AND ANSWERS

T. L. MARPLE (*Union Oil Co. of California*): This question is directed to Dr. Kraus. Does he consider the process of separation of Na^+, K^+, Rb^+, Cs^+ on zirconium tungstate to be primarily an ion-exchange phenomenon

or a physical-absorption phenomenon such as the Tswett chromatographic process?

KURT A. KRAUS: I would rather not enter into a discussion of chromatography versus ion exchange, since I believe that the two fields are not mutually exclusive. Rather, ion-exchange materials may be used with a chromatographic technique, and ion exchange as a mechanism presumably is the explanation for many so-called chromatographic phenomena.

ROBERT KUNIN: Does this refer to the aluminum and iron silicates?

KURT A. KRAUS: I think the question of whether, with a given material, adsorption or ion exchange occurs is purely a semantic one. I use the term ion exchange for any two-phase equilibrium of this type involving electrolytes.

HAROLD H. STRAIN: Suppose you extend the curves of the equation for two substances, with curves of different slope, and then change the concentration. Would this actually result in a change of sequence in the regions where the curves cross? I have never observed change of sequence due to change of concentration, although two materials may be caused to migrate so that one zone runs into another. At this stage and concentration, the sorbent cannot distinguish between either substance; therefore I conclude, qualitatively, that your equation may not be valid when the two substances are mixed.

KURT A. KRAUS: In one of our first anion-exchange separations, that of zirconium and niobium, the first HCl concentration selected happened to be exactly at the cross-over point, and no separation resulted. Separations could be achieved either by increasing or decreasing the HCl concentration. I realize such instances are rare. However, I mention this charge-dependent variation of adsorbability because so many people think that, in general, highly charged ions are better adsorbed than ions of lower charge, a statement that of course is erroneous. I would like to point out too that Dr. Rieman has used the variation of adsorbability with charge and eluting conditions in the separation of magnesium and sodium.

WILLIAM RIEMAN: Yes.

ROBERT KUNIN: Is this not true for more than one monovalent–divalent exchange in very dilute solutions? The resins usually prefer the divalent ions in dilute solution, and, as the solutions become more concentrated, the preference disappears, and eventually a reversal may be noted. This is a direct observation from the Donnan membrane theory.

WILLIAM RIEMAN: Dr. Kraus mentioned that an ion of high valence frequently has a smaller affinity for the resin than an ion of low valence. An interesting example of this is found in our separation of tetrametaphosphate from trimetaphosphate. The tetrametaphosphate is eluted first. In this particular case one cannot get the concentration of eluent low enough to elute the two and have them come out with the trimetaphosphate ahead.

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Extraction

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ABSTRACT Countercurrent distribution is of considerable significance in the field of trace analysis wherever separations are required. It, like chromatography, can be made to be one of the most selective separation techniques known, and yet can be used equally well for initial charges of fractions of a milligram or fractions of a kilogram of starting material. This often permits a very small amount of relatively pure material to be quickly isolated from a large amount of crude starting material.

Another favorable feature of countercurrent distribution in trace analysis is the fact that all the material used for the separation can be accounted for and can be recovered, since it is all in solution in some tube or tubes of the apparatus. There is no loss. A distribution pattern gives a quantitative over-all estimate of the progress of the separation.

The characteristics of countercurrent distribution make it especially suitable for investigations where little is known of the chemical properties of the trace substances being studied. Their presence in the mixture, of course, must be known by the fact that they have at least one distinctive property, such as a certain biological activity or spectral characteristic. The technique was developed for this purpose and has proved its effectiveness in a wide variety of fields.

In a symposium dealing with trace analysis one may, at first thought, question whether or not the subject of extraction properly merits the consideration it is being given on this program. It is my intention to try to show that it does deserve a place from almost any viewpoint one may choose.[14]

Trace analysis implies the ability to detect, characterize, and quantitatively measure very small amounts of substance, whether or not the substance occurs dispersed throughout an overwhelmingly large amount of other material. Obviously much of the research of modern biochemistry dealing with hormones, vitamins, enzymes, coenzymes, inhibitors, etc. is forced to be concerned with trace analysis. The problems require the ability to carry out whole research programs of detection, estimation, and chemical characterization of unknown materials with only trace amounts available. For this purpose various forms of extraction have been used throughout the vast amount of work published in the literature and on which our present-day concepts are based.

In the broader sense, extraction deals with the transfer reversibly or otherwise of solute from one or more phases in which it is dissolved or dispersed to a new phase. This operation may be carried out simply because the analytical determination desired cannot be carried out in the original environment that the substance is found in, and removal to some other suitable one is required. Extraction for this purpose has long been intimately associated with analysis. Even so, much remains to be learned about the proper use of solvents and conditions for the purpose. This alone would appear to justify a thorough consideration of the principles of extraction.

Almost as important is the question of analytical specificity. It is always, of course, highly desirable to have an analytical method that gives a measure exclusively of one element or compound. Unfortunately this state of affairs does not exist for many substances. Some kind of separation or fractionation technique is therefore required, which can be used as a preliminary operation before the analysis is made and thereby achieve the required selectivity. The real potentialities of extraction in this connection have been fully realized only in the last ten years, during which time extraction itself in the form of a countercurrent process has been developed to the point where it alone is certainly one of the most selective separation tools known to chemistry.

Extraction itself is not an analytical method, although the term "extraction analysis" has often been used. Actually extraction can

only set the stage whereby some analytical operation such as titration, weight determination, spectroscopic determination, etc. will permit calculation of the information desired.

In trace analysis, one of the primary questions is that of the limiting amount of material that can be detected. Fortunately this is not a problem in extraction. It is only a problem of the sensitivity of the analytical method to be used in conjunction with the analytical procedure. Extraction can be used just as well for the smallest amount of material that can be measured quantitatively as for larger amounts. In fact, as will be pointed out below, the complication of a shifting partition ratio is often avoided in very dilute solution, and ideality is more nearly approached.

Extraction as a science lends itself beautifully to mathematical interpretation. This is true for all operations from the simplest equilibrium contact of two immiscible phases to the most complicated systematic extraction pattern that may be desired. Thus, when a very dilute solution of a given solute is contacted with an immiscible solvent, two phases result, and the concentration of the solute in the upper phase divided by that in the lower will be a constant independent, within certain limits, of the total solute present. Thus equation 1 is the distribution law of Nernst which is known to hold

$$K = \frac{C_1}{C_2} \tag{1}$$

for most solutes provided the concentrations C_1 and C_2 are sufficiently low. It can often be taken for granted that they will be low in trace-analysis work, but, as shown later, it is very easy to determine whether or not the distribution law is adhered to. The total solute in a given phase at equilibrium is CV where V is the volume of the phase. It follows algebraically that, for equal volumes of the two phases, the fraction q of the total solute in the upper phase is related to K by the expression

$$q = \frac{K}{K + 1} \tag{2}$$

while the fraction p in the lower phase is related to K by equation 3:

$$p = 1 - q = \frac{1}{K + 1} \tag{3}$$

Where the volumes are unequal, the simple relationship can still be used by substituting Kr for K where r is the ratio of the volumes in the upper and lower phases.

Let us now consider direct application of these equations to an analytical problem and assume that a solute is in some solution not suited for direct analysis by a method such as weight determination, titration, or spectroscopy. A measured volume of the solution can be shaken with an equal volume of an arbitrarily chosen solvent suitable for the determination. The extract can be set aside and a second and third extraction made. If W_1, W_2, and W_3 (expressed as weight or any analytical unit proportional to weight) are the respective quantities found in the extracts, it follows from equations 1, 2, and 3 that the fraction of the total being removed on each extraction is related to the partition ratio by equation 4:

$$1 - \frac{W_2}{W_1} = \frac{K}{K+1} \tag{4}$$

When W_2/W_1 is greater than approximately 0.7, a range is reached where the evaluation becomes less accurate. A more reliable range may be reached by increasing the volume of the extract. The approach becomes impractical for partition ratios less than 0.1.

If the partition ratio is really a constant and the analytical method refers to only one solute (or a mixture of solutes with very similar partition ratios), the same relationship between the second and third extract will hold: e.g., that in equation 5:

$$1 - \frac{W_3}{W_2} = \frac{K}{K+1} \tag{5}$$

These are the most useful equations to consider in applying simple extraction to an analytical problem. They are attractive because of their simplicity and general applicability. Obviously such formulas are ideal for empirical work where the nature of the solute or solutes is completely unknown and a research problem is involved.

With these simple formulas to serve as a background for further discussion, it is obvious that extraction can always be made to fulfill the demands made on it, provided partition ratios can be influenced at will in a selective way by the proper choice of solvents and other additives.

One of the best-known examples of this comes in the extraction

of an acid from an aqueous solution into an organic phase. One chooses an organic phase that is known to dissolve the acid freely. An acid with slight solubility in the organic phase is added to increase the partition ratio. If this is not sufficient, inorganic salt is added to the aqueous phase to increase the partition ratio further. These steps increase the partition ratio through a combination of effects in large part connected with a decrease in the percentage of the acid in the ionic state in the aqueous phase. Where the solute of interest is a base, the same approach and reasoning holds except that an inorganic base is used instead of an acid to increase the partition ratio.

Many acids and bases do not behave as expected. Certain of their salts greatly prefer the organic phase. Thus the hydrochlorides of many alkaloids are freely extractable from aqueous solution by chloroform. In fact, this anomalous behavior is used as a basis for a very effective analytical method. The alkaloid hydrochloride forms a complex with the chloroform which has a partition ratio greatly favoring the chloroform.

Complex formation in order to influence extraction in the desired way is a very large field indeed. Great strides along this line have been made in the past several years particularly in the analytical determination of inorganic ions.[21] This field is so large that no attempt will be made here to cover specific cases. In general, a solvent favorable for partitioning the complex must be found and the conditions worked out that are most favorable to complex formation in the aqueous phase. These latter conditions include adjustment of *p*H, choice of temperature, addition of salt or other solutes which influence the total solvent action of the aqueous phase.

Actually some sort of complex formation in solution often occurs and much more widely than is commonly realized. Few solutes behave as if they were completely ideal and do not appear to exist in solutions as if they were entirely dissociated from each other and from the molecules surrounding them. However, there is a large class of substances with outstanding tendencies to hydrogen-bond, chelate, coordinate, or combine in some way with other solutes to form complexes. These include dimethylglyoxime, phosphotungstic acid, phosphomolybdic acid, certain halogen acids, trichloracetic acid, higher fatty acids, salicylic acid, aromatic and aliphatic sulfonic acids, trioctylamino-8-quinolinol, 5,7-dichloro-8-hydroxyquinoline, dithizone, thenoyltrifluoroacetone, and perchloric acid.

These are only a few of the many complexing agents commonly employed in analytical work today. Apparently a new group of these substances with outstanding possibilities are the perfluoro fatty acids.[24] It would seem probable that many others remain to be discovered.

The selectivity of a system can often be increased by bringing to bear some type of chemical equilibrium, the most common of which appears to be ionization. If the solutes to be detected are acidic or basic, introduction of the proper buffer [8] into the system will often give very high selectivities. The equilibrium involved can be as follows where the dashed line indicates the interface. A buffer can be

Organic phase	HX
----------	⇵ ---
Aqueous phase	$H^+ + X^- \rightleftarrows HX$

used to fix the value of the hydrogen-ion concentration, and thus through the ionization constant the fraction of un-ionized acid that is free to partition is controlled. Often the partition ratios in such systems are among the most selective known.

Obviously with bases a similar equilibrium will occur. In partition work designed to detect traces of quinoline antimalarials, it was necessary to control the *p*H to ± 0.02 *p*H unit in order to duplicate a partition ratio. This gave high selectivity as one might expect.

When the solutes to be detected are so closely related that a truly selective separation cannot be carried out by simple extraction, it may prove profitable to turn to a countercurrent process to improve the over-all selectivity. Naturally before it is decided to use this approach the possibilities in simple extraction have already been explored, and this should have given all the basic data required for choice of the best countercurrent procedure.

If countercurrent distribution is selected as the procedure, it is simply an extension of the single-stage operation to a multiple-extraction process. Equipment is now readily available for making multiple stepwise extractions in a systematic way with very little effort. The amount of time required for a separation will depend on the difficulty of the separation and the selectivity of the system.

Perhaps too much emphasis in the last few years has been placed on separations requiring hundreds or thousands of stages. These have been very striking and have led to the precise characterization of a considerable number of key substances in biochemistry which have

heretofore resisted all attempts by other methods. It is often not appreciated that considerable time and effort have been exerted even with the best of equipment.

With due regard for the importance of such separations when all other approaches fail, it would seem best to emphasize the over-all utility and convenience of countercurrent distributions which require relatively few transfers, something in the range of 10 to 30 transfers.

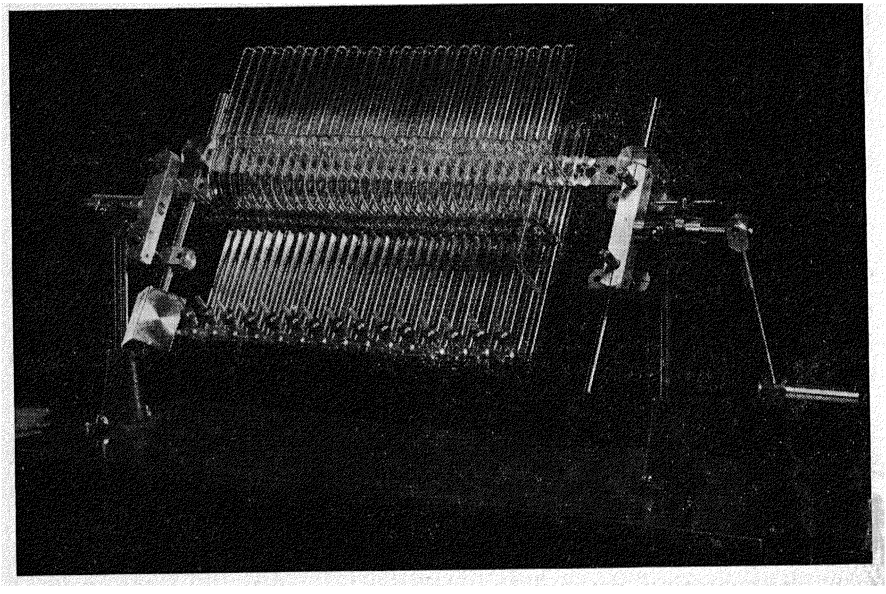

FIG. 1. A hand-operated 30-tube apparatus.

Such distributions usually do not require more than 1 to 2 hours of work including the analysis.

For such work a hand-operated train of 30 to 50 tubes is very convenient. Such an apparatus is shown in Fig. 1.* The different procedures for operating this extraction train have been amply described in the literature [10-12] and will not be treated again here. From an analytical standpoint, the fundamental procedure may be the most satisfactory since, if doubt should occur in the interpretation, the run can be easily extended to higher numbers of transfers.

The results of a countercurrent distribution where the fundamental procedure [11] has been used are best recorded graphically as shown in Fig. 2, which is a calculated hypothetical pattern. One can deduce very quickly from such a graph whether or not sufficient separation

* Available commercially from H. O. Post, 68-22, 60th Street, Maspeth, New York.

has been achieved. This can be done with very little mathematical calculation if both upper and lower phases in a sufficient number of the tubes have been analyzed. The result permits direct calculation of the partition ratios in the tubes analyzed. If the ratios are constant across an isolated band, this band then represents a single substance at least as far as the separating power of the method at the number of

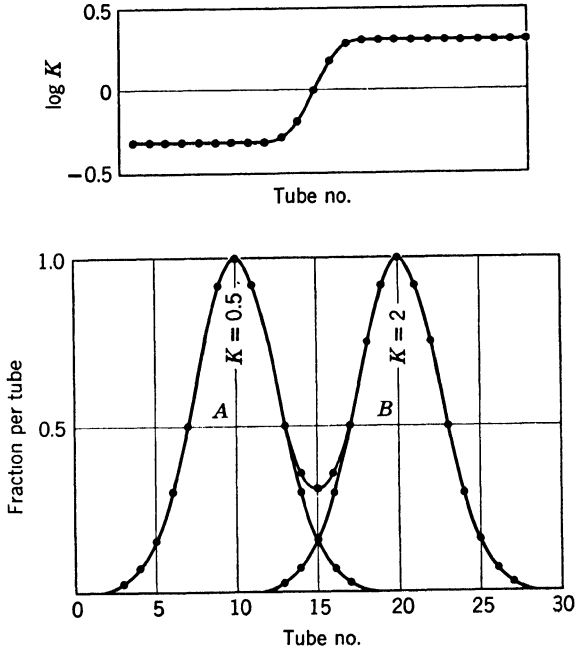

FIG. 2. A hypothetical distribution pattern.

transfers used is able to reveal. Where there is overlapping of the bands, a plot of partition ratios as in Fig. 2 gives good confirmation.

One who is mathematically minded can go further in interpreting the data. Since the process represents systematic division and subdivision of unit quantity of material, it corresponds to a binomial expansion (equation 6), where n is the number of transfers applied,

$$(p + q)^n = 1 \tag{6}$$

p the fraction of solute in the lower phase, and q the fraction in the upper phase. From equations 2 and 3, equation 6 can be expanded to equation 7.

$$\left(\frac{1}{K+1} + \frac{K}{K+1}\right)^n = 1 \tag{7}$$

The literature [11,10,12,2] contains many useful procedures for quickly obtaining the binomials needed. These will not be discussed here. For higher numbers of transfers, the mathematics of probability can be used. An adaptation of the equation for the normal curve of error (equation 8) gives an equation which permits calculation of a curve in about 10 minutes, regardless of the number of transfers involved.

$$y = \frac{1}{\sqrt{2\pi nK/(K+1)^2}} \exp\left(-\frac{x^2}{2nK/(K+1)^2}\right) \qquad (8)$$

In equation 8, y is the value of an ordinate corresponding to a tube x tubes removed from the point of maximum concentration on the curve of the band, and n is the number of transfers.

A calculated curve which exactly matches an experimental one indicates that the solute is behaving ideally; e.g., that the partition ratio remains constant and that complete equilibrium is reached at each stage. The details of matching and calculating theoretical curves have been well discussed in the literature [2,10–12] and will not be treated further here.

It may be of some value to point out that, where routine quantitative analysis of a mixture of a few closely related components is contemplated and where the identity of the ones present is known, a complete countercurrent distribution may not be required. For example, the distribution may have revealed two components *A* and *B* as shown in Fig. 2. Since these solutes have behaved ideally, repetition of the entire analysis on another sample for a quantitative estimation would not be required. One could derive the result by analyzing only the two peak tubes at the conclusion of distribution and could probably also reduce the number of transfers required if these should prove laborious. Overlapping bands containing two components can be interpreted with considerable reliability. However, most workers will desire the greater reliability of an ample number of transfers when they can be so easily reached. Although the more simple form of countercurrent distribution has been used to a rather limited extent in routine analysis, it was not developed to its present degree with this field in mind. Its characteristics are much better suited to the requirements of a research problem in which very little is known concerning the properties of the solute or solutes involved.

Examples of such a use are to be found in the study of the metabolic fate of a variety of drugs. Brodie and collaborators [5] and Titus and

collaborators,[29] as well as many others, have made use of the technique in this connection.

Particularly in biochemistry the stability question is very important. It is self-evident that any type of fractionation which itself causes transformation during the treatment cannot be considered reliable. Indeed it often proves difficult to be sure whether or not such a transformation has occurred. Although countercurrent distribution can be carried out so that it presents the mildest of conditions, very fragile substances nevertheless can undergo transformation. When this occurs, careful interpretation of the distribution pattern is helpful, if not decisive, in deciding whether or not a slow transformation has taken place. If a distribution pattern is obtained in which the calculated curve does not match the experimental one and the partition ratios are not of the order they should be for their respective positions on the pattern, then either the solutes do not give a constant partition ratio at different concentration levels or some transformation is taking place during the run. The first possibility will be discussed later. An example of the second is shown in Fig. 3 which is a purity study made by W. Hausmann on a polypeptide antibiotic called "Polypeptin." The experimental curve is broader than the calculated, and there is a steady drift of the K value across the band.

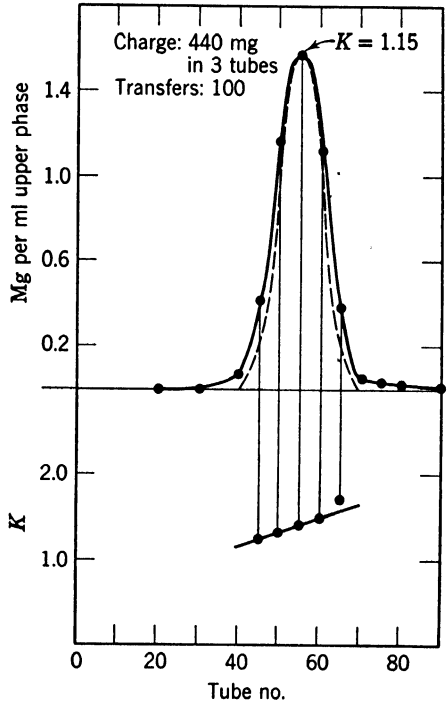

FIG. 3. A distribution pattern showing transformation.

As our concepts advance regarding the larger molecules which play key roles in biochemistry, it becomes increasingly evident that the majority of them have unusual properties. One of these properties seems to be the ability to associate strongly with other solutes to form a complex. When the environment does not present optimum conditions for associating with other molecules, the individual molecules

combine with themselves to form loosely held dimers, trimers, etc. This tendency persists with many solutes even to extremely dilute solution and greatly complicates and enhances the difficulty of separation attempts.

Where the association is predominantly concerned with like molecules a nonlinear partition isotherm results. This gives a skewed-

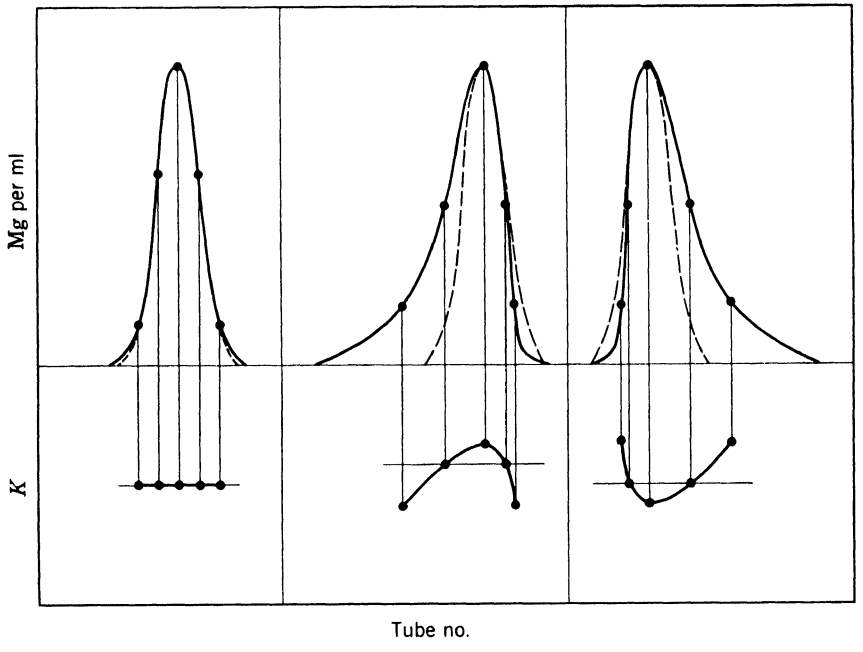

FIG. 4. Hypothetical distribution patterns of solutes with linear and nonlinear partition isotherms.

distribution pattern in countercurrent distribution. The effect can be recognized as shown in Fig. 4.

The left pattern is from a pure solute which does not associate appreciably with its own molecules. The theoretical curve fits the experimental, and the partition ratio across the band is constant. In the middle pattern, the isotherm is such that the partition ratio increases with concentration. This gives a self-sharpening front and a trailing rear. The right-hand pattern is from a solute with an isotherm which gives a decreasing K with an increase in concentration.

This behavior may complicate separation, or it can be taken advantage of in some cases to give an easier separation.[10] Where a linear partition ratio is desired, improvement can be obtained by choosing

a system with stronger associating tendencies.[1] This tends to inhibit the association of the solute molecules with themselves.

It is possible to detect transformation by the above approach even when there is a skewed distribution as Fig. 5 shows. This is an actual

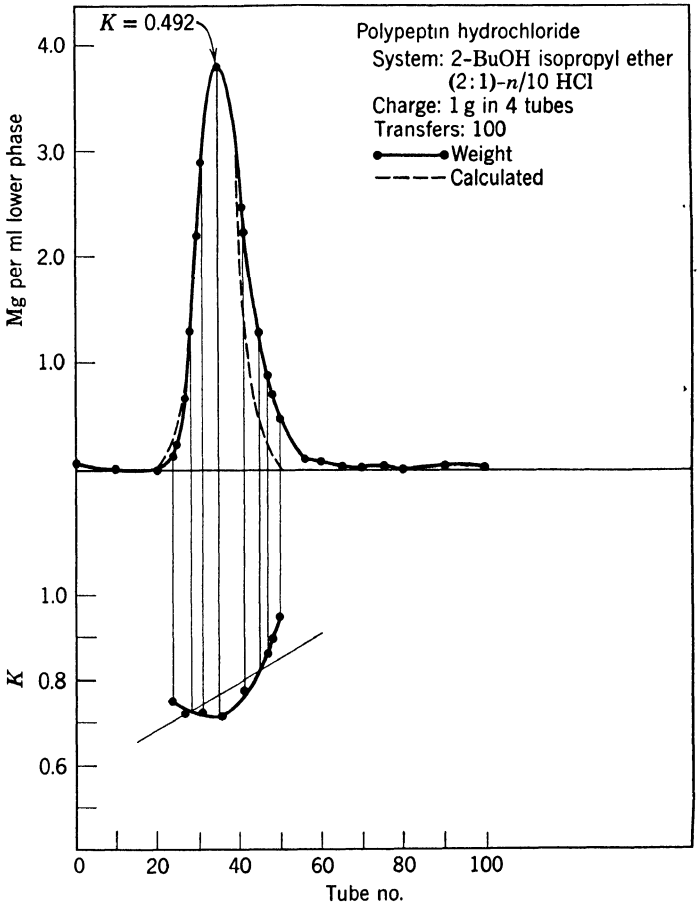

FIG. 5. A distribution pattern indicating transformation and skewing.

experiment with Polypeptin made by Hausmann. In this case the K's on both sides of the band at the same concentration level were not identical.

In the foregoing I have tried to give an over-all viewpoint of some phases of extraction which could be or have been very important in the study of small amounts of unknown solutes. Obviously the time available would not permit complete treatment of the more technical details. These are available in the literature.

In conclusion, I would like to speak briefly of several fields of biochemical research which have drawn heavily on extraction for the advancement made. They have involved the most complicated fractionation possible with countercurrent distribution as well as other techniques. The general type of equipment used is similar to that shown in Fig. 6 but with approximately 200 tubes. The one shown is

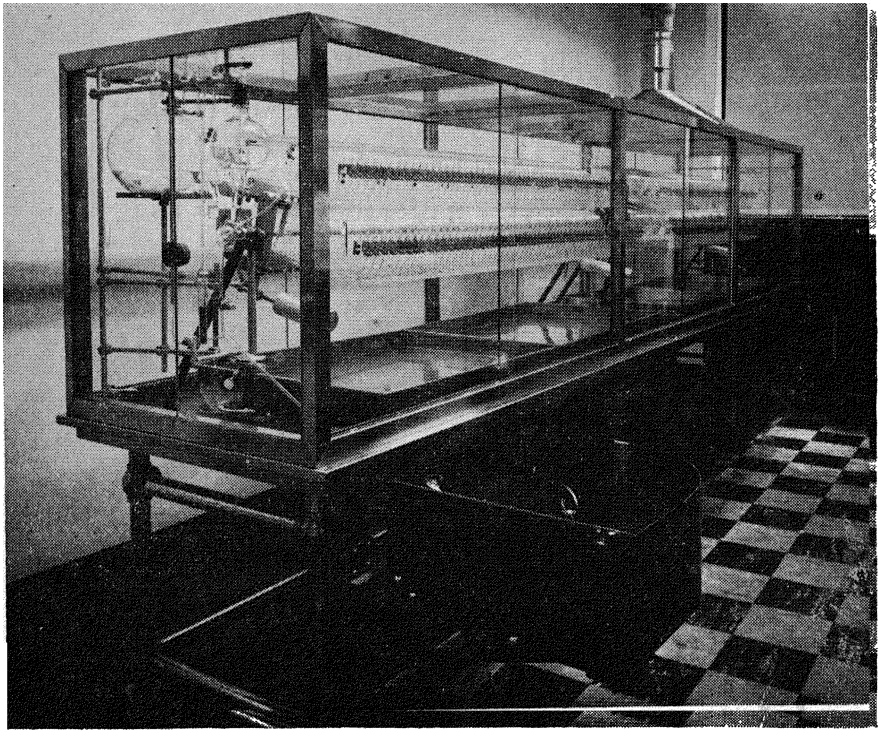

FIG. 6. A 420-tube automatic-distribution apparatus.

an automatic extractor with 420 tubes. It is complete with filling device, robot, fraction collectors, and hood. It has permitted many complex separations to be made. Figure 7, an example of the use that can be made of it, is a schematic flow sheet of the distributions required for the separation of peptides arising on the partial hydrolysis of bacitracin.[20] The top pattern is the initial distribution which separates the mixture into more than 10 families. Each family is then fractionated further or converted into a derivative and fractionated until pure single compounds are obtained. This flow sheet is excellent for bringing out one favorable characteristic of the approach: namely, the all-inclusiveness of the analytical result. In this

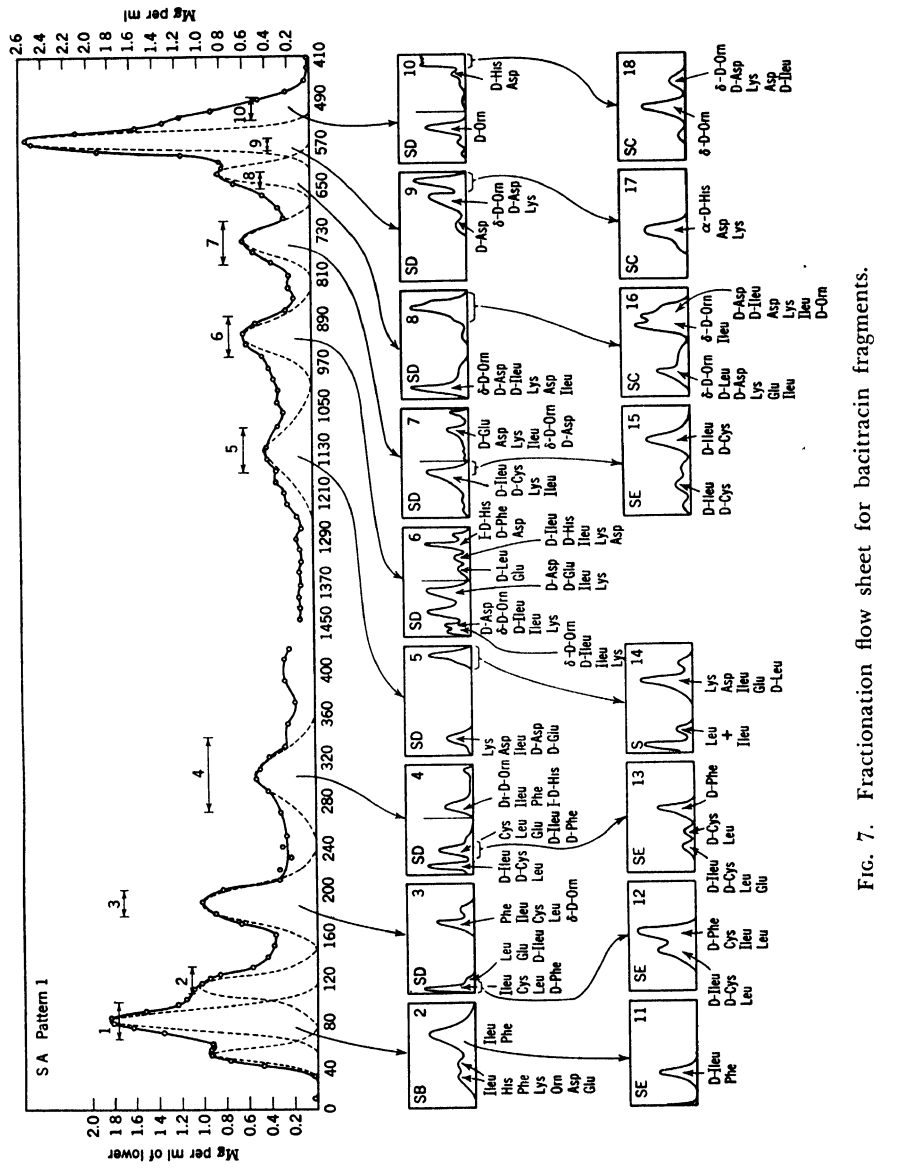

FIG. 7. Fractionation flow sheet for bacitracin fragments.

experiment, the complex starting mixture can be regarded as a mixture of traces of a great many different compounds.

Perhaps one of the most significant analytical contributions that countercurrent distribution has made is connected with purity studies. Regardless of how good the chemical investigation of a natural prod-

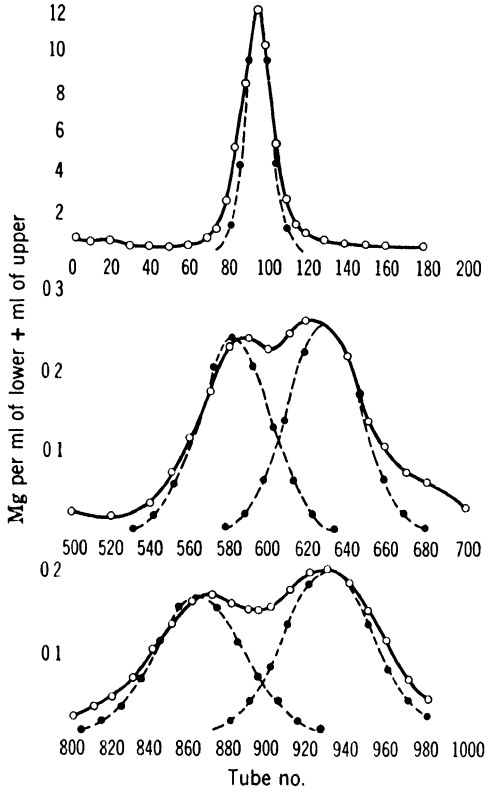

FIG. 8. Distribution pattern showing the separation of pork and beef insulins.

uct is, the study is no more reliable than the evidence that can be presented to show that all the data accumulated relate to a single chemical individual. Countercurrent distribution has repeatedly demonstrated that it stands high in the list of tools available for the purpose by separating more than one compound from preparations that were thought to be pure by all other techniques.[9,15,16] It has fractionated and given clear-cut evidence of purity for the individual fractions from many substances, for which no clear-cut evidence has been obtained by any other technique. These have included the gramici-

dins,[15] tyrocidines,[8] bacitracins,[13] polymyxins,[19] the hormones of the pituitary,[26,30,23,4,7,22,27] Hypertensin (Angiotonin),[6,28] and others.

Irrespective of all theoretical considerations regarding the determination of purity, the most pertinent thing to know when large molecules are concerned is how much change in the structure can be tolerated before separation methods would be unable to distinguish the original from the transformed product. Good models for studying this problem are now found in the insulins from different species of animals. It is known that pork insulin differs from pig insulin by replacement of an alanine residue by a threonine, and a valine residue by an isoleucine.[18] Their molecular weights are nearly 6000.[17] However, an artificial mixture of the two can be separated by countercurrent distribution in a system made from 0.1% acetic acid, *n*-butanol, and pyridine as shown in Fig. 8. The top pattern shows the divergence from the calculated band at 196 transfers, the middle pattern at 1264 and the lowest pattern at 1850 transfers. This result can be considered a demonstration of one of the highest degrees of selectivity yet reached by any separation technique that is applicable to this type of substance and that can be reliably related to substances of such known large size.

I am quite aware that very little has been said thus far about the application of extraction to trace analysis of inorganic elements, and that there is considerable interest in this field. However, the principles of extraction have been much more widely used in organic and biochemical fields, and in most of the references mentioned part of the objective of the work has been to establish principles. These same principles would be applicable in the inorganic field. Not only can good separations be made but information regarding the way the elements are held can be derived. This would be particularly true if they are involved in some type of complex.

DISCUSSION

Discussion Leader
Elwood O. Titus
National Heart Institute

I presume that many in the audience will be interested in the application of countercurrent distribution to inorganic elements. Such application will depend largely on one's ability to complex such sub-

stances with other materials that will permit their distribution. I hope that some suggestions by which this may be accomplished, based on your experience, will be offered from the floor.

For my part, I will limit myself to three illustrations, which will give, perhaps, a sense of reality to some of the problems one encounters in countercurrent distribution. These illustrations will perforce be

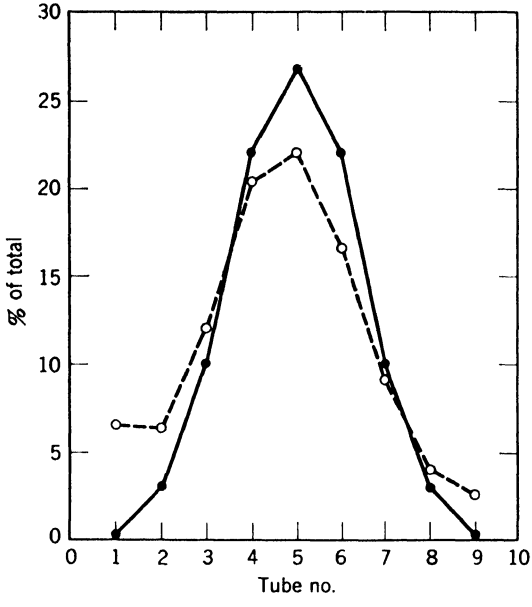

FIG. D1. Countercurrent distribution of 2.5 μg of 5-hydroxytryptamine (5HTA) isolated from brain in *n*-butanol and 0.25 *M* pyrophosphate, *p*H 9.5. Concentrations of 5HTA were measured by ultraviolet fluorescence. ○ Experimentally determined 5HTA. ● Theoretical distribution of a pure substance.

drawn from biochemical work. However, I think the principles can be applied to other situations.

Figure D1 represents the countercurrent distribution of 5-hydroxytryptamine (serotonin) isolated from the brain by Dr. Udenfriend and his collaborators. Although in this tissue the substance occurs only in minute amounts, it appears to play an important role in brain function, and changes of a few micrograms can have profound effects.

In this instance 5-hydroxytryptamine was assayed by its characteristic ultraviolet fluorescence spectrum in the spectrofluorophotometer developed by Drs. Bowman and Udenfriend. The good agreement between the distribution of the fluorescent substance and the theoretical distribution of authentic 5-hydroxytryptamine served to identify

the brain substance in spite of the fact that only a few micrograms were available.

When really adequate analytical instrumentation is at hand, the simplicity and mathematical precision of countercurrent extraction provide a most useful means for the identification of microquantities of material.

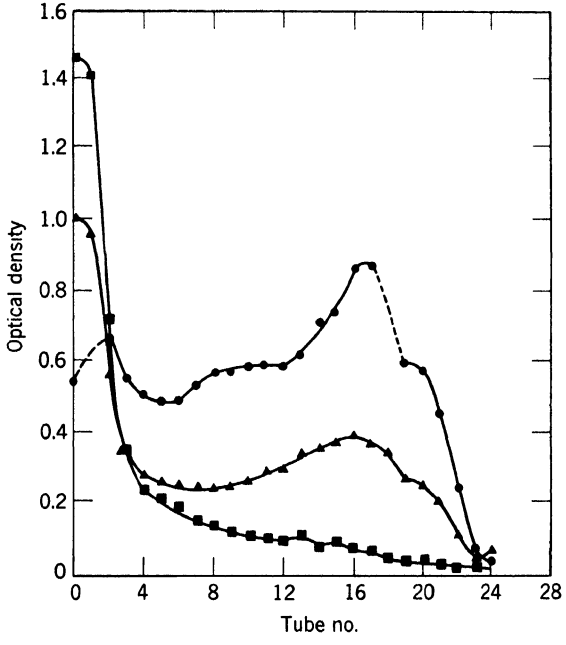

FIG. D2. ▲ Countercurrent distribution of N1-isopropyl-*p*-chlorophenylbiguanide in $CHCl_3$ and borate buffer, *p*H 10.3. ● Contents of tube 16 redistributed in same system. ■ Contents of tube 3 redistributed in same system.

The second illustration represents a situation that can lead to misinterpretation of countercurrent distribution data if the underlying phenomena are not recognized. Here a pure substance, in this instance the antimalarial drug N1-isopropyl-*p*-chlorophenylbiguanide, can exist in aqueous solution as an equilibrium mixture of tautomers, one of which is more nearly completely extractable into chloroform than the other.

Figure D2 illustrates the countercurrent distribution of a pure sample of this substance. The separation of the two forms by distribution is opposed by the tendency for the isolated tautomers to return to the equilibrium mixture, so that the anomalous distribution patterns shown here are obtained.

It is interesting that the high sensitivity of the extraction procedure is one source of the present difficulty, since such a relatively minor chemical change as the tautomeric shift of the double bonds in the biguanide can markedly affect the partition coefficient.

The situation wherein a substance is undergoing reversible changes during the distribution process can, of course, frequently be recognized. The obvious corrective measure is to lengthen the time of each

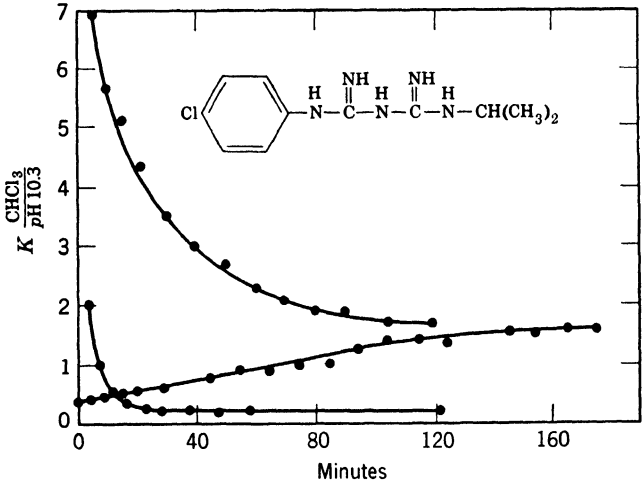

FIG. D3. Partition coefficients of N¹-isopropyl-*p*-chlorophenylbiguanide in chloroform and borate buffer, *p*H 10.3, as a function of time under varying conditions. See discussion in text.

equilibration so that both the transfer of material across phase boundaries and the chemical changes in solution reach complete equilibrium. It is not always appreciated, however, that tautomeric changes can sometimes be very slow.

This is illustrated by a series of experiments carried out in the same chloroform buffer system used for the distributions described above.

To obtain the lower curve of Fig. D3, the biguanide was dissolved in the aqueous phase and allowed to stand. At intervals as shown, samples were withdrawn and shaken for 30 seconds with the chloroform phase in order to measure the partition coefficient. It is apparent that, after about 30 minutes in buffer, the substance reaches a tautomeric equilibrium in which relatively little of the chloroform soluble form exists.

When such a solution in buffer was shaken continuously with the chloroform phase in a sealed apparatus and the partition coefficient

measured at intervals, the middle curve was obtained. Here the removal of the more chloroform-soluble entity has necessitated a readjustment of the equilibrium in water. Because of the slow rate of the latter process, the achievement of final over-all equilibrium required about 2½ hours.

About the same time was required when the free base was dissolved in the chloroform phase and continuously equilibrated with the aqueous phase, as shown in the upper curve.

Under these circumstances it is apparent that it would have been necessary to carry out each equilibration of the Craig apparatus for 2½ hours (60 hours for a conventional 24-plate run) in order to obtain a distribution pattern conforming to the theoretical behavior of a pure substance.

This example presents a somewhat extreme case, but very similar phenomena have been encountered in a number of isolations of natural products.

Finally I would like to mention the application of countercurrent distribution to a situation that occurs not infrequently in studies of intermediary metabolism. For highly potent physiological agents which can be administered only in minute amounts the use of C^{14}-labeled material is often a necessity. Fractionation of the labeled metabolites can be carried out on a trace level readily enough and can provide much useful information, but in many instances it is impossible to deduce structures for such metabolic products from data obtained at the trace level. Recourse must then be had to more conventional chemical means of identification.

In order to provide sufficient material for this, we have found it expedient to administer an unlabeled substance to a large number of animals and to pool the urines or tissues under study. In a separate experiment, the radioactive precursor is administered to a single animal, and the labeled degradation products are fractionated by any suitable microprocedure until they are obtained as radiochemically pure entities. An individual radioactive metabolite may then be added as an indicator to a small aliquot of the pooled products and fractionated by countercurrent distribution. The aliquot chosen must be sufficiently small so that the bulk of material does not absorb the radiation which is used to guide the distributions. The distributions carried out with the labeled aliquot are then exactly duplicated on a greatly expanded scale with the large bulk of unlabeled material. These parallel experiments are continued until material is obtained

that satisfies the criteria for homogeneity and that distributes in accord with the radioactive indicator.

This procedure considerably expands the amount of information obtainable from tracer studies alone. It is in reality an extension of the principles of the well-known isotope-dilution technique, but it avoids the necessity for inspired guesses concerning the structure of unknown metabolites which is inherent in conventional isotope-dilution procedure. Its whole success obviously depends on the fact that the countercurrent distributions of the crude material will be identical, regardless of the scale on which they are done. Other methods of fractionation than extraction can, of course, be used in this procedure, but with few other techniques can the course of fractionation be so precisely predicted when the volumes involved are greatly expanded.

QUESTIONS AND ANSWERS

ROBERT T. HALL (*Hercules Powder Co.*): Can you suggest any general criteria that would be helpful in deciding whether to use countercurrent distribution or partition chromatography, in solving a separation problem? I realize that the specific problem is one of the biggest factors, but nevertheless I thought it might be of interest to compare these two techniques for separation.

LYMAN C. CRAIG: Your question is a very important one but difficult to answer except in a rather general way. The choice of a method, of course, will depend on the technique available in the laboratory, the stage of development of that technique, the experience the workers have in each technique, etc. The rather elaborate technical know-how required for each technique, in order to bring out the highest separating power, places quite a demand on a single research worker to be completely at home with each one. We have tried a wide variety of separation techniques but have been primarily interested in developing countercurrent distribution. In more recent years we have been thinking of the proper integration of the most effective techniques, particularly countercurrent distribution, ion-exchange chromatography, paper chromatography, and zone electrophoresis.

ROBERT T. HALL: What I have in mind is, for example, in partition chromatography, one must consider the effect of the supporting material. In using methanol on silica gel as an immobile phase, it is certain that more than just partitioning between the two phases is involved. Therefore, the countercurrent-distribution method requiring no support or adsorbent, would avoid problems associated with them. Are there other similar types of considerations that might be helpful?

LYMAN C. CRAIG: The choice of a technique is somewhat a matter of personal preference. I can, however, mention a few of my own ideas.

The first preliminary investigation in an unknown field should be done

by a few stages of countercurrent distribution. If the pure solute of interest is reached at this stage, one is lucky. If not, after this preliminary fractionation, one should apply chromatography, ion exchange, or zone electrophoresis. If any of these lead to an apparently pure solute, one should return to countercurrent distribution again for confirmation of the thesis of purity.

It is possible to pick fields in which one technique is superior to the others. For instance, in the carotenes, as Dr. Strain showed in his talk, chromatography has given separations not reached by any other technique. The analytical separation of the amino acids by ion-exchange chromatography in the method of Moore and Stein has about reached the ultimate in this field. Many separations reported by countercurrent distribution such as in penicillin, the pituitary hormones, and insulin have not as yet been reached by any other technique.

However, in any one of these fields, superiority may in part be a question of the amount of labor and emphasis put on a particular technique. If we would concentrate on the carotenes, we might be able to duplicate the separations reported by Dr. Strain. Conversely, he might be able to do better than we with the large polypeptides if he tried hard enough.

I might say that very much more man power has been put into the development of the various chromatographic techniques than has gone into the exploration of extraction.

J. GORDON ERDMAN (*University of Pittsburgh*): I should like to ask you about the rate of equilibration needed for the extraction of these high-molecular-weight materials. Is a longer period required for the insulin separation, for example, than for the amino acid separation?

LYMAN C. CRAIG: In our experience it does not take longer for the larger molecules.

This raises a very interesting point in the theory of chromatography. We do not understand the factors that control the interchange of solutes between two phases in contact, but we do know that, for phases that simply flow past each other without breaking the interface, the rate of solute interchange is very slow. This poses a problem in connection with the commonly accepted theories of chromatography.

KURT A. KRAUS: I would like to amplify one point relating to the relative advantages of ion exchange and solvent extraction. If the materials to be separated have large molecular weights, there is severe doubt that ion exchange will, in the long run, be the method of choice. For large solutes, rates of exchange become very slow, and penetration into the resins becomes difficult except perhaps at very low cross-linking. As a result also, capacities become very small for high-molecular-weight materials. Of course, there have been many ingenious developments in the handling of large-molecular-weight substances by ion exchange, such as the work at the Harvard Medical School, the work of Moore and Stein and W. E. Cohn. Nevertheless, for large-molecular-weight materials, solvent extraction appears to me easily competitive.

LYMAN C. CRAIG: I might add that, in the hands of Moore and Stein and their collaborators, the separation of certain higher-molecular-weight peptides and low-molecular-weight proteins has been possible with suitable ion exchangers with lower cross-linking.

R. L. BOWMAN (*National Heart Institute*): I would like mention made of the future of gas chromatography. It is being a little overlooked. Perhaps it is important, and someone might care to comment on that.

HAROLD H. STRAIN: I mentioned gas chromatography as one of the divisions of chromatography. It certainly has many advantages, but most of the advantages lie in the fact the separated substances in the gas phase can be detected very easily compared to the same quantities of substances in solution. Otherwise there is no advantage except possibly speed.

J. T. HORECZY (*Humble Oil and Refining Co.*): What do you think, Dr. Craig, about continuous-type extraction?

LYMAN C. CRAIG: I think the continuous type of extraction, that is, stage-continuous, which is a mix-and-settle affair, certainly has its place in production. This type of extraction is not for analysis or small laboratory-scale work. It only separates a mixture into two fractions. These then must be subdivided. Countercurrent distribution may separate in one run nearly every component in a mixture whether it has two or ten components.

The literature contains hundreds of accounts of the separation of mixture of organic substances by countercurrent distribution, but comparatively few with the continuous type of extraction.

In part, this is due to the variability of the continuous type of extraction depending on the load, the rate of operation, and other factors. The larger production operations with the continuous type could certainly profit from careful control by the technique of countercurrent distribution.

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Chemical Microscopy

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ABSTRACT The importance of crystallography as an aid in trace analysis is based on the fact that crystals of a given compound, no matter how small, always have identical refractive indices, unit cell dimensions, interfacial angles, etc. If a very small crystal of a given compound can be isolated and characterized by microtechniques, then that compound can be identified. Such samples may be single crystals of airborne dust, occlusions in a metal or in a corrosion film, decomposition products or other reaction products, contaminants in food, particles dispersed in plant or animal tissue, etc. Since nearly all solids are crystalline, there exists the possibility of identifying by crystallographic procedures any very small particle of solid matter.

The sizes that can be isolated and identified in this manner depend to some extent on the tools used. These tools are usually microscopy (light, electron, and now, X-ray) and diffraction (electron and X-ray). The light microscope can identify, by means of refractive index and other crystallographic properties, single crystals weighing as little as one microgram (10^{-6}). The electron microscope, to some extent by particle shape but more generally by electron diffraction on single particles, extends this range by, at least, a factor of 10^{-6} to a lower size limit of about 10^{-12} gram. X-ray diffraction falls in an intermediate range since data can be obtained on single crystals weighing about 10^{-9} gram.

Furthermore, diffraction is not limited to single-particle studies but finds applica-

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tion in the identification of thin films only a few atoms thick. In the same manner, microscopy can be used to identify polycrystalline or amorphous particles in these same small size ranges by shape, color, and refractive index measurements.

INTRODUCTION

Speaking generally, the use of the microscope in trace analysis is limited to heterogeneous samples in which the desired component, though present in trace amounts, maintains its identity as a separate phase. The microscope is usually not good for the analysis of trace constituents of solutions, whether solid or liquid. In fact, its use is based almost entirely on the recognition of particular portions of a physical mixture. The component present in trace amounts must be separated or concentrated mechanically, or it must be distinguishable as such to the eye of the microscopist.

Solutions may be analyzed microscopically under certain special conditions: (1) if the desired component can be crystallized, or (2) if it imparts color or fluorescence to the system which can be measured with high sensitivity.

Various physical concentration procedures may be used to increase the sensitivity or ease of microscopic analysis. These range from the Pasteur technique of mechanically picking out the desired component to the use of a magnet. Other methods of separation include sublimation, chromatography, ion exchange, flotation and centrifugation or differential solubility. Figuratively speaking, the desired component may be "isolated" by immersion of the sample in a medium of the proper refractive index, by use of polarized light and crossed polars, or by appropriate staining methods.

The major advantages of the microscope for trace analysis are:

1. Small sample size.
2. Results in terms of compounds present rather than elements.
3. Additional facts about sample: e.g., heat treatment, polymorphic form, particle size and shape, nature of dispersion.
4. Speed of analysis.

The major disadvantage is the general inapplicability of the microscope to the study of trace constituents in homogeneous systems.

IDENTIFICATION BY MORPHOLOGY

Many materials can be almost instantaneously identified microscopically by observation of their morphological characteristics. Each

of the natural or synthetic fibers can be quickly identified as well as bacterial cells, pollen grains, metals and alloys, many crystalline materials, plant and animal tissues, etc.

Where necessary, the electron microscope can be used to extend the limit of resolving power of the light microscope. The latter is limited, in terms of recognition of a small particle, to a minimum size of about 1 micron, whereas the corresponding limit for the electron microscope is about 100 times smaller. The corresponding weights of 1-micron and 100-angstrom (0.01-micron) particles are, respectively, 10^{-12} and 10^{-18} gram, assuming cubes of density equal to unity.

The virtue of the microscope as a tool in trace analysis lies in the ability to recognize and identify fine particles having a weight under 10^{-6} gram when such materials are a very minor portion of the entire sample.

STUDY OF MORPHOLOGY

The study of morphology is the study of external form or shape. The human eye and brain are constantly using morphology of objects, animate and inanimate, in everyday life to identify friends and relatives, to choose the proper key to unlock the front door, to find a car in the parking lot, to choose the best piece of filled chocolate from the candy box, etc. The method is not infallible, as everyone knows who has tried in vain to unlock a car or house almost identical with his own or who has greeted a presumed-to-be old friend and been rebuffed by a cold look of nonrecognition. Still with training to develop the powers of observation and with a good memory a man can recognize and name with certainty a surprisingly large number of people, cars, airplanes, trees, flowers, animals, etc.

In the same way a microscopist is able to identify a surprisingly large number of metals and alloys, bacteria, plant and animal fibers, pure crystalline compounds, animal cells and tissues, diatoms, starch grains, pollen grains, etc. The sizes of these individual particles or fibers range from about 10^{-6} down to perhaps as low as 10^{-18} gram. Each single particle embodying all of its characteristic morphological features is readily identified. Furthermore, with a knowledge of its density and measurement of its physical dimensions the weight can usually be calculated with high accuracy. The accuracy of this weight figure is greater, the more nearly the physical shape resembles a regular geometrical form and the more accurately the density is known.

The microscopic identification of textile and paper fibers is a good

example of the use of morphology in analysis. The scales on wool fibers, nodes on flax, serrated cells in esparto, pitted baglike cells in nonconiferous woodpulp, smooth high birefringent cylinders of nylon, isotropy in glass wool fibers, etc., enable the microscopist to identify these and dozens of other fibers almost at a glance (Figs. 1 and 2). In a similar way, metals and alloys (Fig. 3) can be quickly recognized by

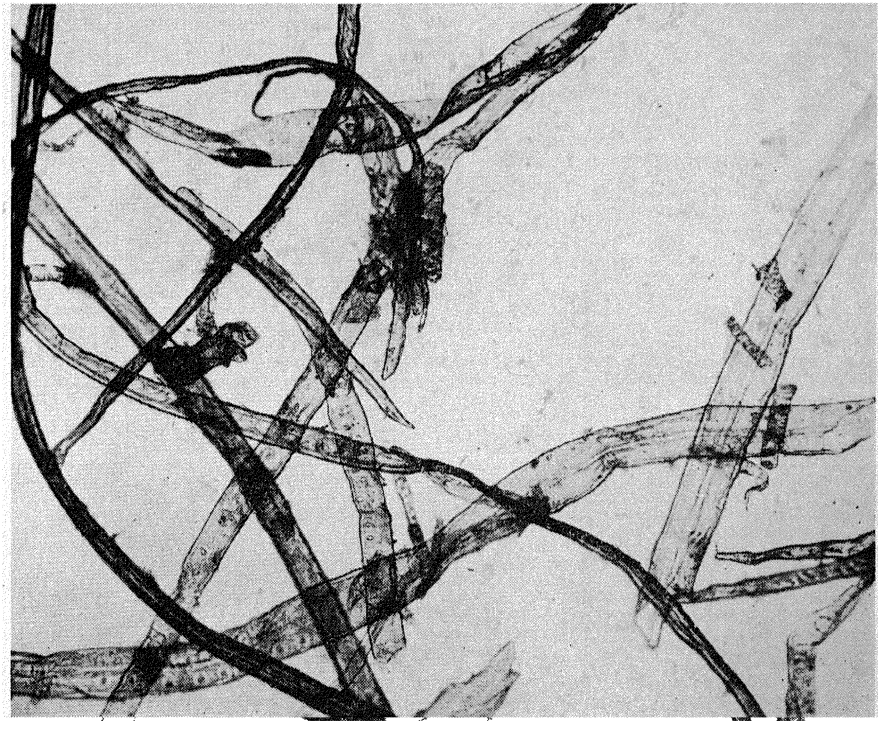

FIG. 1. Fibers of coniferous chemical woodpulp.

the metallographer, bacteria by the bacteriologist, human tissue sections by the pathologist, etc.

These techniques are very useful in air-pollution studies. Samples of suspended dust in the atmosphere over a large city can be quickly collected and as quickly analyzed microscopically. Figure 4 is a photomicrograph of atmospheric debris collected in dust-fall jars in Chicago; the field of view shown contains cotton and wool fibers, bits of paper and wood, flyash, soot, part of a pigeon feather, quartz and other minerals, as well as bits of paint, iron oxide scale, and particles of glass. Many of the particles identified individually weigh less than 10^{-10} gram.

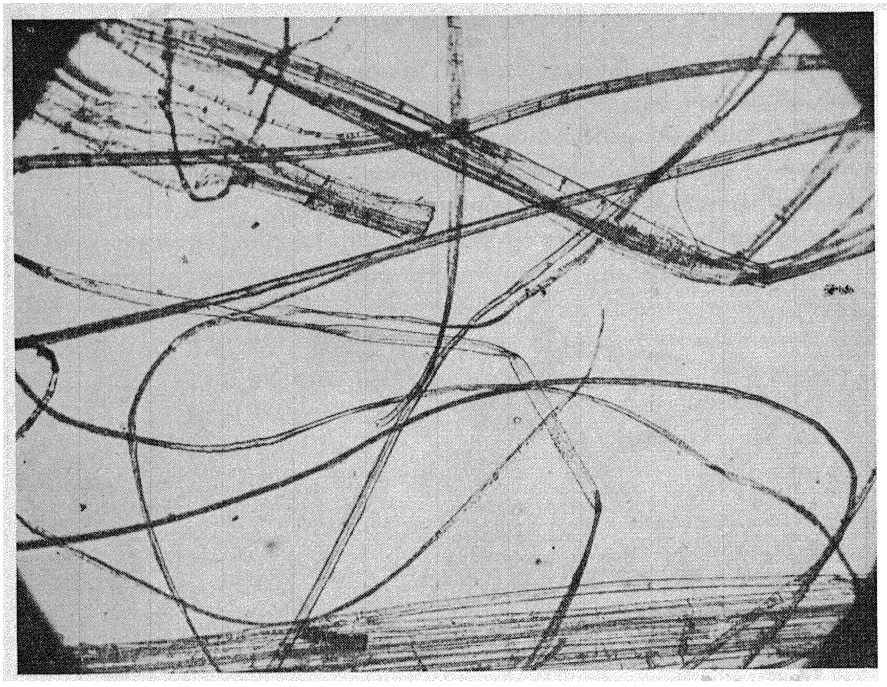

FIG. 2 Fibers of flax.

FIG. 3. Polished and etched section of pearlitic steel.

Occasionally the analyst (metallographer, pathologist, etc.) will make the job of identification easier by using the electron microscope since many metal structures, diatoms (Fig. 5) and especially bacteria, are too fine to be easily resolved using the light microscope. The analyst may also use staining as an aid in identifying tissues, fibers, or bacteria, or he may employ one or more optical methods to identify a given component or to check a mixture for the presence of a given component.

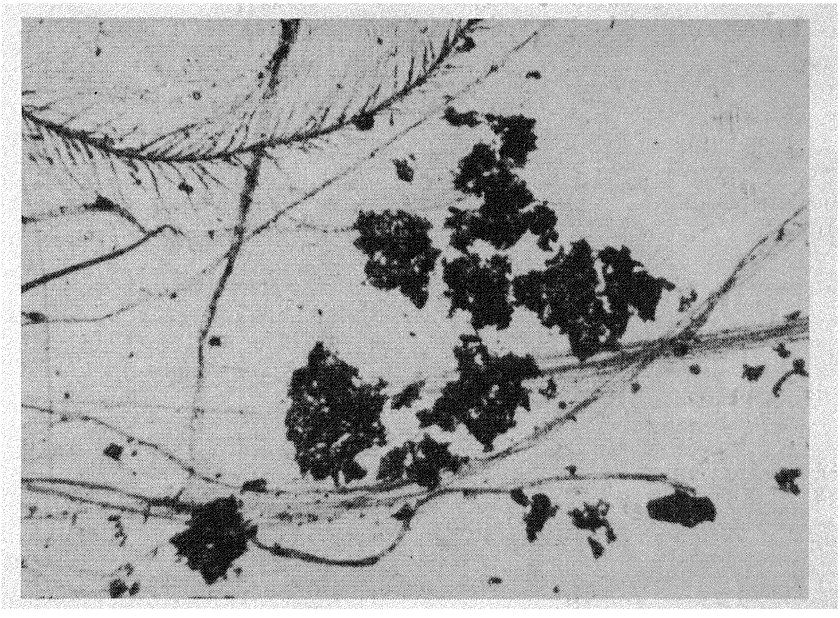

FIG. 4. Settled dust from a dust-fall jar in Chicago. Note the fly ash and fibrous material, including portion of pigeon feather.

The polarizing microscope, the phase-contrast microscope, and the interference microscope may be used to improve the visibility of certain preparations and to bring out details that might otherwise be nearly invisible. The polarizing microscope is especially valuable for differentiation between isotropic and anisotropic components or even for the detection of one component of very different birefringence in a mixture of birefringence substances. Figure 6 shows a photomicrograph of a mixture of isotropic and anisotropic materials taken with slightly uncrossed polaroids. This sample of kitchen cleanser contains an isotropic gritty component, birefringent needles of sodium carbonate and other birefringent particles of soap. All three components are thus easily differentiated by either shape or behavior between crossed polars. The phase contrast microscope and the in-

terference microscope both increase contrast although the latter is more useful for quantitative determination of minute masses.

Proper choice of a mounting medium can also be of great help in picking out minor components of a mixture. In the analysis of diamond powder, for example, since diamond has a very high refractive

FIG. 5. Electron micrograph of diatomaceous earth.

index (n_D = 2.417), it is possible to check very quickly for contamination by mounting the sample in a sulfur–selenium mixture having a refractive index near 2.41 (about 60% Se and 40% S is a suitable medium). All the usual contaminants (adulterants) such as quartz, corundum, glass, carborundum, and even rutile (ϵ = 2.903, ω = 2.616) will appear with strong contrast. Mounting in a medium having an index near 1.65 will quickly pick out quartz, glass, and corundum by their low contrast compared with diamond (Fig. 7); however, carborundum and rutile would also have high contrast in this medium. As an added check in this case the preparation can be examined

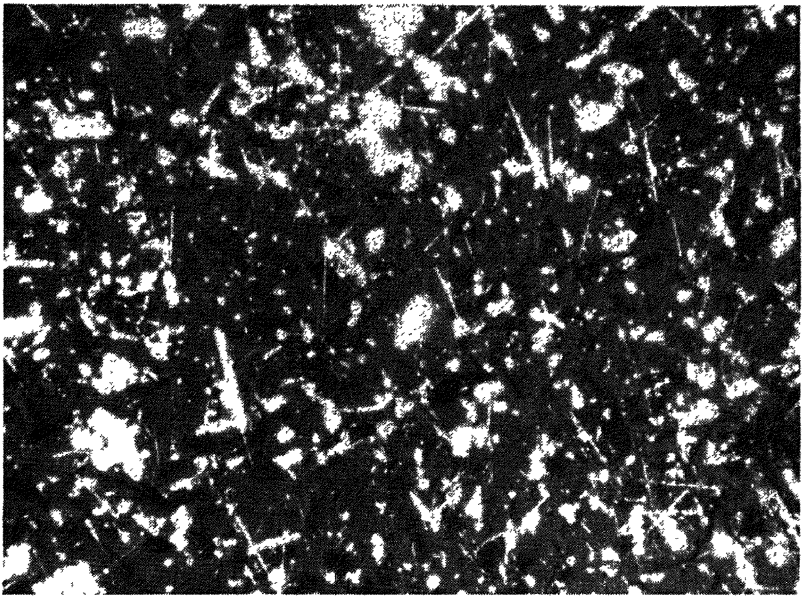

FIG. 6. Sample of kitchen cleanser between slightly uncrossed polaroids. Birefringent particles are white; isotropic particles are black.

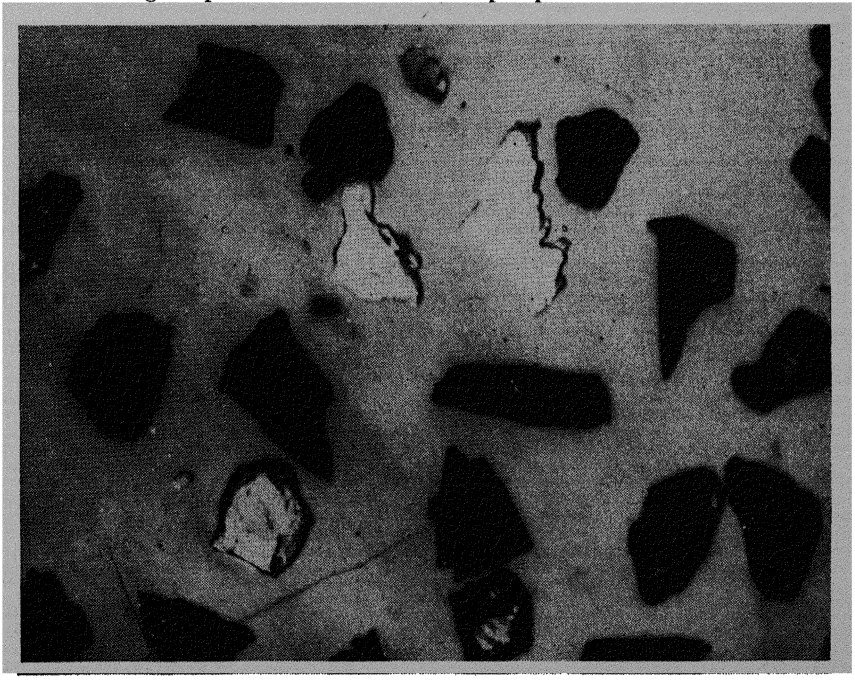

FIG. 7. Crystals of diamond (black) and quartz (white) mounted in a refractive-index medium close to that of quartz.

quickly with crossed polars since diamond is isotropic and most mineral contaminants are anisotropic.

Another specialized technique for rendering small percentages of a minor component readily visible is dispersion staining.[1] This technique is based on the use of a mounting medium having dispersion of the refractive indices very different from that of the component being analyzed but with identical refractive indices at one wavelength. All light of this one wavelength is then transmitted by the particle, whereas light of other wavelengths is partly scattered. The net result is a particle with a colored border. Very small percentages of particular compounds can be identified in this way although, of course, a specific mounting medium must be prepared for each component; quartz, for example, mounted in nitrobenzene gives a blue "dispersion staining" of all quartz particles.

Obviously, even with all optical aids the use of morphology alone as a means of identification has limitations. At best, the procedure functions only with an atlas of photomicrographs for each system of components (e.g., fibers, starches, pollen grains, metals). At worst, each microscopist must set up his own procedure and study each component in the system separately so that he can then recognize and identify any one at any later date. Furthermore, the morphological characteristics are, in general, descriptive and hence do not lend themselves to tabulation.

In certain cases, supplementary numerical properties, such as interfacial angles and refractive indices, can be measured. When this is possible, the analytical method becomes more generally useful. Tables of crystallographic data are often based entirely, or almost entirely, on morphological properties. The five volumes of Groth,[2] for example, include the interfacial angles for nearly every one of the 5000 compounds listed; Winchell's compilations of crystal data for both organic [3] and inorganic [4] compounds and especially the Barker index [5] are basically compilations of morphological data for crystals. Even the more recent book by Donnay and Nowacki,[6] though based on X-ray determination of unit cell parameters, is useful in this connection since interfacial angles can be readily calculated from the unit cell dimensions.

The law of the constancy of interfacial angles is one of the basic laws of crystallography. Crystals of a given compound may, and usually do, vary widely in external shape (called habit), but each and every crystal of a given compound will show the same angle between corresponding faces and edges (Fig. 8).

The calculation of interfacial angles from the cell dimensions or axial ratios determined by X-ray diffraction is very simple. The axial ratios are always expressed as $a:b:c$ for the biaxial systems (orthorhombic, monoclinic and triclinic) and $a:c$ for the uniaxial systems (tetragonal and hexagonal). Ammonium dihydrogen phosphate, a tetragonal crystal, has an axial ratio, $a:c = 1.002$. From this ratio we

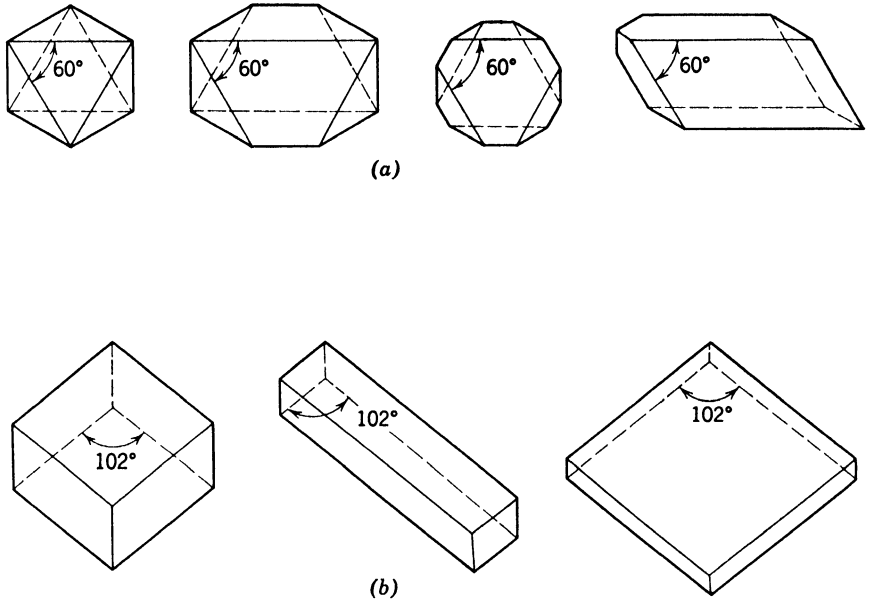

FIG. 8. Common distortions of (*a*) the cubic octahedron and (*b*) the rhombohedron illustrating the law of constancy of interfacial angles.

know that the distance between atoms along the c axis is 1.002 times the spacing between atoms along the a axis. The actual spacings as measured by X-ray diffraction are given as $a = 7.51$ A and $c = 7.53$ A. Either the axial ratio or the cell dimensions can be used to calculate the interfacial angle by means of simple trigonometry. The tangent of one-half the angle about c is 1/1.002 (or 7.51/7.53) or 0.998. The observed interfacial angle is then $2(44.94°) = 89.88°$. This angle will be constant for all crystals of reasonably pure ammonium dihydrogen phosphate wherever, or however, crystallized.

Other crystals even though tetragonal and, at first inspection, identical, will be found to show significantly different axial ratios and interfacial angles. Potassium dihydrogen phosphate, isomorphous with $NH_4H_2PO_4$ has an interfacial angle of 93.60° corresponding to

$a:c = 1:0.939$; another, ammonium dihydrogen arsenate, a corresponding angle of 90.24° with a ratio of $a:c = 1:1.004$; etc.

X-ray diffraction data, either single crystal or powder diffraction, can be obtained on very small amounts of material. Although the amount sufficient depends to some extent on the scattering power of the elements present in the sample, at least one sample of powder (actually a product of lattice decomposition on the surface of a single crystal) weighing about 10^{-10} gram gave sufficient powder diffraction lines for certain identification. With mixtures, of course, larger amounts ranging up to something like 10^{-8} gram are required. Single crystal data can be obtained on any crystal large enough to orient microscopically on the X-ray goniometer: about 10^{-8} gram.

It is often a surprise to calculate the actual weight of a single particle or crystal on which all of the necessary crystallographic properties can be determined. A single particle measuring as little as 5 microns in diameter can be manipulated at will. Its refractive indices may be measured with intermediate washing to eliminate traces of the previous index medium; it can be moved from slide to slide; it can be photographed, X-rayed, measured, and, after all this, saved for later study. Its weight, if it were a sphere having a density of one, would be 6.5×10^{-11} gram.

A few representative analyses of this type made in the analytical laboratories of the Armour Research Foundation include:

Sample 1: Glass sphere 3.2 microns in diameter (3.6×10^{-11} gram); measured refractive index = 1.586.

Sample 2: Polycrystalline aggregate (10^{-7} gram) showed 30% CaO, 30% $Ca(OH)_2$, 35% calcite and 5% aragonite by X-ray diffraction.

Sample 3: Polycrystalline aggregate (10^{-7} gram) showed 85% calcite, 10% aragonite and 5% $Ca(OH)_2$ by X-ray diffraction.

Sample 4: Polycrystalline aggregate (10^{-8} gram) showed 90% NaCl, 10% MgO by X-ray diffraction.

Sample 5: Polycrystalline coating (10^{-10} gram) showed $Pb(N_3)_2$ by X-ray diffraction.

Sample 6: A single crystal of ignited oxide (10^{-10} gram) dissolved in water on a slide and immediately reprecipitated to give many minute calcite crystals 1 to 2 microns in size between 10^{-11} and 10^{-12} gram in weight, identified by obvious morphology and refractive index; original particle CaO.

Oftentimes the morphology of a given component may not be sufficiently unique, or it may be too thoroughly dispersed throughout the sample to be visible as discrete particles. In these cases, concentration

by crystallization (usually precipitation) may be useful. The procedures to be presently described will, it should be noted, apply to systems wherein the desired component is homogeneously dispersed throughout the sample as, for example, ions in a solution. The procedures are not in themselves highly sensitive since the sample must be relatively concentrated; however, the total weight of sample may be extremely small, usually less than a microgram.

Chamot and Mason [7] have described a number of precipitation reactions covering all the common and most of the uncommon cations and anions. These procedures involve addition of standard and specific reagents to a small aqueous test drop with resulting precipitation of characteristically shaped crystalline precipitates. The basis for identification in each case is the specificity of the precipitation reaction and comparison of the crystals so obtained with the written description and photomicrograph.

Corresponding procedures have also been applied to some organic systems usually by the use of reagents which form addition compounds. The test sample and the reagent can usually be melted together or individually but immediately adjacent to and touching each other. Picric acid forms deeply colored molecular addition compounds with most of the polynuclear aromatic hydrocarbons; hence very small samples containing a few per cent of either the hydrocarbon or picric acid can be characterized as such by melting in contact with picric acid or a polynuclear aromatic (respectively) as a reagent. 2,4,7-Trinitrofluorenone [8] can also be used as a test reagent for many aromatic derivatives.

The common requirement of these precipitation reactions is careful standardization of concentrations, times, and temperatures, since otherwise the precipitate might not form at all or, at best, the morphology might be changed and become thereby unrecognizable. Other methods of crystallization not requiring a specific reagent can also be used: Recrystallization from a solvent (Fig. 9) or from the melt or vapor (always under controlled conditions) give characteristic shapes easily recognized and remembered. The latter methods, recrystallization from the melt and vapor states (Figs. 10 and 11) have been described under the general title "Fusion Methods." [9] These methods have found wide application because they are rapid and require little technical background and only small samples.

Basically, the procedure is to heat a small sample of the compound (10^{-5} gram is about the minimum practical weight) on a microscope slide beneath a cover slip, watching for sublimate on the underside

of the cover slip. If a sublimate appears, heating is stopped, and the sublimate is studied for characteristic crystal angles and other morphological features. After study of the sublimate, or if no sublimate appears, the preparation is heated until melting occurs. On crystallization of the melt, the crystal shape and size, presence of gas bubbles, shrinkage cracks, polarization colors and the occurrence of polymorphic transformations are noted. These and other obvious de-

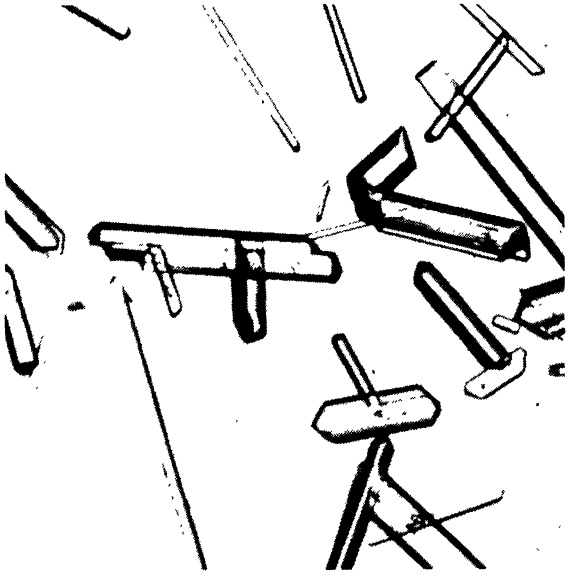

FIG. 9. Crystals of *p,p'*-DDT crystallized from thymol on a microscope slide.

scriptive properties are sufficient to characterize uniquely each of the members of relatively small groups of compounds (perhaps as high as 200 to 300).

During World War II, fusion methods were successfully applied to the identification of high explosives.[10] After careful study of each of the many possible systems, the analysts trained in these procedures were able to identify any one of the 60-odd high explosive compounds or mixtures in a few seconds on samples weighing a fraction of a milligram.

A number of miscellaneous procedures should also be described, among them a most interesting test method developed by Crozier and Seely[11] of the University of New Mexico. This procedure was developed for the purpose of testing suspended dust samples for particular contaminants; for example, sodium chloride. The suspended dust

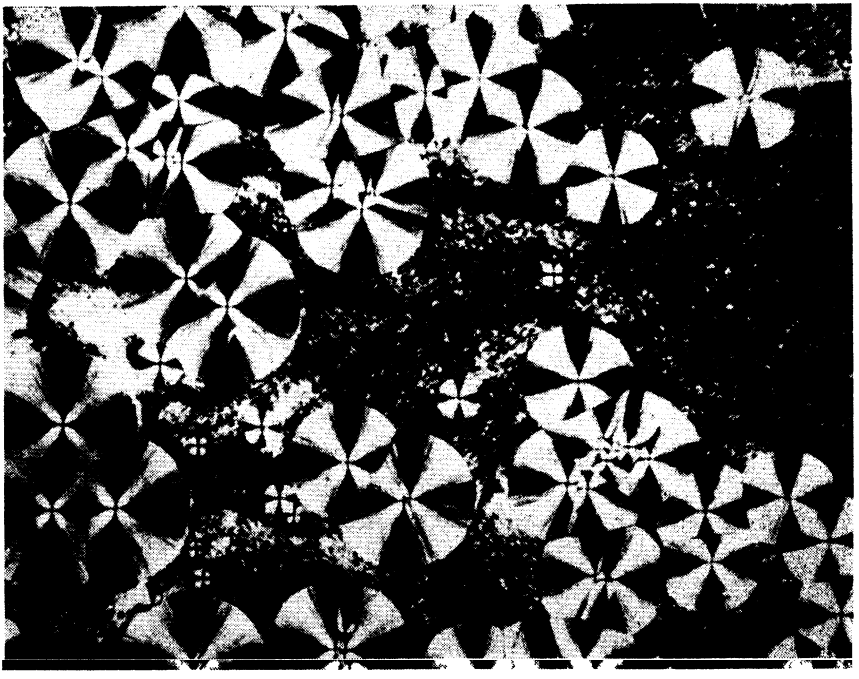

FIG. 10. Cholesteryl acetate crystallized from the melt.

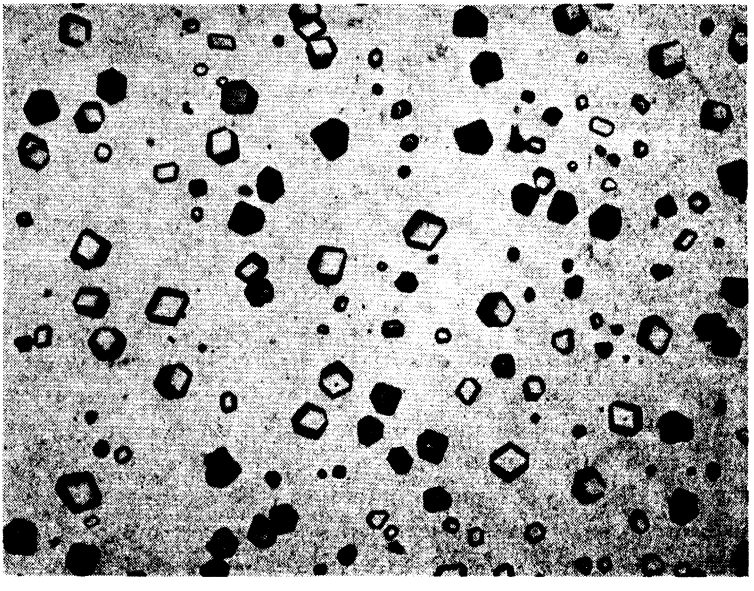

FIG. 11. Sublimed crystals of hexamethylene tetramine.

is impinged onto a microscope slide coated with a gelatin film containing a specific reagent in solution. To test for sodium chloride, a gelatin film impregnated with mercurous fluosilicate is used. After a short time, a number of white halos of mercurous chloride will become apparent in the gelatin film, each halo surrounding the original location of a single chloride particle. The number of halos will, therefore, indicate the number of sodium chloride particles originally present, and the heaviness of the halo the size of the original particle. Extremely fine particles, perhaps as small as 10^{-14} gram can be detected in this manner. The same procedure is applicable to soluble sulfates, carbonates, phosphates, nitrates, etc., and to any of the soluble cation salts, e.g., iron, lead, etc., which form insoluble salts.

Although this general procedure has apparently not been applied to the detection of very fine particles of organic compounds, there is no reason why such methods could not be developed. A film of polystyrene impregnated with picric acid would be an ideal detector for fine airborne particles of anthracene and other polynuclear aromatics. Many other combinations of film and test reagent will suggest themselves as the need arises.

MICROBEAD TESTS

The mineralogists have, for many years, used bead tests [12] as a means of qualitative identification of many of the cations. The procedure used by them usually consists of a borax or sodium metaphosphate bead in a platinum wire loop; a small amount of test substance (usually 10^{-4} gram or less) is dissolved in the bead by heating, and the resulting color imparted to the bead constitutes identification. Oxidizing or reducing atmospheres may be used to aid in the identification since significant color differences may result (Table I).

Although this test is already a microprocedure, it is possible through use of the microscope to extend the sensitivity by several orders of magnitude. The increase in sensitivity is directly proportional to the decrease in volume of the test sample, since the microscope is capable of observing the color in much finer beads. At the same time it is obvious that the thickness of the test sample must not be appreciably less than the thickness of the mineralogist's bead, since otherwise the intensity of the color will be lessened and may become indiscernible. The solution is to form a cylindrical "bead" in a small opening in a metal plate. The plate, preferably gold, about 1 mm thick and about

TABLE I

COLORS OF BORAX BEADS *

Cation	Oxidizing Flame		Reducing Flame	
	Hot	Cold	Hot	Cold
Antimony	Pale yellow	Colorless	Pale yellow	Colorless
Chromium	Yellow	Green	Green	Green
Cobalt	Blue	Blue	Blue	Blue
Copper	Green	Blue	Pale green	Brownish
Iron	Yellow to orange	Greenish to brown	Bottle green	Pale bottle green
Lead	Pale yellow	Colorless	Pale yellow	Colorless
Manganese	Violet	Brownish to violet	Colorless	Colorless
Molybdenum	Pale yellow	Colorless	Brown	Brown to black
Nickel	Violet	Reddish brown	Opaque grey	Opaque grey
Titanium	Pale yellow	Colorless	Yellowish grey	Brownish
Vanadium	Yellow	Green	Brownish green	Yellow to green

* This table is adapted from the excellent text by O. C. Smith.[12]

1 in. by 2 in. in other dimensions may have a large number of very fine holes about 100 microns or less in diameter; about 10 microns, if possible, would be the approximate lower limit. Plates having holes about 100 microns in diameter may be prepared by drilling; holes smaller than 100 microns are most easily made by casting the metal plate in a graphite mold having the desired number, arrangement, and sizes of tungsten wires protruding from the base. Removal of these wires after casting then leaves the desired openings in the plate. In any case, with an opening in the plate 100 microns in diameter, a single crystal of MnO_2 only 10 microns in average diameter and weighing about 10^{-9} gram would impart a readily visible color to the borax glass. Smaller openings would require proportionately smaller quantities; a 10-micron opening would require a single particle weighing about 10^{-11} gram.

The opening in the plate is filled with borax by placing a few milligrams on top of the plate over the opening. On heating from below in a Bunsen flame, the borax melts and then runs into and fills the hole. The excess borax is then broken away, and the sample, a single measured particle, is placed on top of the borax at the center of the opening. Again the plate is heated by holding it in the oxidizing portion of the Bunsen flame until the sample dissolves into the borax.

The color is then observed after cooling by placing the plate on the stage of the microscope and observing with a strong transmitted light.

This same procedure may be used to detect those elements that can be made to fluoresce. It is generally better, however, to use sodium fluoride or lithium fluoride as the glass-forming matrix in this case. The only other change in procedure from that described above is the obvious use of an ultraviolet light source, preferably one that generates a good intensity in the region of 2540 A. Table II lists the fluorescent colors obtained with a few of the elements.

TABLE II

FLUORESCENT COLORS IN FLUORIDE BEADS *

	Matrix	
Cation	Sodium Fluoride	Lithium Fluoride
Bismuth	Blue-white	Orange
Columbium	Blue-white	None
Titanium	Light green	Dark green
Tungsten	Light bluish yellow	Light blue
Uranium	Brilliant lemon yellow	Brilliant blue

* These colors are characteristic of response to short ultraviolet radiation around 2540 A.

There is some possibility that this technique for qualitative detection and identification may be used for quantitative analysis in the region of 10^{-10} gram. Quantitative analysis in this range depends on the preparation of standards and control or measurement of the physical dimensions of the test sample. A series of fluoride bead standards can be readily prepared by filling a series of openings in the metal plate with fluoride and adding to each a definite weight of test substance. The weight of each is again calculated by measuring the necessary dimensions of a definite physical shape of a salt of the desired element. For example, small well-formed crystals of sodium chromate decahydrate can be grown from water, and individual crystals containing as little as 10^{-11} gram of chromium can be measured and "weighed" by calculation with an accuracy of ± 10–20%. There is, of course, an obvious advantage in choosing a salt in which the percentage composition of the desired cation is very low.

A number of crystals of gradually increasing weight are used to give fluoride "beads" of gradually increasing and known percentages of the desired element.

MICROCOLORIMETRY

In one instance, small spheres of red iron oxide glass were analyzed quantitatively and individually by comparing the colors of the spheres with the colors of standard glass spheres. In this case, the standard iron oxide glass can be made up in bulk, and therefore very accurately, and then ground to fine particle size in a ball mill (to an average particle size of about 10 microns) and fashioned into spheres by passing the powder through a blast-burner flame. The latter is accomplished by sifting the powder into the gas stream to the burner by means of a T-tube connection. The powder is collected after passing through the flame on a sheet of glossy paper or a pane of glass and contains each of the original particles in its equivalent spherical shape. A large sample of several hundred particles of each composition is mounted on a slide in a refractive-index medium of about 1.65 (Aroclor, a chlorinated diphenyl used as a plasticizer and manufactured by Monsanto). Such a preparation contains spheres having all diameters from about 1 to perhaps 100 microns.

The individual spheres to be analyzed are then compared with spheres of the same diameter in the standard slides. By this means, individual spheres as small as 2 to 3 microns and containing only 5% Fe_2O_3 can be analyzed quantitatively. This amounts to about 10^{-11} gram of iron.

PREPARATION OF MILLIMICROGRAM ANALYTICAL STANDARDS

Methods such as those given above can be used to create standards in the range 10^{-6} to 10^{-12} gram. Since single particles of known composition having reasonable shapes can be "weighed" by calculation from measured dimensions and known density, and since such particles can be manipulated at will, they may be placed in a sample matrix for microanalysis. The technique is general in that it can be applied to organic or inorganic systems and to water-soluble or water-insoluble systems. Each particle containing a known weight, perhaps only 10^{-12} gram, of the component in question may be added to a solution, placed on a film, centered on the end of a needle, or otherwise located for analysis. It may be manipulated as is or it may be mixed with an inert carrier material for easier handling.

The relationship among the particle weight, particle density, par-

ticle size, and weight of "active" component is given in Fig. 12. The term "active" signifies the portion of the particle of analytical interest; e.g., the 15.2% chromium in a particle of sodium chromium decahydrate when analyzing for chromium or the 52.6% of water in the same compound when analyzing for this component.

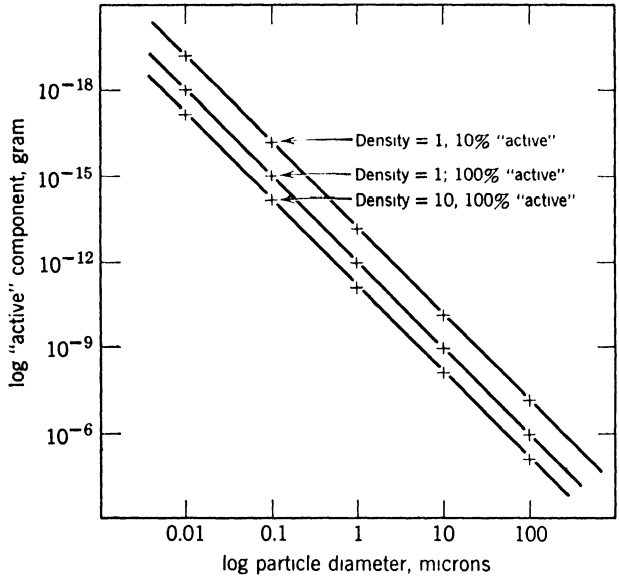

FIG. 12. Relationship between particle size (as cubes), percentage composition and weight of "active" component and density of particle.

ABSORPTION MICROSCOPY

In the same way that visible light is absorbed by fine particles and may be used qualitatively for identification, so also is electromagnetic radiation in other frequencies absorbed characteristically. X-ray, ultraviolet, and infrared absorption may all be used provided suitable instrumentation is available.

The Perkin-Elmer model 12-C single-beam infrared spectrometer with the reflection-microscope attachment is a commercially available instrument for both ultraviolet- and infrared-absorption measurements on very small particles. Single crystals, never larger than 10^{-6} gram are used, the object being to obtain an absorption pattern characteristic of the material under study. The sample size varies with the frequency used. In the far-infrared samples, about 10,000 square microns in area and not more than 10 microns thick (about 10^{-6} gram)

are required; however, in the near-infrared and ultraviolet samples about 500 square microns in area and about 10 microns thick (about 10^{-8} gram) are required. Infrared-absorption patterns are most generally useful since both organic and inorganic compounds normally show many sharp and measurable absorption bands which characterize not only the compound itself but also the polymorphic form of that compound.

Another general use of absorption measurements is in the study of plant and animal tissue sections. Here the sample must be studied either photographically or by means of an infrared- and ultraviolet-sensitive photomultiplier tube which furnishes a visual image on a fluorescent screen. The preparation must be illuminated with monochromatic light, usually of a wavelength known to be strongly absorbed by the desired element or functional group. Strong absorption will be shown in those portions of the preparation containing the desired component. Since other functional groups may also absorb at the same frequency, several more observations are made at other frequencies where the absorbances for the desired component are known.

X-ray absorption is less specific than ultraviolet and infrared absorption, since absorbance of X rays is a direct function of the atomic number. In a tissue, or other thin section, such as a metal film, the X-ray microscope or an X-ray radiograph will show gradations in absorption determined by the distribution of elements throughout the sample. Concentrations of high-atomic-weight elements would cause high absorption; areas of low absorption would indicate the presence of low-atomic-weight elements. The techniques of measuring X-ray absorption are most useful for determining the distribution of a known element in a thin section: e.g., distribution of tin in an aluminum–tin alloy.

CONCLUSION

The principal value of chemical microscopy in trace analysis lies in the study of very small amounts (10^{-6} to 10^{-18} gram) of material in itself a relatively pure component. Each such component is recognized by its physical properties, principally morphology. The quantitative analysis is carried out by "weighing" the particle or particles by measurement of the physical dimensions and calculation of the mass from the known density and measured dimensions. The trace analyst should also be interested in chemical microscopy since masses of pure compounds in the range down to about 10^{-10} gram can be weighed as

the red swatch of fabric (upper left), shows the presence of the oleophylic portion of the water-repellent finish while an acid-type dye stain in the blue swatches (lower left and right) shows the presence of the formaldehyde resin portion. Both of these stains are sensitive. However, when we look at the individual fibers under the microscope,

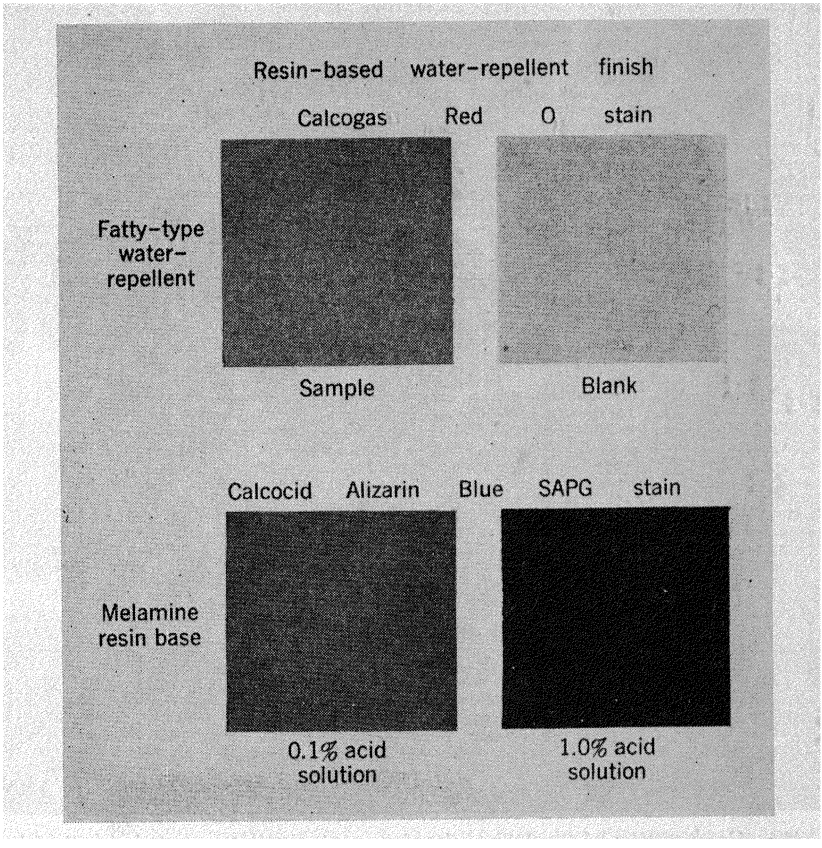

FIG. D1. Resin-base water-repellent finish on cotton fabric, stained to show the resin.

the process of magnification is accompanied by a diluting effect which makes the stain technique viewed under the microscope a much less sensitive one. A similar situation is encountered in the examination of damaged or defectively woven fabrics. The defect is very evident in the macrosample, but, when we attempt to examine it under the microscope in order to determine the ultimate unit of the defect, we often lose it completely.

There are cases where the material in question can be isolated and we can handle as little as 10^{-6} gram of a material with the light

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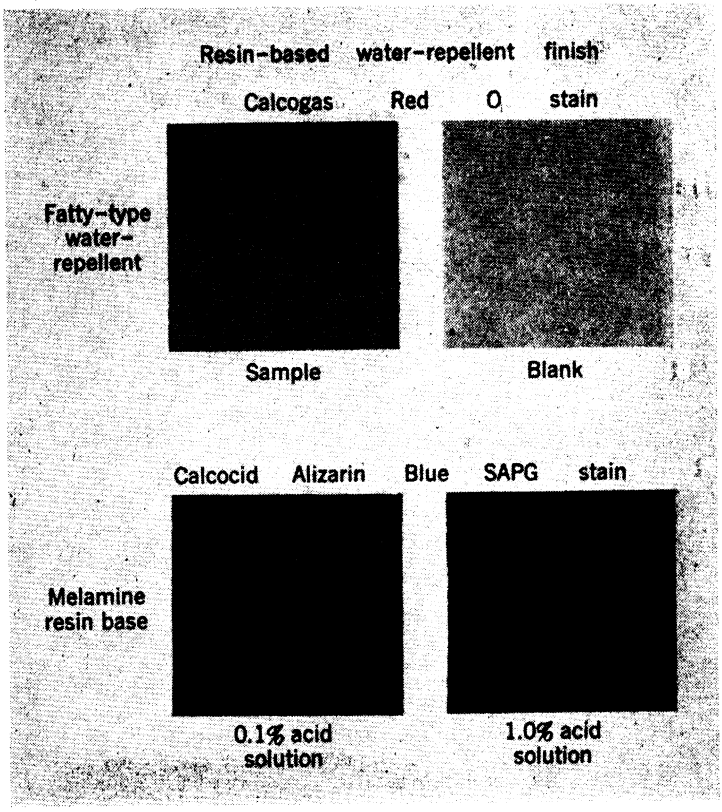

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There are cases where the material in question can be isolated and we can handle as little as 10^{-6} gram of a material with the light

microscope, as was stated by Dr. McCrone, and with the electron microscope as little as 10^{-12} gram. The real advantage of the microscope, and it does not matter whether it is a light or electron microscope, is its ability to give us further physical information about our

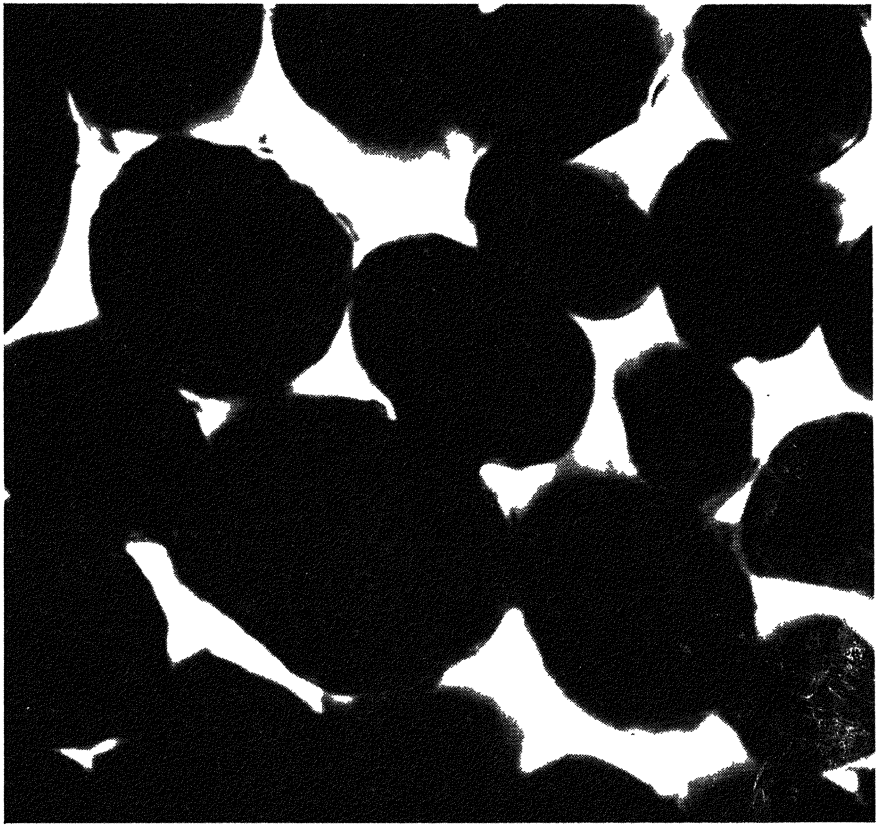

FIG. D2. Cross section of wool fiber showing even distribution of dye. Magnitude 1200×.

sample over and above the fact that this or that constituent is present. This added information is the relative distribution of components of a system. Figure D2 shows a cross section of a dyed wool fiber as seen through a microscope which shows an even distribution of the dye. The microscope is not capable of showing differences in dye content in the various portions of the fiber. Figure D3 shows another wool fiber showing poor distribution of the dye. We see evidence of the bilateral structure of wool, and we see some evidence of dye localization between fibril bundles. If we were to look at this fiber with an

electron microscope, we would see even greater localization or lack of distribution of the traces of dye that produce the over-all dyeing effect leveled out by our limited normal vision. Another example is shown in Fig. D4. This is an ultrathin cross section of a paint film

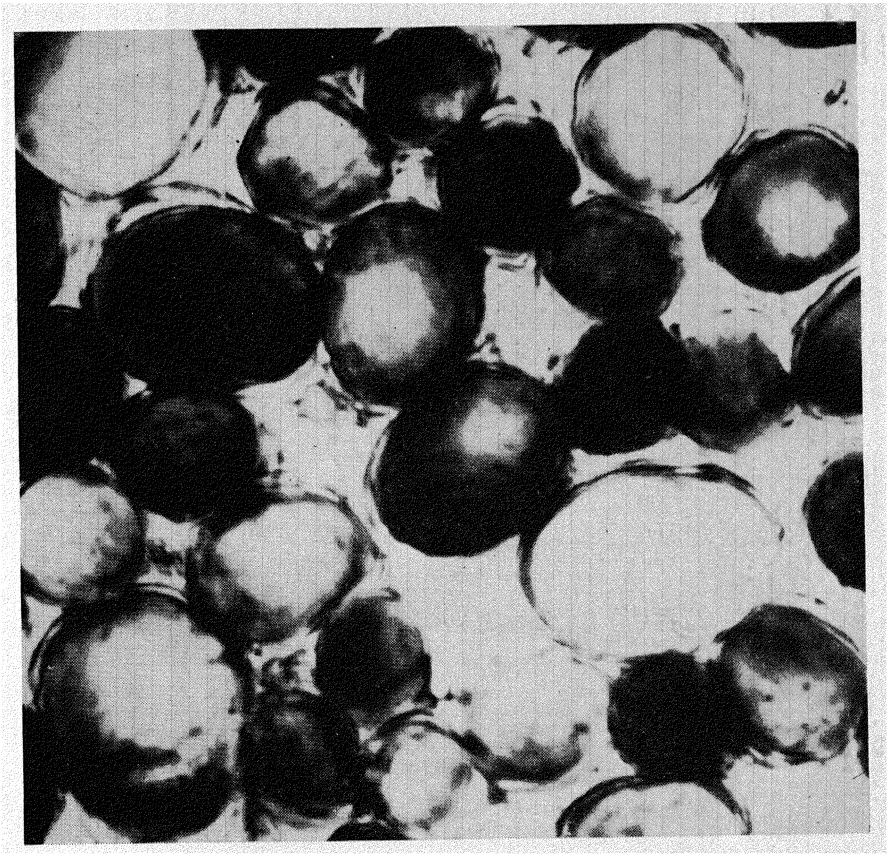

FIG. D3. Cross section of wool fiber showing uneven dye distribution. Magnitude 1200×.

as seen through the electron microscope. I think the nonuniformity of the distribution of the pigment particles is the most striking feature. It explains not only some of the hiding power and flow difficulties but also some of the weathering characteristics encountered. Although the pigment-to-vehicle relationship is certainly not one of trace amount, we are showing the location of trace amounts of pigment in the bulk sample.

We can pick up minute coatings of resin finishes on fiber as is shown in Fig. D5. By proper mounting, we can emphasize the resin

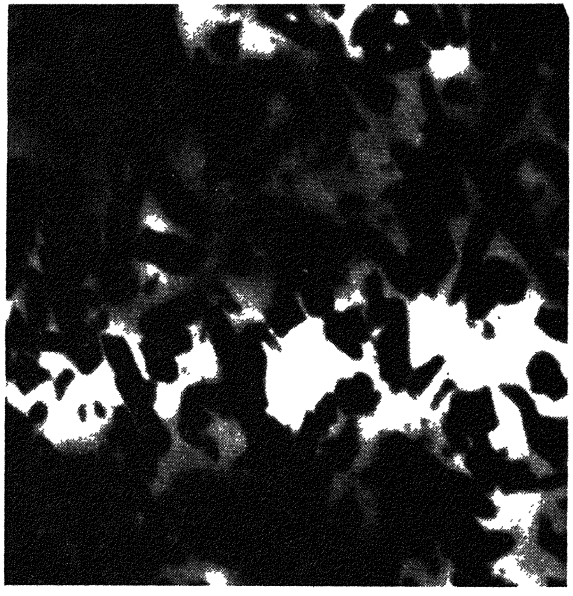

FIG. D4. Electron micrograph of ultrathin cross section of a paint film. Magnitude 20,000×.

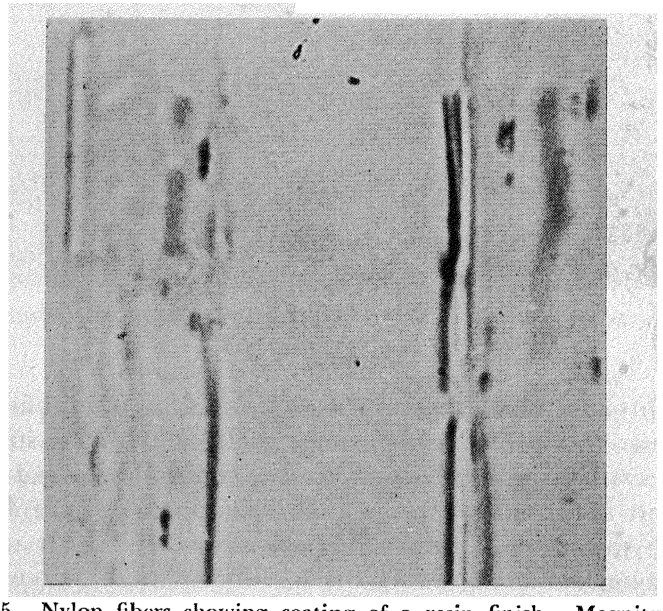

FIG. D5. Nylon fibers showing coating of a resin finish. Magnitude 1500×.

coating on nylon fiber. The phase microscope will pick up still smaller deposits of resin as shown in Fig. D6. We can go one step further and show finish applied to a single pigment particle. A 0.2-micron particle as we see it in this electron micrograph (Fig. D7) would weigh approximately 2×10^{-14} gram. The coating on these TiO_2 particles, which is barely visible, is present in considerably smaller quantity.

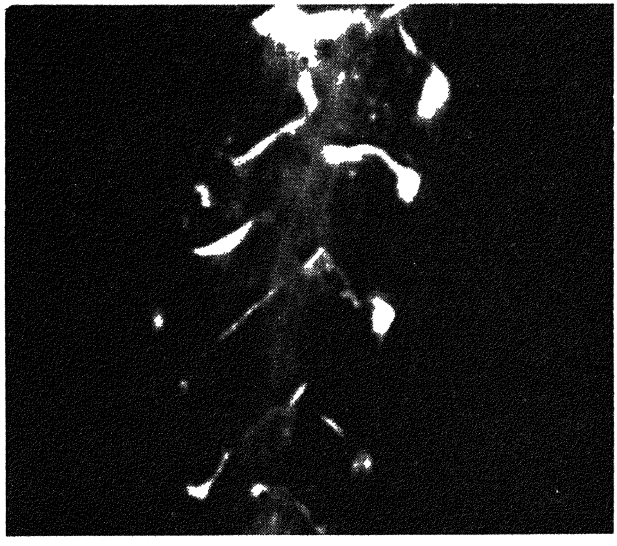

FIG. D6. Phase-microscope micrograph showing resin finish on wool fiber. Magnitude 1500×.

Dr. McCrone mentioned the adaption of the microscope to colorimetry. One other area of microscopy in which Dr. McCrone has done considerable work is fusion techniques. Here again, the microscope is not capable of trace analysis in the true sense, but it does approach it. I think the example will illustrate the added information that can be gained by adaption of a microscope to what we normally think of as macrotechnique. Although microscopical melting-point data have never been universally accepted, they are capable of producing information not normally obtainable by other means. Eutectic temperatures can be used for detecting small quantities of admixtures. The accompanying table lists the melting points and eutectic temperatures of four sulfa drugs. The eutectic temperatures are as characteristic of the combination as the individual melting points are of the individual compounds. The ability to detect eutectic

MICROSCOPICAL MELTING POINTS

In degrees centigrade

Pure Compound, m.p.	Eutectic	Impurity, m.p.
Sulfadiazine, 252	173	Sulfathiazole, 191
Sulfadiazine	224	Sulfamerazine, 234
Sulfadiazine	188	Sulfamethazine, 199

melting will depend on the distribution of the two components with respect to each other. Figure D8 shows a mixture of sulfadiazine and

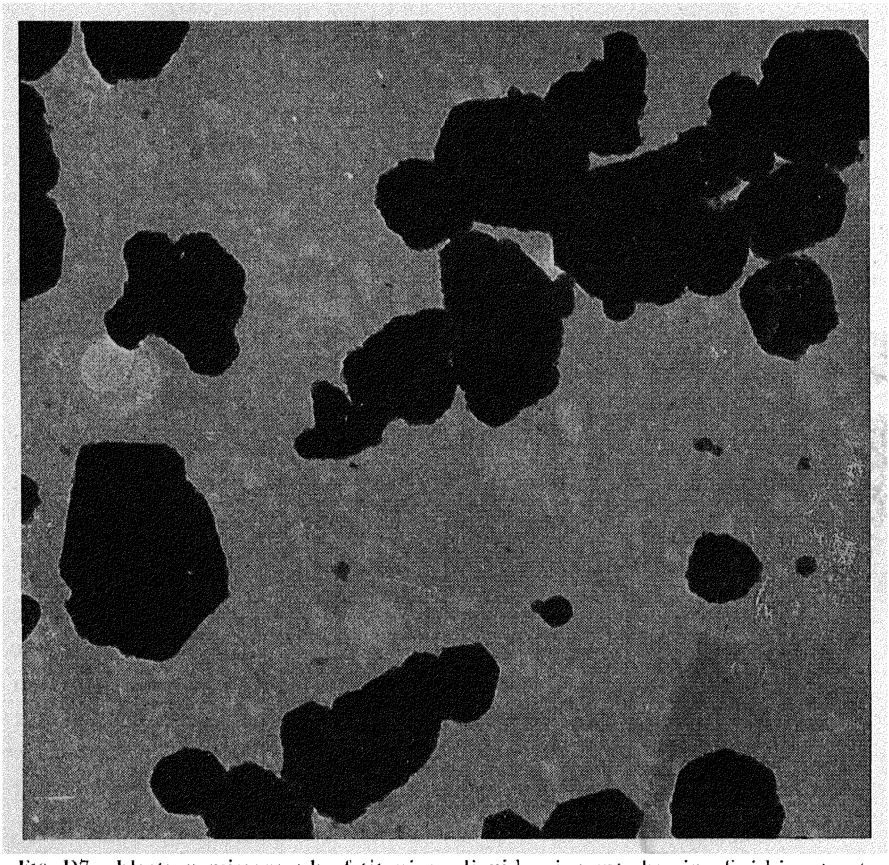

FIG. D7. Electron micrograph of titanium dioxide pigment showing finishing treatment on each particle. Magnitude 35,000×.

sulfathiazole. I think you can see indication of melting of the eutectic combination. True, it is akin to looking for a needle in the haystack when we get down to concentrations of less than 0.1%, but it can be

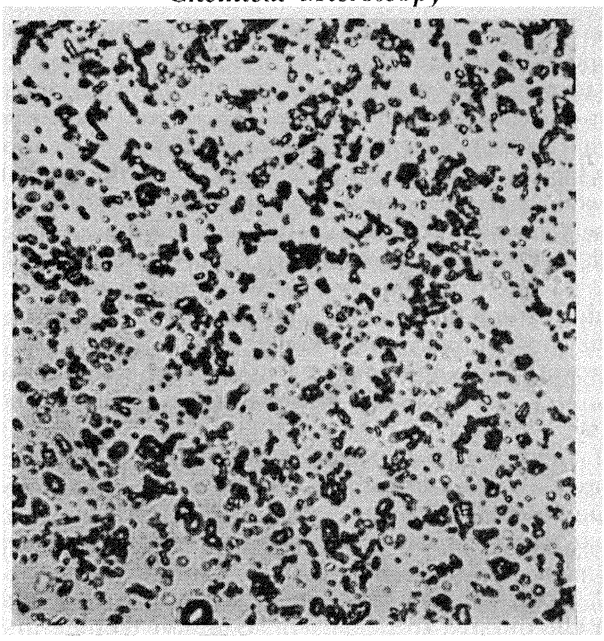

FIG. D8. Optical photomicrograph showing melting behavior of a mixture of sulfadiazine and sulfathiazole. Magnitude 400×.

done and it not only gives us an idea of the substance present but of the amount as well.

QUESTIONS AND ANSWERS

HAROLD H. STRAIN: How many different chemical substances can you recognize by observation?

WALTER C. MCCRONE: This depends. With strictly descriptive properties, a good microscopist may recognize several hundred organic compounds by the characteristic crystals grown from the melt, besides a variety of fibers, synthetic and natural; pollen grains; metals and alloys; etc.; amounting in all to perhaps 2000 to 3000 different chemical substances. On the other hand, if measurement of numerical properties are allowed, the list of chemical substances that can be positively identified increases greatly and is, in fact, limited only by the availability of known tabulated data for comparison: in all, certainly in excess of 10,000 of the most common substances.

HAROLD H. STRAIN: Have you ever looked at a chromatogram after the solution has evaporated? Is the solute distributed over the surface?

WALTER C. MCCRONE: When a chromatogram is examined microscopically after evaporation of the solvent, the solute is sometimes observed to be distributed as crystals and sometimes as an oil; which occurs depends on the solvent, purity of the solute, rate of evaporation of the solvent, and nature

of the solute. Rapid evaporation will tend to prevent crystallization. Inorganic compounds are more likely to crystallize than organic compounds.

J. C. CAVAGNOL (*General Foods Corp.*): Often we are interested in the identification of sugars in small samples. There are tests carried out in the field of colorimetry using a capillary tube of a hundred microns or so in diameter. One is limited to the length of the cell. I was thinking of an example in which several sugars might be present in small quantities with an emulsifier. Suppose you wanted to identify two or more sugars present. Is there hope for such identification under the microscope? Have you done it?

WALTER C. MCCRONE: If one depended on optical rotation, it would be necessary with mixtures of sugars to crystallize the sample and pick out from the crystalline product individual pure crystals of each sugar, à la Pasteur.

J. C. CAVAGNOL: I was thinking of Pasteur's technique as one possibility.

WALTER C. MCCRONE: This can be done if the amount of material is relatively large, say 1 to 2 mg.

DONALD H. ANDERSON (*Eastman Kodak Co.*): I have been surprised at the lack of attention given to infrared techniques in the papers presented. The question about identifying sugars raised by Dr. Cavagnol prompts me to point out that micro-infrared equipment and techniques are easily adapted to this work.

WALTER C. MCCRONE: You are referring, I am sure, to the microscope attachment for the infrared spectrometer. It is also possible to identify each of the sugars by their optical properties in the visible: e.g., refractive indices, optic axial angles, dispersion.

DONALD H. ANDERSON: That is a possibility, but I was referring more specifically to work with a silver chloride lens condensing unit. These units are available for the Baird infrared spectrophotometer, and Beckman Instruments also have a similar attachment. These are both readily applicable to trace analysis where a few micrograms of sample are all that are available. The micro-infrared techniques are particularly important in the analysis of compounds having medical significance.

Within the last few years, a simple lens system has been developed [13] that enables infrared-absorption curves to be prepared on microsamples, using spectrophotometers that have heretofore been considered suitable only for macro work. The unit uses a pair of planoconvex silver chloride lenses about 20 mm in diameter. Silver chloride is used because of its ability to transmit infrared energy. The first lens focuses the beam of infrared energy onto an area about $\frac{3}{4} \times 3$ mm, where the sample is mounted. The second lens collects the rays that have passed through the sample and redirects them along the original path of the beam. The sample is held on a block that is pinned to insure its being properly placed between the lenses. The lenses in turn are held on a metal plate which has pins that engage in a conventional point-and-groove kinematic mount. The entire unit is only a few inches long, weighs only 4 ounces, and can be inserted in the instrument or removed in a matter of seconds.

Calibration curves for quantitative work have been prepared, using fractional microgram quantities of material in the beam. Normally, however, the amounts tested range between 10 and 50 μg. The technique for mounting the samples is the potassium bromide matrix technique described by Stim-

son [14] and Scheidt and Reinwein.[15] The micro dies used and related techniques are discussed in sufficient detail in two articles,[16,17] so that no description is being given here. The method has been applied very widely to both inorganic and organic compounds. The only difficulty so far encountered has been traced to metathesis between potassium bromide and compounds such as amine sulfates. With these and similar compounds, some new brands appear which can be shown to be due to conversion of the sulfate into amine hydrobromide and potassium sulfate. This difficulty can be avoided by the simple expedient of working with the hydrobromides whenever possible.

One of the problems of workers in this field is the isolation of small samples. Paper chromatography is an excellent supporting technique to separate the substances under study. In some instances a single spot on a chromatogram has contained ten times as much chemical as was needed to secure a good infrared record.

Increasing use is being made of infrared measurements in medical research, and it is our belief that the microtechniques will markedly extend this field.

WALTER C. MCCRONE: This subject should have been mentioned, but I left it out because of the shortage of time. It could be generalized. Ultraviolet absorption can be accomplished. If one looks at a biological specimen, illuminating it with light in the ultraviolet or infrared, and uses a monochromator, one can then, by determining the absorption characteristics of individual parts of the sample, tell something of the composition of those parts, or, if one had a single crystal of sugar, something like 10×50 microns, one could determine the entire infrared pattern of the crystal.

A. BENEDITTI-PICHLER (*Queens College*): Another point about the sugars. Around 1938 or 1939 several papers were published (I believe it was Quincy's work) on recognizing sugars and mixtures of sugars by recrystallizing various solvents and examining the morphology of the sugars. It worked quite well. I used it.

WALTER C. MCCRONE: I am sure that this discussion amply illustrates the variety of microphysical properties available for characterization of substances under the microscope. Such substances can be present in very small percentages but must be in themselves pure; single particles weighing less than 10^{-10} gram can usually be positively identified often at a glance.

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5

Spot Tests

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ABSTRACT Spot-test methods of analysis owe their importance to the fact that they provide simple, direct means for running qualitative and semiquantitative analyses. From the standpoint of trace-analysis applications, it is important to note that spot tests are the most sensitive of the ordinary microchemical techniques, both from the standpoint of dilution sensitivity and absolute sensitivity. Spot tests are now available for practically all the inorganic ions, and present developments are providing functional group and class tests as well as direct tests for many important organic compounds. It is of special significance, therefore, that trace analysis of both inorganic and organic materials can be made, using spot-test techniques.

Spot tests are the most sensitive of the ordinary microchemical techniques, both in regard to dilution sensitivity and in terms of absolute sensitivity. Although it has not been stated exactly what is understood by the term "trace," the general impression exists that it refers to relatively small amounts of material existing in the presence

of gross amounts of other substance. Regardless of whether traces are considered as minute quantities coexisting with large amounts of matrix, or whether they are thought of simply as micro amounts of substance, spot-test methods are ideally suited for general trace-analysis applications. Identification limits of 1 to 0.001 gamma are common for spot tests, and extremely favorable dilution limits are associated with the tests. Of special significance is the fact that spot tests apply to both inorganic and organic analyses.

The development of spot-test methods of analysis began about thirty-five years ago, when it was shown by Feigl in his doctorate research that remarkably sensitive tests for metals could be obtained by performing selected reactions on filter paper. This work has continued uninterrupted and is now augmented by both the occasional and the dedicated efforts of dozens of other investigators. Even before their systematic development began, spot tests were used in isolated organic and inorganic applications. According to the writings of the elder Pliny, early Romans detected iron in vinegar by means of a reagent paper prepared by soaking papyrus in an extract obtained from gall nuts. The earliest documented account of a spot test is that given by Schiff,[23] who suggested the use of paper impregnated with silver carbonate for the detection of uric acid. The importance of paper as a reaction medium was recognized by Schoenbein in 1861, when he demonstrated that aqueous solutions will ascend strips of paper in such a manner that different solutes appear in separate zones.[22] Goppelsroeder extended these studies by making thorough investigations of the capillary separation of dissolved dyes and other organic substances.[16] Goppelsroeder's work included studies of the capillary rise and wetting by solutions of inorganic salts. The studies of reactions on paper by Feigl were actually inspired by this work, and, when it became obvious that reactions on paper were often completely different in their aspect from reactions taking place in test tubes and beakers, the new field of spot-test analysis was begun.

The scope of spot-test analysis has been broadened since the inception of the technique, so that there is no real or implied restriction that the tests be run on filter paper. Spot (or drop) tests are now considered as including those wet chemical tests that can be run on a single drop of solution. The test solution can be placed on a spot plate, spot-test paper, watch glass, or in a micro test tube or crucible. A drop of reagent is added to the unknown, and a positive test is indicated by the formation of some characterizing color or precipi-

tate. No instrument or other special measuring device is needed to indicate the test results. Although the term spot test refers generally to the detection of a particular ion, functional group, or molecule in a single drop of sample, considerable latitude exists regarding the nature and scope of the tests. Some reactions are run directly on solid samples, gases, or volatilized samples; and some tests disclose groups or classes of compounds, while others may serve to disclose directly the presence of two or even more individual ions.

Probably the most striking feature of spot-test analysis is its elegance. Reactions of the highest possible selectivity and sensitivity are employed, so that identification can usually be secured without recourse to elaborate separation schemes and without depending on time-consuming concentrating or gathering operations. Where nonspecific reagents must be used, "conditioned" reactions are employed so as to eliminate interferences. These conditioning operations are so integrated with the fundamental test procedures that they become simply another routine step. The casual users of such tests are often unaware that the procedure involves more than a single reaction.

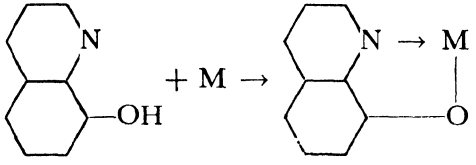
For special problems, such as those met in trace analysis, it is to be expected that eliminating large quantities of material that could cause interference might often become a necessity. The usual approach in such instances is to isolate the sought-for material in some simple, innocuous system. Solvent-extraction, ion-exchange, chromatographic, volatilization, and gathering methods are most often used for such purposes and lend themselves in many cases to ready integration with standard spot-test procedures. Further comments on this phase of the work will be made later.

DEVELOPMENT OF REAGENTS

The improving of sensitivity and reliability of tests is a well-recognized possibility that is an established part in the design of spot-test procedures. In fact, the methods used for enhancing reaction characteristics have largely originated from the work of investigators who were either seeking to perfect new spot tests or who were using spot-test techniques to study reagents and reactions. A general discussion of this subject has been presented in book form,[2] and only a brief orientation will be attempted here. It is of considerable importance, however, that the background of spot-test research be introduced, and for this reason it should be pointed out that two courses of action

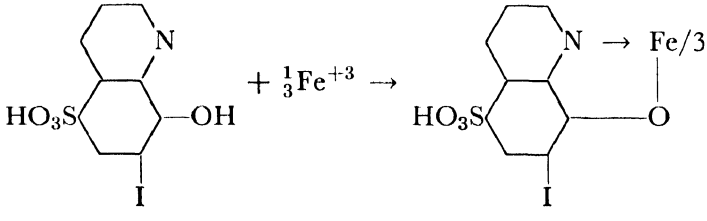
may be followed in developing more reliable and more sensitive tests. The most fundamental approach is to create new reagents. Most new reagents are obtained by making derivatives of promising known compounds. Practically all reagents with high sensitivities and selectivities are organic in nature and so lend themselves to modification.

An excellent example of the dramatic change that can be obtained through modification in structure is given in 8-quinolinol (8-hydroxyquinoline) and 8-hydroxyquinoline-7-iodo-5-sulfonic acid. As is well known, the parent molecule reacts with most heavy metals to form yellow, insoluble chelates of the general type as shown below. The



where M is one equivalent of metal

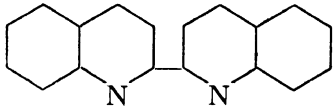
Fe(III) complex of 8-quinolinol differs from most other salts in its color, which is greenish black, although it is water-insoluble like the other 8-quinolinol chelates. Yoe[34] utilized the well-known solubilizing properties of the sulfonic group so as to prepare an iron reagent that would have the desirable characteristics of 8-quinolinol without the innumerable complications associated with its water insolubility and the insolubility of its salts. The 7-iodo-5-sulfonic acid derivative proved to be so satisfactory as a reagent for iron that it is widely known by the trivial name "ferron." Because of the general solubility and lack of interfering color of its various complex metal salts, it can be considered to be a specific reagent for iron. The aqueous solutions



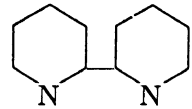
of the reagent are an orange–yellow, and its Fe(III) complex is green. The reaction takes place in acidic (*p*H of 2.0–3.5) solution and in addition to its specificity has a sensitivity of 0.5 gamma of iron at a dilution limit of 1 part in 100,000 when used for spot-test applications.

A second example of the value of systematically studying the preparation and properties of derivatives of important reagents is given for

1,10-phenanthroline and its analogs. The classical studies of Smith and his associates have provided a wealth of information concerning the effect of substituent groups on the reactions of these compounds with iron.[24] The iron reagent 2,2-dipyridyl, which has the same functional groups as 1,10-phenanthroline, has likewise been subjected to careful and fruitful study. Hoste [18] found that the diquinolyl structure (I) is completely different in its reactions from its dipyridyl



I. 2,2-Diquinolyl (cuproin)



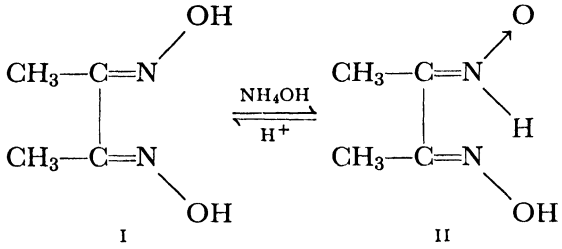
II. 2,2-Dipyridyl

analog (II). The first is a truly specific copper reagent while the parent compound can be considered as an iron-specific reagent.

Through systematic studies, coupled with fortuitous discoveries and intelligent observations, new specific, selective, and sensitive reagents are being made available. It is conceivable that specific and sensitive reagents may eventually be proposed for most inorganic ions and even many organic compounds and functional groups. Such an ideal situation does not now exist, however, and an alternative approach must be followed in providing satisfactory tests.

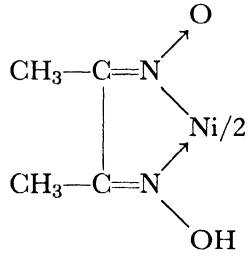
It should be kept in mind that the ultimate need is for specific and selective tests of high sensitivity and not necessarily specific, selective, and sensitive reagents. Though the latter constitute the ideal examples, it is usually far easier to condition reactions of a reasonably selective reagent to attain specificity than it is to find or synthesize a new reagent that is specific.

The conditioning of inorganic reactions may be accomplished in a number of ways. The most obvious approach is to control *p*H. The control of hydrogen-ion concentration is particularly important when used to insure the formation of reactive configurations of organic reagents that exist in tautomeric equilibrium. Furthermore, most reagents used in spot-test analysis are weak acids and as such are often greatly influenced by *p*H variations. The importance of *p*H in establishing reactivity can be illustrated by the reactions of dimethylglyoxime. This reagent reacts with nickel in ammoniacal solutions and palladium (II) in strongly acidic media. In its reactions the reagent first undergoes a nitrone shift, producing a salinogenic hydrogen:



I II

Nickel reacts with II, forming the familiar pink-red chelate:



Under the conditions specified, palladium is sequestered as the ammine complex and so does not interfere. In acidic solutions, insufficient reagent in the nitrone configuration exists to enable the nickel reaction to take place. However, palladium will react because the palladium dimethylglyoximate is so stable that it is able to shift the tautomeric equilibrium of the reagent in opposition to *p*H influences.

A second very simple conditioning method is to oxidize or reduce interfering ions. In the great majority of instances, changing the oxidation state will so alter its reaction characteristics that a troublesome ion will be rendered innocuous. An even simpler approach is to select a reaction medium that is suited for the test reaction but inhibits or disguises possible interfering reactions. As mentioned earlier in this chapter, remarkable differences can often be noted between a reaction run on filter paper and the same reaction performed in a test tube. An extreme example showing the effect of reaction medium was disclosed by West and Hamilton,[29] who found that the dithiooxamide (rubeanic acid) test for copper was enhanced in sensitivity many thousand-fold when the test was performed on human skin rather than on filter paper. Interfering reactions which occur with nickel, iron, and cobalt are not similarly enhanced.

The conditioning of reactions cannot always be successfully accomplished by the simple expedients described above. Very commonly it is necessary to resort to actual separation of constituents in order to approach reaction specificity. Such separations, however,

may be done simply in many cases, so as to preserve the desired elegance of spot-test methods. The nicest approach is to sequester or "mask" interfering ions—this may or may not be classified as a separation, depending on the point of view.

The masking of reactions has come to mean the sequestering or inhibiting of reactions. The action involved is usually complexation but may also be the formation of pseudo salts or the results of competitive reactions. No physical separation takes place, and the only operation required is the mere addition of the masking agent itself. Very simple but universally known examples of masking are found in the use of orthophosphate, pyrophosphate, oxalate, fluoride, citrate, tartrate, and similar complexing addenda as sequestering agents for Fe(III). Through use of such agents, the yellow color of Fe(III) solutions (or, more properly, the color of the chloro complexes of trivalent iron) can be bleached, and most of the reactions of the iron can be prevented. Almost all chemists are now aware of the masking action of the "complexones," such as ethylenediamine-tetraacetic acid, and the wide variety of uses to which this agent has been put. The collected applications of this masking addendum alone would fill many pages of text, and a great volume of work is still in progress, and probably even more work is yet to be started.

Capillary separations, which take place in the pores of filter paper, utilize the preferential adsorption, diffusion, and chemical reactions that are characterized by the paper itself. In some cases the physical state of the paper must play an important role in the separations because of surface effects that influence the behaviors of the various solutes. In other instances the chemical history of the paper may enter into the picture through the influence of trace amounts of residual chemicals remaining in the paper fibers and exerting catalytic or other chemical effects. Whatever the action may be, paper is very often a unique and valuable medium for test reactions, and many simplified tests are based on its use.

One of the more interesting examples of capillary separation is found in a convenient test for traces of permanganate in soluble chromates.[15] A drop of test solution is placed on filter paper, whereupon any permanganate present is reduced by the cellulose. The resulting deposit of hydrated manganic oxide is fixed in the paper and can be detected as such by washing with water so as to remove the chromate which has remained unaltered. As little as 0.3 gamma of potassium permanganate can be detected in the presence of one hundred thousand times as much potassium chromate. A second familiar example

of capillary separation is given by the procedure for the simultaneous detection of molybdate and tungstate using stannous chloride and potassium thiocyanate.[4] When a drop of test solution containing both tungstates and molybdates is placed on spot-test paper moistened with hydrochloric acid, the tungsten is deposited as the yellow tungsten trioxide. A drop each of potassium thiocyanate and stannous chloride are then added, and the tungsten is converted to a blue–green insoluble lower oxide, and the molybdate is reduced and complexed as the stable hexathiocyantomolybdate (V). The red molybdeum complex is acid-soluble, and so the addition of a final drop of hydrochloric acid causes the outward diffusion of a red ring (molybdate), leaving a center blue–green spot (tungstate). Capillary separations were originated as a tool for the study of dyes and similar organic compounds. Many applications in the field of organic chemistry have been described, and there has been a reawakened interest due to the improvement and extension of capillary separations, which have led to what is now known as paper chromatography.

Chromatography in its various forms has long been an important adjunct to spot-test analysis (and spot tests, in the form of streak reagents, have been an invaluable tool in chromatography). Extended treatment of the methods and applications of chromatography and ion exchange are presented elsewhere; so a detailed discussion of them here would be repetitious. The classic work of LeRosen [20] and his students on the development of streak reagents is specially cited, however, to emphasize the close association of the separation and detection procedures.

Like chromatography and ion exchange, extraction methods of separation are often an integral part of spot-test analysis. Extraction methods, likewise, are so important that they are discussed separately and so need relatively little mention here. There are certain developments in the field of liquid–liquid extraction, however, that are of particular interest when considered along with spot-test techniques, and some additional comment here seems justified. It is particularly important to recognize the unique value of solvent extraction as a means of isolation and concentration. Trace amounts of material, both organic and inorganic, can be selectively extracted. Furthermore, almost any desired efficiency can be obtained through use of multiple-extraction operations.

Extraction techniques have been combined with the use of spot-test and colorimetric reagents, such as dithizone, sodium diethyldithiocarbamate and thiocyanates, to give unitized operations of sepa-

ration and measurement. Recent work has shown that it is also possible to employ the selective action of solvent extraction as a separate action and then follow this directly with the detection or determination of the extract without removing it to an aqueous system. For example, West and Hamilton [30] have demonstrated that Sb(III) can be extracted as the iodide complex into benzene and the antimony then detected in the benzene layer by addition of rhodamine B. This reagent is normally not at all specific for antimony when used in aqueous media; however, when the prior extraction operation is utilized, a specific reaction is obtained, and increased sensitivity can be secured. It is important to note here that the extraction must be a separate operation in order to secure the specific action—although the antimony salt of rhodamine B can be extracted as such, other metal salts of the reagent are also extractable, and little gain in selectivity is obtained over simple tests run directly on aqueous systems. A second example of applied extraction techniques combined with direct color development in the nonaqueous system is given by the test for gold proposed by West and McCoy.[32] They have shown that gold can be isolated and concentrated through extraction of Au(III) chloride into *n*-butyl alcohol and the metal then detected and/or determined by color development in the alcoholic medium by means of α-naphthylamine.

A technique for making separations on a single drop of sample has been advanced by Weisz.[26] The method depends on the use of a "ring oven" and has been applied to classical separations employing precipitation reactions. Soluble salts, or salts extracted through the action of acids, bases, or complexing agents are diffused through paper to an outer ring where the solvent (water) is evaporated by a heated ring. The transported salts are concentrated at the point (ring) of evaporation, and an efficient, rapid separation from the main precipitate or residue is obtained. Preliminary work in the laboratories at Louisiana State University have shown that the "ring-oven" technique can be readily extended for use with many of the solvent-extraction procedures—this permits an extension of the "ring-oven" method and offers numerous advantages in applying both liquid–liquid and liquid–solid extractions to spot tests. All advantages derive from the fact that the solvent is quickly and easily evaporated, leaving the extract in a concentrated form immediately available for direct spot-test analysis on the supporting paper.

The "ring oven" described by Weisz is shown in Fig. 1. The exact dimensions are not critical but the heating block itself should have an

outside diameter of approximately 50 mm and an inside diameter of about 25 mm. The block can be made conveniently from brass, but the upper surface, at least, should be gold- or platinum-plated to prevent attack by the acidic solutions that would normally come in contact with the ring top. The block should be heated by means of a "Nichrome" or similar heating element controlled by a powerstat. The operating temperature (upper surface) should run in the range of 125° to 150° C. A retaining ring should be provided to hold the filter paper firmly against the hot surface. This ring can be a glazed ceramic tile or it can be gold- or platinum-plated brass. The outside diameter of the retaining ring should be approximately the same as that of the heating ring. The inside diameter, however, should be 4–5 mm greater than the inside ring diameter of the heating block. A guide should be provided through which capillary tubes can be inserted and centered on the filter paper surface. The capillaries are used to add reagents and wash liquids and should be inserted to deliver at the exact center of the ring area so as to insure equal diffusion in all directions to the evaporation surfaces.

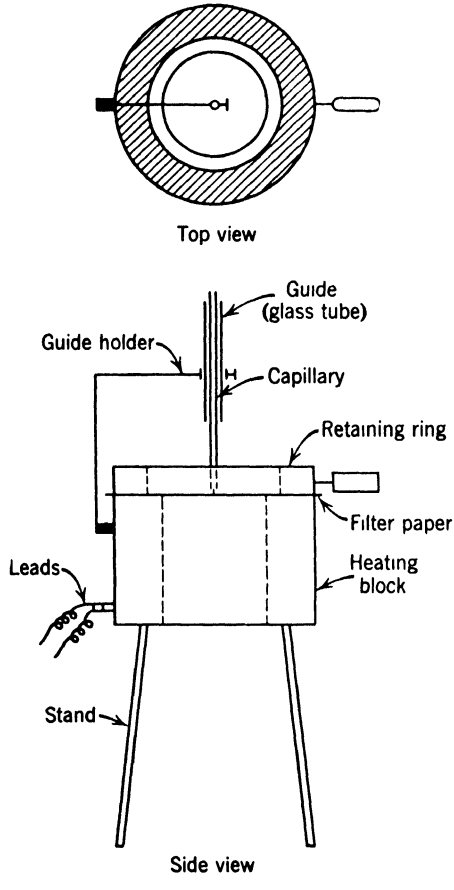


FIG. 1. Ring oven.

Operation of the ring oven can be illustrated by the simple example of the classical separation of Cu(II), Fe(III), and Ni as reduced to a single drop scale of operation. A sheet of 50-mm filter paper (e.g., Whatman no. 41 or S&S no. 585) is used as the medium for the necessary precipitation reactions, as well as the means for making subsequent separations. A 10-cu mm portion of a slightly acidic test solution is added as near the center of the paper as possible. The paper is then placed over a source of hydrogen sulfide and the spot exposed

to sulfide (the gas should pass through the paper to be sure all of the copper is precipitated). After complete precipitation of the sulfide is assured, the paper is transferred to the warm ring oven and placed so that the reaction area is in the center of the open ring. The retaining ring is then placed on the paper and a 10-cu mm portion of 0.1 *M* hydrochloric acid is added to the spot by inserting a capillary, containing the acid, through the guide tube. The tip of the capillary is allowed to rest gently on the paper surface, and the acid flows into the paper due to the forces of gravity and capillary attraction. The precipitate of copper sulfide remains fixed in the pores of the filter paper during the acid treatment, but the iron and nickel are solubilized as the chlorides and washed outward until they reach the ring surface of the oven. The precipitate is washed with a second portion of 0.1 *M* hydrochloric acid and then with two 10-cu mm portions of ethanol. About 30 seconds should be allowed to elapse between washings. As the wash liquids diffuse to the heated ring, the solvents evaporate, leaving the transported iron and nickel salts deposited in the fibers of the paper as a fine, clearly established circle.

After the nickel and iron have been washed free from the copper sulfide deposit, the paper is removed from the ring oven and the area containing the precipitate cut free by means of a cork borer. The disk containing the copper sulfide is then placed on a fresh piece of filter paper and centered on the ring oven. Two 10-cu mm portions of 2 *M* nitric acid are then added to dissolve the precipitate and transfer the copper to the fresh paper where it is washed to the ring surface and deposited. The paper is removed and cut into halves. One half is treated with a drop of a saturated alcoholic solution of dithiooxamide. Copper is indicated as a semicircular dark-green or black line. The other half of the paper can be used to confirm the presence of copper using α-benzoinoxime.

The original filter paper, containing the deposited ring of iron and nickel chlorides, is cut in quadrants. One quadrant is treated with a drop of 1% alcoholic dimethylglyoxime. The paper is then held over an open bottle of ammonia, and the presence of nickel is shown by the appearance of a red line. A second quadrant can be treated with dithiooxamide to confirm the results (a violet line is obtained with nickel although in its absence a light orange line may form due to a reaction with iron). The remaining quadrants are used for detecting iron. The potassium thiocyanate and potassium ferrocyanide tests may be used.

For some spot-test problems, it may be necessary to concentrate

trace materials by precipitation. Simple precipitation is seldom of sufficient sensitivity, however, and precipitation of traces will usually involve the use of "gathering" or "collecting" agents. It is of interest to note that most such operations involve organic rather than inorganic precipitants and are applied only to inorganic separations. Feigl has pointed out earlier [3] that the collector action of precipitates *in statu nascendi* is of great potential value in trace analysis, and for tests of purity even the most sensitive reactions may fail because of extreme limiting proportions. In fact, the induced precipitation effects of fresh precipitates are an absolute necessity in trace analysis. Many collectors are recorded in the literature and can be applied for spot-test investigations. Among the general gathering agents, silver oxide can be cited for use as a collector for metals forming hydrated oxides, and copper sulfide is a likely choice for metals precipitable as sulfides. The latter example serves to illustrate the unique value of gathering on the basis of the reported [25] concentrating of mercury. As little as 0.02 gamma of mercury can be concentrated from a dilution of 1 part in 2,500,000,000 through use of copper sulfide scavenging.

Volatilization methods have proved to be of value in isolating both inorganic and organic compounds for spot-test analysis. Traces of inorganic substances can often be volatilized and collected, and many more examples of such operations exist than is generally realized. As might be expected, spot tests for osmium may take advantage of the volatility of perosmic acid.[5] As little as 0.008 gamma of osmium can be separated and detected, and the isolation process makes the tests specific. Germanium is difficult to detect with certainty because of the large number of interferences encountered with the available reagents. Careful distillation of the Ge(IV) chloride serves to isolate it from most sources of interference. Arsenic is commonly separated and detected by volatilizing it as the hydride and detecting the gas with suitable reagents. Chloride can be detected by forming the volatile chromyl chloride, which is isolated as such and subsequently identified by the diphenylcarbazide reaction with the chromium.[7] Among other metals separated through volatilization are selenium, mercury, antimony, and rare earths.

Needless to say, many organic compounds are isolated and/or detected by utilizing their volatilities or the volatilities of their decomposition products. For example, thiophen can be detected [8] by volatilizing it from a benzene solution by isothermic distillation at 80° to 90° C. The vapors are caught in a sulfuric acid solution of isatin

with the subsequent formation of blue to blue-green indophenin. The most common examples, however, are those in which decomposition products are evolved and detected. The test for glycerol based on its conversion to acrolein can be cited as an illustration. When glycerol is heated with a dehydrating agent, acrolein is formed,

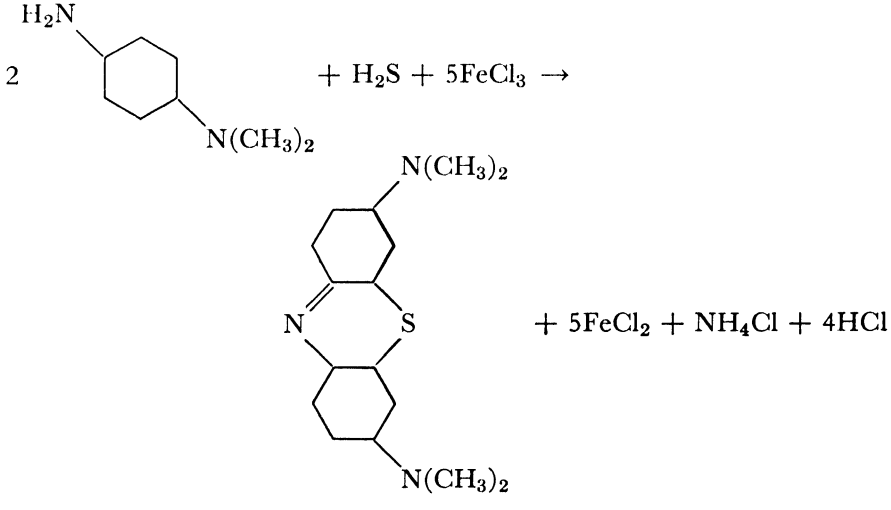
$$CH_2(OH)CH(OH)CH_2(OH) \xrightarrow{KHSO_4} CH_2{=}CHCHO + 2H_2O$$

The acrolein evolved can be detected by means of a test paper impregnated with sodium nitroprusside and piperidine.

REACTIONS FOR INORGANIC SPOT TESTS

A number of reaction types are of value in general spot-test work. In trace analysis, the high sensitivity required limits the probable choice of tests to certain reaction types that are characterized by their consistently high response to minute amounts of reactants. A survey of the most important classes of reactions is presented to serve as a guide in the preliminary sorting of possible trace-analysis reagents.

Direct Synthesis of Organic Compounds Other than Salts. The test for nitrite employing the Griess reaction is a familiar example of the direct synthesis of distinctive organic compounds. The reaction is extremely sensitive (limit of identification = 0.01 gamma; dilution limit = 1:5,000,000) and lends itself well to trace-analysis applications. Because a synthesis involving organic materials reacting with inorganic ions is relatively rare (except for the formation of salts), it is to be expected that such reactions are inherently specific. As further



evidence of this, the test for sulfide based on the synthesis of methylene blue can be cited. The reaction is completely dependent on the presence of sulfide and so must be free from interferences. Again, this reaction is very sensitive and supports the conclusion that reactions of this type are well suited for trace work.

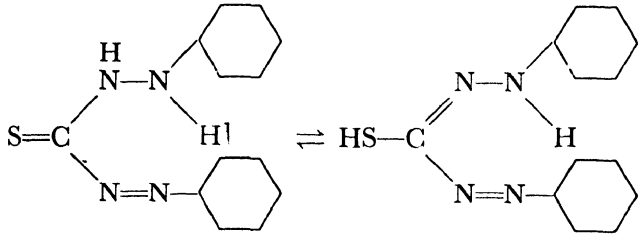
Formation of Inner-Complex Salts. The great majority of reactions used for spot-test analysis utilize reagents that react to form chelate compounds. Most chelates are colored, and many are so intensely colored that they are of great value in detecting and determining trace materials. The classic example of a chelating addendum of high sensitivity is diphenylthiocarbazone (dithizone). Although the reagent is not specific, its reactions can be "conditioned" so that many highly selective procedures are possible. The importance of diphenylthiocarbazone is evidenced by the great number of publications that have appeared dealing with its use. Welcher devotes over 86 pages [27] to the review of dithizone and dithizonates. The versatility and value of the reagent for trace analysis is indicated in Table I.

TABLE I

IDENTIFICATION LIMITS OF SOME METAL–DIPHENYLTHIOCARBAZONE REACTIONS

Metal	Limit of Identification, γ	Source
Bi	1.0	Welcher [27]
Cd	0.01	Welcher
Cu	0.03	Feigl [7]
Co	0.04	Welcher
Pb	0.04	Feigl
Hg	0.25	Feigl
Ag	0.05	Feigl
Zn	0.025	Feigl

In its reactions, diphenylthiocarbazone may function in either keto or enol form, as shown in the formula. As would be expected, the



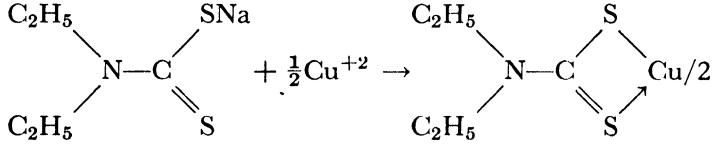
tautomeric equilibrium can be shifted by changing the *p*H of the medium, and this serves to "condition" the reactions. Through

control of hydrogen-ion concentration, combined with proper use of masking agents and other "conditioning" methods, the selectivity and sensitivities of the reagent can be altered to meet special needs.

Any mention of dithizone should include mention of the characteristic solubility of its chelates. The reagent is ordinarily made up in chloroform or carbon tetrachloride solution. In its reactions with metals, the chelates formed are so soluble in the nonpolar solvent in which the reagent is introduced that very efficient extraction of the product results. This extraction is often of peculiar value in spot-test or colorimetric applications. Of special importance is the general use of dithizone extractions in concentrating traces of metals for final detection and determination with the various techniques applicable to analytical measurement of minute amounts of metals.

As a general rule, reagents forming inner-complex salts are very sensitive, and the reaction products are soluble in nonpolar solvents. Such reagents are consequently significant for indicating the presence (and concentration) of metals and, in addition, are useful for separating metals through extraction. The reactions are seldom highly selective. A reagent of the inner-complex forming type does not necessarily give highly colored chelates, nor does it necessarily yield water-insoluble, extractable salts. A number of inner-complex salts are completely colorless and very soluble in water. Such compounds are of importance in trace analysis, not as color-developing agents but as masking agents. Examples of these complexing addenda are citrate, tartrate, malonate, and ethylenediaminetetraacetate.

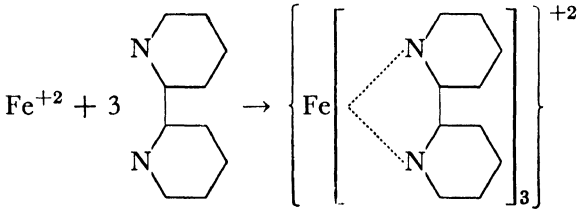
In connection with the discussion of inner-complex salt formation, sodium diethyldithiocarbamate is worthy of mention. This reagent reacts in a very sensitive manner with Cu(II), yielding a brownish yellow inner-complex salt. The product is readily soluble in a number of organic solvents and lends itself to the spot-test detection, colorimetric estimation, and the extraction and isolation of traces of



copper. Of special interest in connection with the present discussion is the contribution of Clark and Hermance. These investigators showed [1] that an insoluble white zinc salt of diethyldithiocarbamate could be used as an impregnating agent for preparing a spot-test reagent paper. Not only did they provide a convenient means of de-

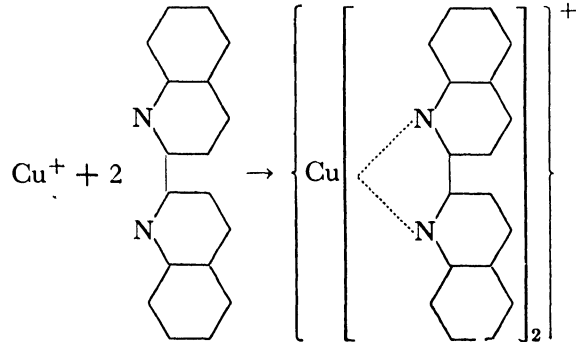
tecting copper, but they also demonstrated the value of impregnated papers as a means of increasing test sensitivities. The enhanced sensitivity can be attributed to the fact that the reaction takes place directly on the reagent surface, and there is no dilution and little diffusion. The increase in sensitivity may often exceed a factor of ten and thus becomes of special value in spot testing for traces.

Formation of Penetration Complexes. This reaction class includes a number of reagents of very high sensitivity. Also, this type of reaction may be entirely specific and so is ideally suited for trace analysis. Complexing addenda involved in the formation of penetration complexes may be unidentate (e.g. pyridine) or multidentate: The latter are the most important for reactions of high sensitivity. Probably the best-known example of this type of reaction is that occurring between Fe(II) and α,α-dipyridyl.[13] The reaction is sensitive to as



little as 0.03 gamma of iron and is essentially specific. The only deleterious effects are from other metals that tend to form ammine complexes. Large amounts of such metals may form weak colors with the reagent and also tend to interfere by competing for the reagent and thus reduce the test sensitivity.

The analogous 2,2′-diquinolyl also forms a penetration complex.[18] In the case of the diquinolyl, however, the reaction is with Cu(I) rather than Fe(II). In fact, the reaction with copper can be considered as being the best-known example of a specific test. No "condi-



tioning" of the reaction is required because of the specificity of the reagent. The reaction (shown in the formula) serves to disclose the presence of as little as 0.05 gamma of copper. If the test must be run in the presence of colored ions which might inhibit the discernment of the test results, the complex can be extracted into an organic solvent for examination in an isolated system.

Redox Reactions. Although not usually very selective, redox reactions often exhibit high sensitivities. The many tests based on the use of benzidine, in which the reagent is oxidized to a blue meriquinoid compound, serve to illustrate the general nature of redox reactions. Any oxidizing agent with sufficient redox potential can be expected to affect the formation of the "benzidine blue." In all cases the reaction product is the same, and so it is not possible to distinguish one oxidant from another.

Nonspecific reagents, such as the type considered here, may be applied in such a manner that possible interferences are avoided. For example, gold is often detected by reducing it to the metal. The reactions are often quite sensitive but are difficult to apply because of the many interferences that are encountered. West and McCoy have proposed a simple method for securing specificity in a gold test employing α-naphthylamine. They suggest[32] that chlorauric acid be extracted from an aqueous solution, saturated with potassium chloride, using *n*-butyl alcohol as the solvent. The extracted metal is then detected directly in the nonaqueous solution using α-naphthylamine as the reagent. The extraction procedure serves to isolate and concentrate the metal. Most of the selectivity is derived from the extraction, but conducting the reaction in the nonaqueous solution also aids in eliminating possible interferences, because there are very few inorganic color-developing reactions in such systems.

Redox reactions are often an integral part of test procedures involving syntheses or catalyzed and induced reactions. One of the most valuable of such tests is the periodate–tetrabase procedure for the detection of manganese.[6] Mn(II) is oxidized in acetic acid solution by means of periodate:

$$2Mn^{+2} + 5IO_4^- + 3H_2O \rightarrow 2MnO_4^- + 5IO_3^- + 6H^+$$

By adding a chloroform solution of tetrabase (tetramethyl-*p*-diaminodiphenylmethane), an intense blue oxidation product is formed which will disclose as little as 0.001 gamma of manganese. The oxidation in this case must be catalytic because it depends on the action of permanganate, not periodate. The permanganate in oxidizing the

tetrabase is reduced to Mn(II) but is reoxidized immediately by the periodate and so undergoes no permanent change.

Catalyzed and Induced Reactions. As illustrated above, catalyzed and induced reactions can be both sensitive and selective. A review of the spot-test applications of such reactions has been presented elsewhere,[25] and a general discussion of their analytical importance is also available.[2] As a matter of emphasis, nevertheless, one or two examples will be cited here. The above references should be consulted for details.

One of the classic examples of catalysis is the so-called "glow" reaction. If platinum metals are deposited on some surface such as an asbestos sheet and then heated, they can be made to glow by holding the spot in a stream of water gas or hydrogen. The gas is adsorbed on the surface of the deposited metal and there reacts with the oxygen of the surrounding air. The heat of the reaction produces a visible glow with as little as 4×10^{-8} gram of platinum. Similar sensitivities are obtained for the other platinum metals (except osmium, which is too volatile), and the test is specific for the platinum metal group.

A second very important example is the catalytic sodium azide–iodine test for sulfides. A mixture of azide and iodine shows no visible evidence of reaction over periods of many months. If even a minute trace of sulfide is introduced, however, there is an immediate increase in reaction rate, as shown by a very rapid disappearance of the brown color of the iodine and a rapid evolution of nitrogen. The reaction catalysis probably depends on the formation of an intermediate complex between sulfur and iodine, which then reacts rapidly with the azide:

$$S^{-2} + I_2 \rightarrow [I{-}S{-}I]^{-2}$$

$$\underline{[I{-}S{-}I]^{-2} + 2N_3^- \rightarrow 2I^- + S^{-2} + 3N_2}$$

$$2N_3^- + I_2 \rightarrow 2I^- + 3N_2$$

The test is very significant because it is sensitive to as little as 0.3 gamma of sulfide. Also both organic and inorganic sulfides can be detected, and insoluble as well as soluble salts exhibit the catalytic effect. The reaction is specific for sulfide sulfur—sulfate, sulfite, and free sulfur are without effect, and selenium or tellurium do not interfere in any way. The reaction is used for quantitative analysis[17] as well as for spot-test identification work.

Various authors have applied catalytic reactions in quantitative determinations of trace materials. The high sensitivities combined

with the selectivity or even specificity of such reactions make them very useful for such applications.

REACTIONS FOR ORGANIC SPOT TESTS

It must not be overlooked that traces of organic materials may be as important as traces of inorganic substances. The detection and determination of small quantities of organic groups or compounds pose many special problems. The main difficulty, however, in the development of organic spot tests and other simple, direct approaches to organic analysis has probably been the general belief that such methods would be so subject to interferences and so restricted in sensitivity that they did not justify attention. The appearance of a separate volume [8] devoted to organic spot tests should serve as evidence that such methods can be developed, and it is to be hoped there will be increased activity in the application and perfection of such methods.

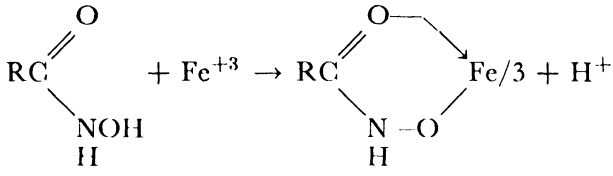
Spot tests for organic analysis may be applied for identifying elements bound to carbon and for making various preliminary tests. Most of the reactions for such applications resemble the inorganic tests discussed above and so will not be treated further here. Instead, a summary will be given to show the types of reactions to be used in detecting various functional groups and for detecting certain individual compounds.

Chelation Reactions. The importance of chelation reactions in the detection and determination of metals is widely known. It should be clear that the reverse process is also of value, so that nickel becomes a logical reagent for detecting the α-dioxime grouping.[19] This approach is quite valid, and a number of tests have been proposed on such a basis. It is important to note that these reactions can be applied to existing groups and, in addition, may find use after a suitable configuration has been established by preliminary conversion of some other grouping. For example, carboxylic acids can be converted into hydroxamic acids by treatment with hydroxylamine after preliminary conversion to the acid chloride:

$$RCOOH + SOCl_2 \rightarrow RCOCl + SO_2 + HCl$$

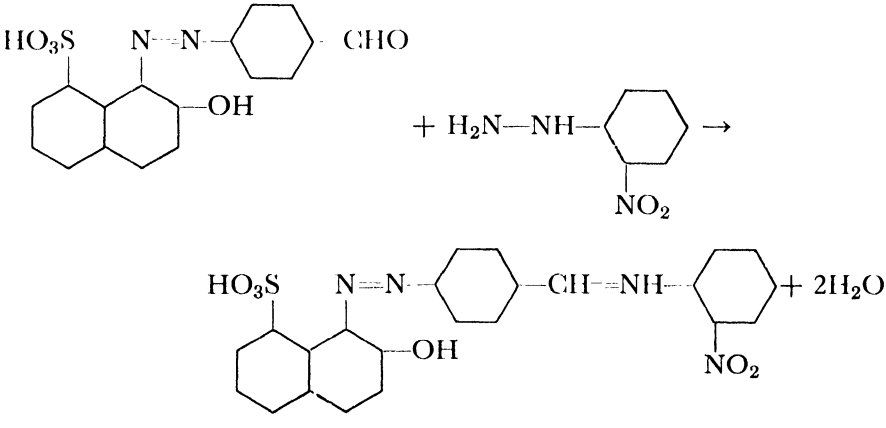
$$RCOCl + NH_2OH + 2NaOH \rightarrow RC(=O)NHONa + NaCl + 2H_2O$$

The sodium hydroxamate is treated with acid and the resulting hydroxamic acid reacted with Fe(III) to produce the characteristic red-violet complex of the iron chelate: [10]



The sensitivity of the test ranges from 2 to 20 gammas for most of the carboxylic acids.

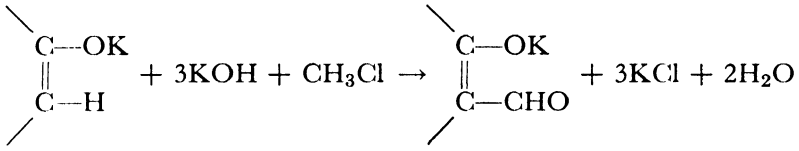
Condensation Reactions. Many tests for organic compounds are based on condensation reactions. Such reactions are simple and afford efficient means of detection where colored products are obtained. Acyl- and arylhydrazines will condense to form colored hydrazones with diazotized *p*-aminobenzaldehyde. The reaction serves as a good means for detecting the hydrazine group and has a limit of identification for most compounds of about 1 gamma. The reaction involving *o*-nitrophenylhydrazine is shown in the equation. The color change



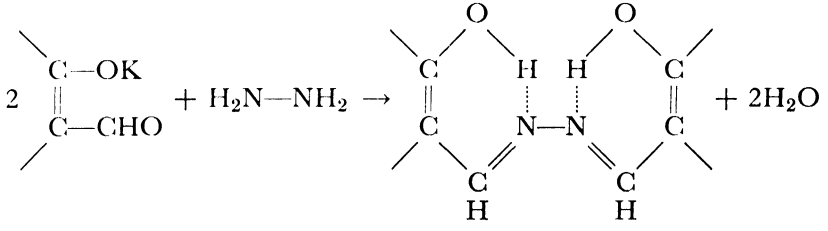
is from yellow (reagent) to violet (hydrazone).

Organic Synthesis. Rather complicated organic syntheses may be utilized as the basis of spot-test procedures. It is important to note that, for purposes of detection, high yields or even pure reaction products may be unnecessary, provided that reproducible reactions occur to such extent that distinguishable compounds are obtained. A relatively straightforward example is the utilization of the Tieman–Reimer reaction in the detection of phenols.[14] Phenols with unoccu-

pied ortho positions can be heated with chloroform in the presence of alkali to produce *o*-hydroxyaldehydes, as shown below. Although



the yield of this reaction is low, enough aldehyde is obtained from most phenols to provide sufficient material for the final step in the synthesis—the condensation with hydrazine to form the corresponding aldazines, as shown. The products are characterized by their low

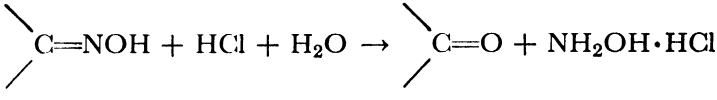


water solubility and a strong orange fluorescence. The test can be performed in a relatively short time, and high sensitivities are obtainable, in spite of the fact that the reactions are nonstoichiometric and slow.

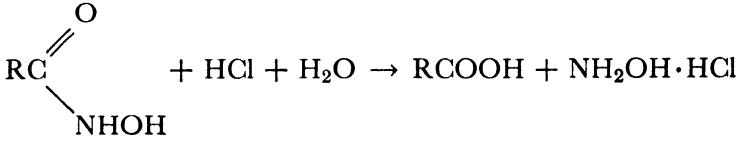
Redox Reactions. As might be anticipated, oxidation–reduction reactions play an important part in organic spot tests. Some tests depend on a single step to produce a distinctive reaction product, whereas others may require an oxidation or a reduction as the key step in a complicated series of reactions. The more complicated reactions often lead to selectivities unobtainable with the simpler reactions.

A test for oximes and hydroxamic acids has been proposed,[12] which is based on the following steps:

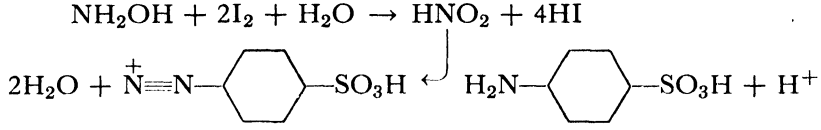
1. Formation of hydroxylamine hydrochloride,



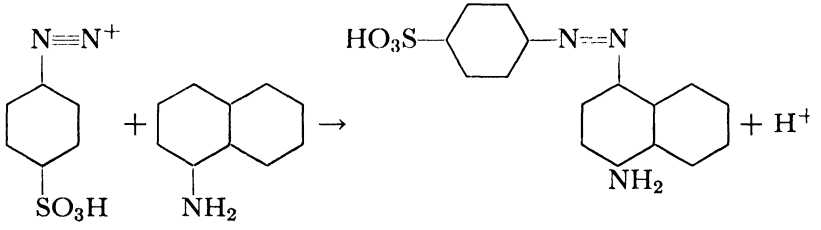
or



2. Oxidation of hydroxylamine and diazotization,



3. Coupling with α-naphthylamine to develop test color,



Excess iodine is removed by addition of sodium thiosulfate before the final step. Although the procedure seems involved, it requires no time-consuming operations. The test is sensitive and specific.

Catalytic Reactions. As mentioned previously [28] catalyzed and induced reactions serve as very sensitive tests for both organic and inorganic compounds. The iodine–azide test for sulfide applies to both branches, serving as an extremely delicate reaction for disclosing thioketones and thiols in addition to its use in detecting soluble and insoluble inorganic sulfides. The chemistry of the catalysis has been presented in the discussion of inorganic tests and need not be repeated because of the similarity of the reactions.

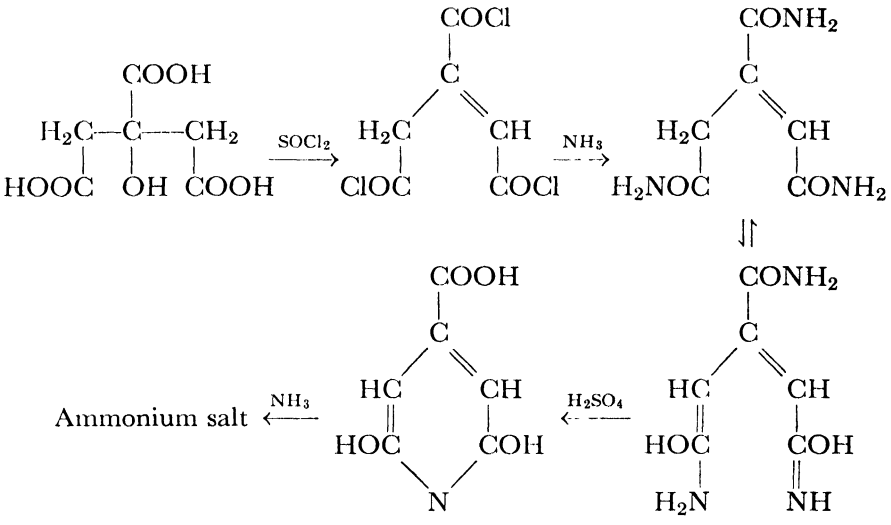
Detection of Individual Compounds. In many instances it is possible to detect certain molecules as such. The procedures for such tests usually represent a combination of functional group reactions that are so selected or so conditioned that the net result is a selective or specific test for the compound in question. In many cases completely isolated reactions may occur and so serve as a distinctive indication of the presence of a compound.

A rather remarkable test for oxalic acid is based on its inducing action on the chromic acid oxidation of indigo.[28] Neither oxalic acid nor indigo are appreciably oxidized by chromic acid when existing alone, but, when even a trace of the oxalate is added to a mixture of the other two reactants, immediate oxidation takes place. The remarkable specificity of various enzyme reactions is also worthy of note. The peroxidase activity of the hemoglobin in blood catalyzes the

oxidation of benzidine by hydrogen peroxide.[21] This is the basis of a universally employed general test for fresh or dry blood.

A test for pyrogallol has been found which depends on the formation of an intense blue color when pyrogallol is added to a solution of resorcinol containing a small amount of zinc ion. Upon the addition of ammonia, a distinct color develops in the presence of as little as 0.05 gamma of the pyrogallol.[31]

A more complicated procedure is involved in the test for citric acid which involves its conversion to ammonium citrazinate.[9] Although the reactions may seem involved, citric acid is readily converted into ammonium citrazinate by treatment of the acid with thionyl chloride to form aconitic acid chloride which yields the triamide upon heating with ammonia. The tautomer of the triamide is converted by sul-



furic acid to the citrazinic acid, which is subsequently treated with ammonia to form the highly fluorescent ammonium salt.

A rapid test for salicylaldehyde can be made by converting it to water-soluble phenolates, which display a strong blue-green fluorescence in ultraviolet light.[14] The test is specific if advantage is taken of the volatility of salicylaldehyde so as to isolate it from possible interferences. Simple isothermal distillation serves to make the desired separation.

A final example may serve to show the simplicity of certain tests for given organic compounds. A very elegant test for formaldehydes has been proposed,[33] which is based on the demasking of a tetracyanonickelate–dimethylglyoxime equilibrium mixture. The reagent

is prepared by dissolving nickel dimethylglyoximate with potassium cyanide:

$$Ni(HD)_2 + 4CN^- \rightarrow Ni(CN)_4^{=} + 2HD^-$$

$$Ni(CN)_4^{=} \xrightarrow{HCHO} HC(H)(OH)(CN) + Ni(HD)_2\ (red)$$

where HD^- is dimethylglyoximate ion. When formaldehyde (0.5 gamma or more) is added, the cyanhydrin is formed, releasing nickel and permitting the reprecipitation of the red nickel dimethylglyoximate.

DISCUSSION

Discussion Leader
Frank J. Welcher
Indiana University

You have just heard an excellent review of the fundamental principles used in the development of spot-test analysis. For the remainder of the time I would like to confine my few comments to what might be regarded as a plea for a wider extension of the use of spot-test methods. For the most part these tests are simple and highly sensitive, and may be applied to very small quantities of materials. For these reasons they merit special attention.

My reasons for calling to your attention the advantages of spot-test analysis are based on the fact that our many analytical techniques have become so highly specialized and so complex that frequently methods outside our own specialty are not understood or are simply overlooked. There appears to be a definite tendency to ignore the very simple methods with the unconscious feeling, perhaps, that accuracy and precision are in some way related to complexity. In any event, analysts seem to prefer the use of more complex methods when simpler methods are often adequate. For example, great emphasis may be placed on the application of infrared spectroscopy or other instrumental methods to the determination of organic structures rather than the use of simple chemical reactions which may be observed by means of spot tests. Not that the latter are universally applicable,

for they are not, but they frequently are of service, and may be carried out with a minimum of equipment and a minimum of time.

The matter of sensitivity has been indicated in some of the examples that Dr. West has given us. He has pointed out that quite a few of these tests have sensitivities extending down to quantities of approximately a thousandth of a microgram, and the dilutions are frequently of the order of one to two and a half million. A number of tests and their sensitivities are shown in the accompanying table.

LIMITS OF IDENTIFICATION OBTAINED BY SOME SPOT TESTS

Cation Detected	Reagent or Reaction Used	Limit of Identification, μg
Aluminum	Morin in ultraviolet light	0.005
Copper	*o*-Tolidine and NH_4SCN	0.003
Copper	Diphenylcarbazone	0.002
Manganese	Tetrabase and KIO_4	0.001
Mercury	Catalysis of formation of Al_2O_3 from aluminum	0.001
Platinum metals	Induced reduction of nickel salts	0.0025
Uranium	Fluorescence	0.001

Furthermore, spot tests may be carried out with quantities of unknown ranging down to one hundredth or two hundredths of a milliliter of liquid, or with exceedingly tiny crystals. Ordinarily these tests are simply carried out, as Dr. West has shown, by adding a drop of the material to filter paper or to the depression of a spot plate, and then treating with drops of suitable reagents.

It is not only the small quantities of substances that may be detected that is important, but it is the fact that, in the simplicity of the test itself, we frequently have opportunities that are actually unavailable in other forms of microanalysis. We can often eliminate completely certain tedious separation procedures that are essential by other methods. Dr. West has demonstrated that copper may be detected by means of dithiooxamide in the presence of cobalt and nickel, without any preliminary separation, by using malonic acid as a complexing agent. The separation of ions on filter paper is fairly common. The principle is not new. It is a matter of capillary movement in the paper. Feigl originally proposed a method for the detection of copper in the presence of nickel and cobalt with dithiooxamide, using capillary separation. Copper, cobalt, and nickel produce colored complexes with dithiooxamide in neutral or ammoniacal solutions. The copper salt, however, has the lowest solubility-product constant, and its value is exceeded with extremely small quantities of copper. Nickel

and cobalt are precipitated by dithiooxamide either slightly, or not at all, if the test solutions contain free acetic acid. The determining factor is the relative concentrations of the acetic acid and the cation. On the other hand, copper is precipitated quantitatively under these conditions.

In the analysis of an unknown, the concentration of nickel and cobalt in the test solution is not known, and therefore the proper quantity of acetic acid cannot be determined exactly. Therefore, this sensitive test for copper cannot be carried out in a test tube if cobalt and nickel are present, since the latter may also be precipitated. It is possible, however, to detect copper under these conditions when the test is performed as a spot test on filter paper. In this procedure, a drop of the test solution, acidified with acetic acid, is placed on a strip of filter paper that has been impregnated with dithiooxamide. Two zones are formed of differing acetic acid content. In the central zone where the acid concentration is the greatest, only copper is precipitated in the form of a dark-green or black circle. Nickel diffuses farther and forms a blue to violet ring around the central zone.

If cobalt is present, the same procedure is followed, except that the central zone is surrounded by a yellow-brown ring of the cobalt complex.

Sometimes the medium in which the test is carried out is in itself important. For example, hydrazine salts react in neutral or acid solutions with *p*-dimethylaminobenzaldehyde to form an orange or yellow color, which permits the detection of as little as 0.01μg of hydrazine when the reaction is carried out on a spot plate. When the reaction is carried out on filter paper, a red or pink stain is formed, which fluoresces intensely in ultraviolet. This reaction will reveal as little as 0.001 μg of hydrazine at a dilution of 1:50,000,000. Since the salts of *p*-dimethylaminobenzaldehyde do not fluoresce, and since only the solid hydrochloride of the condensation product fluoresces in ultraviolet, it is probable that adsorption occurs on the filter paper, and that the fluorescence is due to an adsorbate.

This example is not an isolated case. The medium may physically contribute to the test, or it may actually enter into the chemical reaction. A very well-known example of this is found in the detection of the permanganate ion in the presence of several thousand times as much chromate. The test depends on the fact that cellulose rapidly reduces the permanganate ion to manganese dioxide when a solution containing permanganate is placed on filter paper. Under similar conditions the chromate is practically unaffected by the action of filter

paper, even at high concentrations. As little as 0.3 μg of manganese can be detected by observing the brown spot of hydrated manganese dioxide, or as little as 0.001 μg by treating the dioxide with *o*-tolidine or benzidine.

The quantitative aspect of spot-test analysis should not be overlooked. This subject has not been fully explored, and I think that most of the methods thus far proposed are of a semiquantitative nature. These, however, can be employed simply, and often are of great value. For example, Evans and Higgs have used semiquantitative spot tests for the identification of alloys in a sorting procedure. These tests are carried out by adding a drop of a suitable solvent to the alloy, and treating the resulting drop of solution with appropriate reagents. These, of course, depend on the metal sought. Thus, for example, aluminon is used to determine aluminum, dimethylglyoxime for nickel, diphenylcarbazide for chromium, and 1-nitroso-2-naphthol for cobalt. These tests are used to detect the various metals present, and, by comparing the colors of the resulting spots with suitable standards, a semiquantitative determination of the metal enables a particular alloy to be identified. The method requires only a few minutes, and contributes in an important way to solving this particular problem.

There has been some work by Liebhafsky and Winslow, I believe, on the measurements of transmitted and reflected light from some selected spots. As I recall, the spot tests studied were those of copper with α-benzoinoxime, and that of silver formed by the reduction of silver salts. It has been reported that, under properly controlled conditions, the transmitted light obeys Beer's law. Thus it appears possible that, by the use of drops or microdrops, exceedingly small quantities of substances may be determined by spectrophotometric methods.

Another interesting example of the use of spot tests in quantitative analysis involves a chromatographic separation of zinc as a preliminary to a colorimetric determination with dithizone. A series of strips of filter paper are used for the separation, and the region into which the zinc diffuses is observed by spotting one strip with a dithizone reagent. This zone is marked and cut from the other strips of paper and the zinc is transferred from the filter paper by suitable means and subsequently determined with dithizone.

I also wish to add a little to the subject of identification of organic compounds. This technique is comparatively new, although it has been used for some time to a limited extent. The work done so far is only very fragmentary, but it appears to offer a great deal of promise.

Personally I have been much interested in investigating many of the reactions using organic compounds for the detection of inorganic ions, and it is my feeling that, by reversing these procedures, many organic functional groups may be detected and determined by use of inorganic substances. This has been covered at least in outline, but perhaps a point or two might be emphasized. First, let me point out that for a technique to be recommended there must be some distinct advantage resulting from it. In this instance it is the simplicity of the method as well as its selectivity that is important. Moses, some time ago, wished to decide quickly whether or not one of the isomeric aminobenzoic acids was anthranilic acid. Anthranilic acid, because of its structure, is capable of forming colored chelates with a number of inorganic cations. By simply using stock solutions of cobalt, nickel, and iron salts found in the laboratory, Moses showed that the unknown sample gave colors identical with those described in the literature for anthranilic acid. The *p*- and *m*-isomers are incapable of such reactions, and so it was concluded that the unknown was anthranilic acid.

When the organic analyst is equipped to make the various physical measurements, and to form the many derivatives used in organic identification, such analytical problems as the above are comparatively easily solved by conventional methods. On the other hand, there are many instances where organic analysis is not a part of the analyst's program, and therefore such simple methods as that used by Moses can greatly reduce the amount of time and effort involved.

There is a further advantage to such spot-test methods, that not only may individual compounds be detected, but often functional groups in the molecule are also revealed, and their position in the molecule indicated. We had occasion a short time ago to differentiate the three isomeric phenylenediamines. In a few minutes, we developed a procedure that enabled us to do this by using the reagents at hand, the only equipment being a spot plate, stirring rods, and test tubes. In this particular instance we used the beautiful purple color formed with *o*-phenylenediamine and acetylacetone. The other two isomers do not similarly react. *p*-Phenylenediamine reacts with hydrogen sulfide in the presence of ferric chloride to give a color analogous to that of methylene blue. The *m*-isomer does not give either reaction, and so by this comparatively simple procedure it was possible to locate definitely the position of the two amino groups.

It seems that there is additional opportunity in this type of analysis to determine the configuration of various groups. As you know,

there are three configurations of the orthodioximes. Of these, the *anti-* form yields the characteristic red, insoluble nickel compound; the *syn-* form is totally incapable of reacting with metallic salts; and the *amphi-* form yields a greenish yellow compound with nickel. Thus, the nickel ion may be used to differentiate among the three configurations of the orthodioximes.

Further, I think that perhaps with some study the effect of steric hindrance might be successfully employed for the evaluation of certain structures. For example, acetylacetone gives a well-known color reaction with Fe(III) salts. When, however, alkyl groups are substituted in the 3-position, the results obtained depend on the character of the group substituted. Radicals like the isopropyl group or the *sec*-butyl group prevent the iron reaction, but straight-chain radicals, such as the normal propyl group, or chains in which the branching occurs more remote from the 3-carbon atom, such as the isobutyl radical, do not interfere with the iron reaction.

Feigl suggested an interesting application of the use of metal salts for the establishment of organic structures. Aliphatic and monocyclic hydroaromatic orthodiketones having the group $-CO-CO-$ react with hydroxylamine to form the orthodioximes, which react with nickel salts to yield red or yellow compounds. It is thus possible to detect diketones of this type, and to distinguish the aliphatic (or monocyclic hydroaromatic) diketones from the aromatic diketones, such as benzil, phenanthraquinone, and camphorquinone. The latter are orthoquinoidal in character, and consequently are not oximated by hydroxylamine. Furthermore, compounds containing a methylene group adjacent to a carbonyl group may be detected by a similar method, since the methylene group is oxidized to the carbonyl group by means of selenium dioxide. Hydroxy ketones having the structure $-CH(OH)-CO-$ are also oxidized to the corresponding diketones. This grouping, in contrast to the $-CO-CH_2-$ group, is easily oxidized to the orthodiketones by oxidizing agents such as chromic acid and potassium ferricyanide.

Selenious acid also oxidizes unsaturated hydrocarbons to the corresponding orthodiketones. Also alcohols containing the grouping $-CH(OH)-CH_2-$ are oxidized to the diketone by means of selenious acid. In all cases the diketone formed may be converted to the orthodioxime, which in turn is detected by means of nickel salts. It appears that a suitable scheme might be worked out whereby the various structures indicated above may be detected singly or in the presence of one another by a simple application of spot-test techniques.

Perhaps the foregoing discussion is so fragmentary that it does not have the appeal that I would like it to have, but I believe that, with more interest in this type of analysis, the many isolated applications that have been reported could possibly be brought into a more useful comprehensive scheme.

QUESTIONS AND ANSWERS

HAROLD H. STRAIN: If you cannot provide us with a universal detection reagent, perhaps you could provide us with a half a dozen. We will settle for that.

PHILLIP W. WEST: We are working on some of those now (*laughter*).

DISCUSSANT: I have seen your spot test, Dr. West, on another occasion, and I recall that you reported that sometimes it does not work with some individuals, and it seems to be the skin itself that has something to do with the success of this test. I wonder if you have any comment on that.

PHILLIP W. WEST: That is a good question. I am happy you asked that. I find that I usually look around and try to find a good-looking girl to come up and run the test. Usually it works particularly well on women's hands; the skin is soft. I have also wondered a bit more about this. The skin is the best medium; the second best, gelatin. Gelatin does almost as well as human skin. When I gave the demonstration I carefully said "human skin." I might as well finish the story. About a week ago it suddenly occurred to me after all these years that I ought to look into the matter a little more. One thing that occurred to me was this: I found that when I am perspiring a bit the test works better on my own skin. My skin is soft; I am a good subject. I thought that maybe chloride had something to do with it. I got into what you might call a bioassay technique for investigating this. It occurred to me a dog does not perspire. My neighbor's dog had long ears, eight to ten inches long, and soft skin. I got her into the kitchen and rubbed a penny on one ear. No test. I went over to the other ear and put a little chloride on there and it worked beautifully. I suppose I could close the session now by making the comment that, if it were Cicero who had been doing the experiment, he might have coined the phrase "lend me your ears."

FRANK WELCHER: If there is no further discussion, thank you very much.

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6

Colorimetry

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ABSTRACT Colorimetric methods of analysis are based upon measuring the light-absorptive capacity of systems containing, or chemically equivalent to, the desired constituent(s). Such methods are among the oldest analytical procedures. Currently, they rank near the top in terms of their over-all utility.

The application of a colorimetric method in a specific case usually involves two principal steps: (1) the separation of the desired constituent from interfering constituents, and/or the development of a suitably colored system, and (2) the measurement of the colored system. The first step is largely chemical and the second entirely physical.

Developments in colorimetry during the last few decades have been very extensive. Thus, Snell's small book of 1921 contained only 150 pages, but the Snell and Snell treatise of 1948–1954 is in four volumes, with a total of 2470 pages. Primarily, the chemical advances have centered in discovering new reagents and in obtaining a better understanding of their functioning in specific color-forming reactions. Physical advances have been most noteworthy in the development and refinement of photometers, particularly photoelectric instruments.

This chapter presents a perspective of the current status of colorimetric methods of analysis; both their merits and their limitations are summarized.

The program of this symposium shows that a variety of methods have been developed for the detection and/or estimation of constit uents present in materials in trace amounts. One of the oldest and best-known of such methods has long been designated by chemists as colorimetry. The general objective of this chapter is to give one author's perspective of the contemporary place of colorimetric methods of analysis.

DEFINITIONS

Because of persistent confusion in usage of terms, perhaps mention should be made first of certain definitions employed in this chapter. The term colorimetry has come to have rather distinct meanings in physics and in chemistry. To physicists it implies the measurement of color as color, without reference to what the colorant is or to its amount. Ordinarily the operation consists in determining a color match for the sample in terms of equivalent stimuli. To most chemists the term has long implied the identification of a colorant and/or the estimation of its amount.

For the purpose of this chapter I am using the term colorimetry in the old chemical sense; however, I prefer the expression visible absorptimetry. One may then use other expressions, such as ultraviolet or infrared absorptimetry, to refer to other regions of the spectrum. Two other words, photometry and spectrophotometry, are now much used by chemists in place of colorimetry. They are used later in this chapter, in a particular sense, in the discussion of instruments. A few other absorptimetric terms are mentioned in the next section.

Both chemists and physicists are concerned fundamentally with the light-absorptive capacity of substances. *Light* is the aspect of radiant energy of which a human observer is aware through the sensations arising from the stimulation of the retina of the eye. The range of wavelengths of radiant energy involved constitute the visible region of the electromagnetic spectrum. Then expressions such as ultraviolet, black, invisible, and infrared light are inappropriate. *Color* consists of the characteristics of light other than spatial and temporal inhomogeneities.[10] It is an experience of the observer, and, as such, a psychophysical phenomenon, rather than a property of the object observed. In both aspects of colorimetry we are concerned essentially with (1) a source of radiant energy, (2) the object that is to be observed or measured, and (3) a detector for the unabsorbed radiant energy. Each of these items is discussed later in the consideration of the present status of the subject.

LAWS OF ABSORPTION

Two laws fundamental to quantitative determinations in colorimetry are based on experimental study of the absorption of light by homogeneous, transparent solids and solutions. The mathematical derivation of the laws is given in various elementary textbooks of physical chemistry.

It will suffice here to state that Bouguer's law * expresses the relationship between the thickness and the light-absorptive capacity of a medium. For homogeneous systems there is no known exception to this law. Beer's law expresses the relationship between the concentration and the light-absorptive capacity of a solution. Later on, mention is made of deviations from this law, particularly as they may involve error in the determination of the amount of desired constituents.

A commonly used combined expression of these two laws for solutions is

$$A = abc = \log 1/T$$

in which A is absorbance, a is absorbance index (or absorptivity), b is thickness (usually in centimeters), c is concentration of solute in the solution, and T is transmittance. Many instruments are now graduated in absorbance and/or transmittance. Transmittance is expressed as

$$T = P/P_0$$

in which P_0 and P are, respectively, the radiant flux incident upon, and emergent from, a transmitting sample.

As the absorbance index equals A/bc, its numerical value depends both on the magnitude of b and c and the units in which each is expressed. This constant, or index, characterizes the light-absorptive capacity of a system. The molar absorbance index, a_M, implies a thickness of 1 cm and a concentration of 1 M for the solution.

Often other variables affect the measured value of the absorbance or transmittance. Examples are the wavelength at which measurement is made, the solvent used, the spectral band width isolated, and the temperature. Obviously, all such variables should be clearly stated in any publication involving such measurement.

* In older textbooks, referred to as Lambert's law.

SYSTEMS SUSCEPTIBLE TO COLORIMETRIC MEASUREMENT

If one is to detect or estimate a constituent colorimetrically, there must be a color to observe or measure. This color may be that of the desired constituent, or, more likely, that of some other substance having a known chemical relationship to the desired constituent. Examples are cited later. If the desired constituent, or its chemical equivalent, does not itself possess a color, then a colored system has to be developed. In this section attention is directed to some of the important relevant problems involved. Most of them are chemical in nature.

In dealing with polycomponent samples often the most difficult problem is separation. The constituent(s) separated may be (1) the desired constituent, (2) something chemically equivalent to it, or (3) interfering constituents. All of the common methods of separation are used. They are not considered here, but papers for this symposium do deal with several of the more recently applied techniques. It may be mentioned, of course, that all such operations are to be avoided, if possible. They all take time, equipment, and skill, and often they are the source of error.

Although some constituents may be determined in gases and solids, the most important application of colorimetric methods has been to solutions. Consequently, the following discussion is limited to this type of system.

Desirable Properties of Solutions. For colorimetric measurement any solution should fulfill, insofar as possible, certain requirements. These concern the kind of results obtainable and the ease and convenience of the operation. Among the desirable properties involved, the following are most important:

1. Self-colored. Such constituents do not require the use of color-forming transformations, processes to be explained later.

2. Intensely colored. This requirement concerns the numerical value of the molar absorbance index, a_M. Other conditions being comparable, the higher the value of a_M, the more sensitive the method. In some cases there seems to be need for a method capable of determining one part of a constituent in a billion parts of solution.

3. Stable. Any system must be stable long enough to permit measurement. Although indefinite stability is preferable, many relatively instable systems are measurable under carefully specified conditions.

4. pH-insensitive. The nature or the intensity of many colors is *p*H-dependent. Although this difficulty often is easily overcome by suitable buffering of the solution, or by careful measurement with a *p*H meter after adjustment of the *p*H, such limitations render the method less easy to use and more time is required.

5. Temperature-insensitive. Fortunately, few colored solutions are appreciably affected by the usual variation of a few degrees likely to occur in a laboratory. In case of significant change, precautions must be taken, such as working in a constant-temperature room or with thermostated absorption cells.

6. Color-match permanent standards. Unless the known standards and the unknown solutions color-match under different sources of illumination, a standardized comparison procedure must be adopted for a given illuminant.

7. Conform to Beer's law. The Bouguer–Beer equation may be written

$$c = A/ab$$

The absorbance A is measured; the cell thickness b is known; and for known systems the molar absorbance index a_M may be determined. Then, if the system conforms to Beer's law, the concentration c may be calculated upon measuring the absorbance (or its equivalent, log $1/T$). Later, reference is made to systems not conforming to Beer's law, and to what may be done in such instances.

Systems Susceptible to Colorimetric Measurement. Relatively few inorganic substances, whether molecular or ionic, are satisfactory as such. Some, such as nitrate, nitrite, and chloride ions, are colorless in solution. Others, such as Fe(III), Mn(II), and Ni(II) ions do not possess sufficient color for the usually required sensitivity.

In contrast, hundreds of organic substances possess sufficient color. However, often individual compounds are not easily determined because of the presence of other compounds which interfere. For many of these the separative techniques discussed in this program are extremely useful, or it may be possible, if conditions are favorable, to resort to a simultaneous polycomponent spectrophotometric procedure. Hundreds of organic compounds are colorless, of course, or only slightly colored.

The search for reagents to enhance colors of low sensitivity, or to develop colors with colorless substances has been going on for more than a century.[14] Currently, this area is being widely studied. The treatises by Welcher [25] and by Snell and Snell [22] and the monograph

by Yoe and Sarver [27] give a good idea of what has been accomplished. Inspection of these and other reference works shows that both inorganic and organic reagents are used for inorganic and organic desired constituents. In any case, such color-forming reactions, if ideal, would meet at least the following requirements: (1) be free of interference, (2) use a colorless reagent, (3) develop the color rapidly, (4) react stoichiometrically, (5) be independent of a particular sequence of using two or more reagents, (6) yield a system having maximum absorption in the most desirable wavelength region, (7) avoid special treatment, such as extraction into an immiscible solvent, and (8) employ a reaction of known mechanism.

To my knowledge, this list of requirements is not met by any reactant reported thus far, and few reactants approach the ideal. Thus, the long search for a strictly specific reagent remains unrewarded, although a few highly selective reagents have been discovered, such as 2,2′-biquinoline for Cu(II) ion. Research is slowly yielding both better reagents and more information on the nature and means of control of variable factors affecting the use of the reagents.

The following partial equations illustrate the general direction in which chemistry is being applied to develop colors, the examples being for inorganic reagents functioning as oxidants, reductants, or complexants:

$$Mn^{+2} + IO_4^- \rightarrow MnO_4^-$$

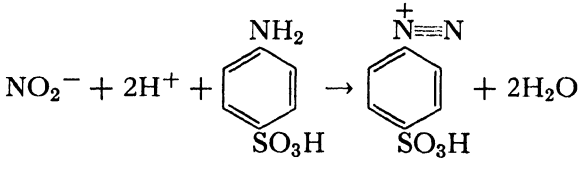
$$H_4[Si(W_3O_{10})_4] + SnCl_4^{-2} \rightarrow \text{W-blue}$$

$$H_3PO_4 + 12H_2MoO_4 \rightarrow H_3[P(Mo_3O_{10})_4] + 12H_2O$$

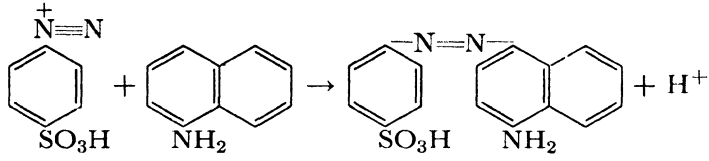
$$Cu^{+2} + 4NH_3 \rightarrow Cu(NH_3)_4^{+2}$$

$$Co^{+2} + 4SCN^- \rightarrow Co(SCN)_4^{-2}$$

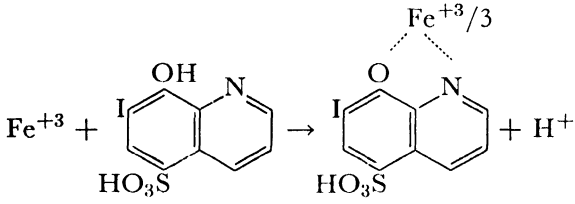
Coupling and chelation are two widely used processes employing organic reactants. In water analysis, for example, the nitrite ion first diazotizes 4-aminobenzenesulfonic acid. Then a colored product is



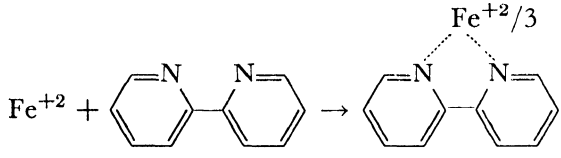
formed by coupling the diazotate with 1-naphthylamine. Careful control of *p*H in both steps is important.



The use of 7-iodo-8-hydroxyquinoline-5-sulfonic acid for Fe(III) ion and of 2,2″-bipyridine for Fe(II) ion illustrate chelation. The respective reactions are



and



Relatively close control of *p*H is necessary in the first case, but a much wider range is permissible in the second.

Interferences. Action of some constituent, other than the one desired, to give an error in the determination of the latter is known as interference. Colorimetric methods are like many others in that interferences of various kinds remain one of our major analytical problems.

Although the nature of interferences varies from case to case, the following kinds are well known: (1) the interferer itself is colored; (2) the interferer yields a color with the color formant; (3) the interferer yields a precipitate with the color formant; (4) the interferer complexes the desired constituent in a way to prevent action of the latter with the color formant; and (5) the interferer, through oxidation or reduction of the desired constituent, prevents color formation.

Both chemical and instrumental approaches find use in dealing with interferences. The interference may be prevented in many instances by suitable chemical action, or it may be taken into account in some way in the measurement in many other cases. For example, satisfactory results may often be obtained by operations or processes such as the following: (1) determine the amount of the interferer, and correct for its effect; (2) separate the interferer (or the desired constituent) by one of the general methods of separation available; (3) complex the

interferer in a stable, nonreactive form; (4) change the interferer to an unreactive oxidation state; (5) avoid interference by suitable adjustment of the *p*H; (6) avoid the interfering color by making the measurement in a suitable region of the spectrum; and (7) compensate the interference by adjustment of the color standards, or by appropriate instrumental calibration.

MEANS OF COLORIMETRIC MEASUREMENT

The number and variety of means available for colorimetric measurement are not exceeded, and perhaps not equaled, by those of any other kind of method. Some are extremely simple and inexpensive; some cost a few hundred dollars; and a few of the best spectrophotometers cost as much as $15,000.

The following instrumental elements are essential, whatever the means of measurement: (1) an illuminant, such as light from the sun, an incandescent tungsten filament, a phosphorescent tube, or, rarely, a monochromatic source, (2) an absorption cell containing the colored solution, and (3) a detector for the light not absorbed by the solution. The significance and present situation of illuminants and detectors is discussed later.

I prefer to consider all such instruments as absorptimeters, with two subdivisions, namely, comparimeters and photometers.

Comparimeters. Comparimeters comprise devices and instruments in which the measurement is made by comparing the unknown solution with one or more standards of known concentration or color value. A color-matching pair or position indicates the same light-absorbing capacity in the two systems, and hence equal amounts of desired constituent. A comparimeter is in essence a relative absorptimeter.

The four techniques for making comparisons of standard(s) and unknown are standard series, balancing, dilution, and duplication. The first two are most important.

Here we find the oldest of all colorimetric methods. The Duboscq balancing comparimeter was announced in 1854, and the standard series technique must be older. Although the latter is the simplest of all, its continued general utility is evidenced by the persistent sale of standard series sets for determining many different constituents.

Because of general knowledge about these techniques,[6,15,27] it is sufficient here to state that they are adequate if they provide the answer needed. If Hydrion paper, for example, shows the *p*H of a

system within the limits desired, there is no justification for a Cary spectrophotometer for the determination.

The kind of results obtainable varies greatly among these procedures. Thus, the colored paper scale for nitrazine *p*H indicator varies 0.5 *p*H unit from hue to hue through the range of the indicator. In contrast, the recommended standards in 30-cm Nessler tubes for determining residual chlorine in water may run as follows: 0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.07, 0.1, 0.2, 0.3 ppm. A trained observer can distinguish the colors in adjacent tubes in this series.

Presumably any ordinary colorimetric procedure for a single constituent can be set up for a comparimetric technique. Various precautions may have to be observed. Reference works [22,26] contain recommendations for nonconformity of systems to Beer's law, spectral differences between the unknown and standard systems, and other relevant details.

Photometers. In using comparimeters only a color match is determined. In using photometers the actual light-absorptive capacity is determined, usually in terms of transmittance or absorbance. Actually, the radiant flux, P_0 and P are concerned. If the Bouguer–Beer law holds for a given system, the concentration of the desired constituent may be calculated from the measured transmittance or absorbance. In contrast with comparimeters, with photometers one is more deeply involved with the nature and action of illuminants, detectors, and means for isolating particular regions of the spectrum.

1. Illuminants. Sun- or daylight is rarely used as an illuminant. Monochromatic sources may be used. Thus, the mercury arc gives several spectral lines, the strongest in the visual region being at 546 mμ. However, a desirable number of such lines, suitably located, is not readily obtained or controlled. The usual source is an electric bulb with an incandescent tungsten filament. Under ordinary conditions of its use, much less energy is emitted at 400 mμ than at 700 mμ. Consequently, the instrument may be less sensitive in the blue than in the red end of the spectrum. The ideal illuminant for photometers has yet to be produced.

2. Detectors. As detectors of radiant energy unabsorbed by the solution, the human eye and photocells are most common. Their response characteristics vary widely for different wavelengths of radiant energy or colors. The sensitivity of the normal eye peaks near 560 mμ and drops very low at 400 and 700 mμ. Readings in the far-blue or red regions are consequently difficult to make and likely to be unreliable, but the eye is a remarkably sensitive detector under optimum conditions. Visual photometers were most important between 1920 and

1935. Now they have been largely replaced by photoelectric instruments.

Three types of photocells are being used: the photoconductive, the photovoltaic, and the photoemissive. Each type has merits and limitations. As with illuminants, there is no ideal detector. Space precludes mention of more than a few generalities.

As far as I know, American instruments do not employ the photoconductive type of cell. Consequently, it will not be considered here.

Photovoltaic cells, of the copper oxide type, are widely used. Their output is sufficient to make amplification unnecessary. Instruments so equipped are usually the cheapest. The response curve of such a cell roughly approximates that of the eye above 550 to 560 $m\mu$, but in the blue region the cell is much more sensitive.

Photoemissive cells vary widely in their response characteristics. The kind of cathode coating is most important, but methods of production are probably significant too. Potassium hydride cells show maximum sensitivity in the blue region, but they are insensitive above 600 $m\mu$. Cesium oxide cells respond throughout the visible region, but they are far more sensitive in the red than in the blue. Some instruments are equipped with one each of these two kinds of cells, thus making it possible to use the one most sensitive for the region being measured. All such instruments require amplification of the current produced.

Photocells have been used in instruments in a variety of ways.[17] Some arrangements use two and some one. Some employ the cell as a null-point detector, and many others do not. Only an electronic expert is in a position, to evaluate critically all the proposals. Perhaps two decades of conditioning with the General Electric spectrophotometer makes me feel more confident of null-point arrangements. In any event, I cannot overemphasize the importance of checking any instrument regularly with suitable standards.

3. Filters and monochromators. One more essential instrumental variable is important, especially as it may concern the data obtainable. This is the means employed to isolate some finite width of spectral band of a heterochromatic illuminant. It is this element that determines whether the instrument is called a filter photometer or a spectrophotometer.

A filter passes only certain portions of the radiant energy emergent from an illuminant, such as the incandescent tungsten filament in a lamp. Currently used filters are generally made of glass. Single glasses, such as Corning Signal Green, pass a fairly wide band of light, perhaps as much as 100 $m\mu$ in width. Composite filters, made of two

or more glasses cemented together, narrow the band very much. The resulting low transmittance decreases the over-all sensitivity unless the filter effect can be compensated by increasing the radiant energy and/or the sensitivity of the detector. The fairly new interference-type filter passes a nominal band width of less than 20 mμ, with a transmittance of about 30%. It is obtainable with the band centered at almost any desired wavelength.

In spectrophotometers the usual heterochromatic light beam is dispersed into its spectral components by means of a prism or a diffraction grating. Prisms are more commonly used. In either case, the spectrum so formed is incident upon a slit which passes a narrow range of wavelengths. If the prism is fixed, the slit is movable, and vice versa. As prismatic dispersion is not linear, maintenance of a constant band width at all wavelengths necessitates a mechanically variable slit width. In the General Electric instrument, this change of width is accomplished automatically. In grating instruments, which have linear dispersion, the slit is not variable in width and so is less complicated.

The combination of slit(s), prism(s), grating(s), scales for reading wavelengths and slit widths, and means for their manipulation constitute the monochromator in a spectrophotometer. This word is misleading, in a sense, as the light passed approaches monochromaticity only with the narrowest of usable slits. Prevention of stray light in the beam is a problem. Some instruments use a double monochromator, and others rely on internal mechanical baffles.

The width of spectral band passed by a monochromator depends on the type of instrument and its use. That of the Coleman Universal model is stated to be 35 mμ and is constant. The General Electric recording instrument has a constant band width of 10 mμ. The width of the band varies with the Beckman models DU and B. Usually with the DU model it is close to 1 mμ, and with the B model it is perhaps three times as broad. Under the best operating conditions, the Cary model 11 instrument is considerably narrower than the Beckman DU.

4. Instrumental assemblies. As already indicated, filter photometers and spectrophotometers consist essentially of illuminants, filters or monochromators, absorption cells, and detectors. In the evolution of these instruments during the last few decades many different arrangements of these optical, electrical, and mechanical elements have been used. Even so, only a fraction of the spectrophotometric assemblies postulated by Van den Akker [24] have been commercialized.

For one reason or another the mortality rate of many of these instruments has been high. Visual filter photometers are no longer

being advertised. Probably only one visual spectrophotometer is available in the United States; yet the German König-Martens instrument at the National Bureau of Standards has long been used for standardization work. My standard photometric glasses were checked on it. Currently I am trying to assemble for a photometric museum examples of instruments no longer being used.

Some of these instruments have been, and others still are, relatively simple in order to keep them inexpensive. Some are fairly complicated and cost thousands of dollars. With all filter photometers and nonrecording spectrophotometers, one obtains, respectively, a photometric reading for a given filter or wavelength setting. Recording instruments, of course, scan the portion of the spectral region desired. In any case only a few minutes are required; but the instrument of the American Optical Company is practically instantaneous, as the curve is shown on an oscillographic screen. This makes photographic records possible, at given time intervals, for changing systems.

What is best? In general, the answer is the instrument that is cheapest and most easily used, if it will give the kind of results desired. Many thousands of single constituents are determined daily in clinical and industrial laboratories using filter photometers. With large undergraduate classes I have used a half-dozen different instruments of this type for the determination of manganese in steel. In contrast, nothing but spectrophotometers capable of the best photometric accuracy with narrow spectral bands will give data satisfactory for the simultaneous determination of constituents in polycomponent systems. Recording instruments are indispensable, of course, for obtaining rapidly for the whole spectral region the data so often needed to study the over-all effect of variables on a given system.

Nature and Use of Photometric Data. Users of photometers should keep in mind both the kind and limitations of data obtainable with such instruments. In a given spectral region, isolated by the filter or monochromator, one obtains some instrumental reading which is related to the light-absorptive capacity of the solution. Usage differs so much in conventions and practice that one needs some knowledge of them in order to read current publications.

With most present spectrophotometers, the photometric scale generally is graduated in transmittance T and/or absorbance A. Absorptance, $1 - T$, or log A may be calculated. With the General Electric instrument one simply shifts cams to use the scale desired. Each scale has its merits. The international CIE system of color specification uses transmittance.[11] Also such values are entirely satisfactory for calibration curves for quantitative analysis. If plotted on semilog

paper versus concentration, a straight line, starting at 100 for per cent T, shows conformity to Beer's law. Nonconformity does not mean that the curve cannot be used, provided only that enough points are determined to locate the curve. Absorbance is much used. It also yields a straight-line calibration curve, plotting on a linear scale and starting at zero for zero concentration, if the solution conforms to Beer's law. In simultaneous polycomponent analysis, absorbances are needed, if values are to be added or subtracted and simultaneous equations are to be solved. Here it is highly desirable to have conformity to Beer's law, although Stearns [23] has suggested ways of dealing with nonconforming systems. Absorptance was proposed by Ringbom,[20] and its merits were emphasized by Ayres,[1] for plotting curves in which concentration is plotted as the abscissa on a log scale. Log A has been employed by at least one company for several million curves as curve shape then is independent of concentration of the solution.

It is important, too, to keep in mind that the measured value of transmittance, or any related value, may be partly dependent on instrumental conditions, such as slit width and stray light. The detector responds to the total flux incident upon it, no matter what the origin. Thus, measurement of a system with pronounced absorption bands, such as neodymium nitrate solutions, with instruments having very different spectral band widths may yield very different values of transmittance. This is the principal reason that we do not yet have any generally accepted values of molar absorbance indexes which all may use. Also, it indicates the inadvisability of using a calibration curve not obtained on one's own instrument and under one's own working conditions. Yet most authors of papers insist on publishing their curves. Even more uncertain may be the data with filter photometers using wide-band filters.

For the determination of single constituents these values are measured at some particular spectral region. Greatest sensitivity is obtained for the method if the spectral band passed coincides with the region of maximum absorption by the solution. Sometimes, so as to avoid an interfering constituent, one may have to move to another wavelength, with resulting loss of sensitivity. Or, in simultaneous polycomponent analysis, measurement must be at the wavelength for which the absorbance constants were determined for solution of the necessary simultaneous equations.

Occasionally the ratio of two readings, at two different wavelengths, is used for qualitative purposes. Generally, however, a spectrophotometric curve is obtained by plotting the photometric value as ordinate

versus wavelength as the abscissa. Incidentally, engineering codes of recommended practice specify plotting increasing wavelength values from left to right. Instead of wavelength some people prefer wave number, frequency, or logarithm of wavelength. The shape or profile of such a curve is characteristic of the absorbing solute, if it is pure, and the vertical position indicates the amount present.

For a substance possessing prominent band structure, the form of the spectrophotometric curve depends on the wavelength intervals at which point readings are taken with nonrecording instruments. Preferably writers should show the points for a curve, but at least what was done can be stated. In general, writers should state clearly all details necessary to enable another competent in the field to repeat the work.

Occasional checking of instrumental scales, and of analytical working curves, should not be neglected. Because the meter reads on operating a switch is no reason to assume that the reading is reliable. The wavelength scale on my Beckman DU spectrophotometer has been some 10 mμ in error on three occasions. One company, handling thousands of spectrophotometric curves, puts on each sheet a small portion of one of the bands of a didymium glass, thus providing some indication of reliability for the photometric and wavelength scales. I use four glasses certified by the National Bureau of Standards for checking photometric scales, and a mercury-arc lamp for checking wavelength scales. Analytical working curves may be checked by using substances of known purity and following the working conditions specified for the method.

APPLICATIONS OF COLORIMETRIC METHODS

Colorimetric methods came into significant use only about 1850,[14] although the first color-forming reagent is said to have been mentioned by Pliny about 60 A.D.[18] Progress was slow from 1850 to 1900, but during this time there appeared such well-known methods as the use of thiocyanate for iron, phenol-2,4-disulfonic acid for nitrate, and 4-amino-benzenesulfonic acid and 1-naphthylamine for nitrite. Some think that these methods have not been improved upon. The last half-century has witnessed the discovery of new color-forming reactions, and the development of new and better instruments, at a steadily increasing rate.

Simple gravimetric methods were used by Lavoisier, and no doubt earlier, as the equal-arm balance is a very old instrument. The first

titrimetric method was described by Descroizilles [4] in 1806. These two techniques, together, have formed the core for teaching elementary quantitative chemical analysis for more than a century in the United States. I have committed myself, in a major percentage of my book,[15] to continuing emphasis upon these classical procedures. The reasons are not relevant here. However, I also devoted 10% of the book to colorimetric methods, quite apart from separations, which have no essential connection with any kind of measurement.

As far as general industrial and clinical applications are concerned, colorimetric methods seem today to be substantially on a par with gravimetric and titrimetric methods in practical importance. Every issue of the principal journals devoted to analytical chemistry contains new methods and improvements in, or further applications of, old methods. My reviews represent one evaluation of such developments.[16]

The constituents that may be determined by colorimetric methods are both varied in nature and large in number. The best cross-sectional sampling of the many kinds of applications, to both inorganic and organic constituents, is the four-volume treatise by Snell and Snell.[22] At least a dozen different single volumes have appeared. In coverage some are rather general, and some are specialized. Of those general in nature, Yoe's book [26] gave the subject much impetus a quarter of a century ago. Later examples are those by Lange,[12] and by Charlot and Gauguin.[3] As examples of specialized books, Sandell's [21] contains selected methods for many of the metals; Haywood and Wood's smaller work [8] contains composite procedures for the minor elements in a number of alloys; and Fister's [5] and Mehl's [13] collections cover selected spectrophotometric methods for clinically important systems.

MERITS OF COLORIMETRIC METHODS

Analysts still await the discovery of a type of method that will be the panacea for all their troubles. That is, it would be applicable to any and all situations, and it would be best in all analytical senses of this term. I believe that, as yet, each of the methods discussed in this symposium, as well as any others, has its place. Likewise, each of them, including colorimetry, has its merits and limitations.

Mention has already been made of the wide applicability of colorimetric methods. These applications include (1) elements, such as residual chlorine in water, (2) ions, both cations and anions, such as nitrite in water and thiocyanate in blood, and (3) molecules, such as ammonia in water and hemoglobin in blood.

There are additional important points of merit. The technique of measurement is often very simple, as in the use of *p*H test papers. It may be very exacting, as with the simultaneous polycomponent analysis of instable systems not conforming to Beer's law. Whatever is necessary in the way of separations, has, of course, no connection with the operation of colorimetric measurement.

Very often little time is required, providing there are no separations nor exacting control of color-forming processes. If separation is necessary, then the over-all procedure may be long.

The total range of equipment is large. For the determination of many single constituents the equipment required may be relatively cheap, as with Nessler tubes and standard series sets. Filter photometers cost a few hundred dollars, and the Beckman model B spectrophotometer is only approximately twice as much. Recording, narrow-band spectrophotometers are expensive instruments. One needs a narrow-band instrument of high quality for reliable work with simultaneous polycomponent analysis. This last application is an almost unique approach for handling certain systems, such as the chlorophylls in leaves.

Colorimetry was included on this program as one of the methods applicable to trace amounts of constituents, and the technique has its greatest merit in this direction. Thus, the determination of chlorine and of ammonia in water go down to as low as 0.01 ppm, and a few methods are better in this respect. Inherently, the magnitude of the molar absorbance index is the determining factor. However, one may control, within limits of the apparatus, the thickness of the absorption cell. The intensity of the illuminant, along with its spectral distribution, and also the sensitivity of the detector are important. Only recently have multiplying photocells been incorporated in expensive spectrophotometers. The peak sensitivity in a given case should be determined experimentally, care being taken to use refined techniques in any preparative processes and to maintain the optimum working conditions for the measurement.

Colorimetry has applications outside the range of trace amounts of constituents, although these uses are not of primary importance here. Thus, some constituents are determined in the general range of 50 to a few hundred parts per million by using relatively insensitive reagents. An interesting different development is the extension of this range, by means of differential measurements, to include certain constituents that are major components of materials. The theory for this application has been developed by Hiskey,[9] and applications to some industrial materials have been reported by Bastian.[2]

Wide variation characterizes the kind of results one may obtain with colorimetric methods. The *p*H test-paper technique is relatively crude. Many other visual comparimetric results are probably no closer than one part in 20; that is, the uncertainty is ±5% of the amount present. With careful use of the most reliable spectrophotometers, this value may approach one part in 100. The best of such instruments, properly operated, will reproduce transmittance readings to within perhaps 0.2% at 80 to 90%. The Hiskey–Bastian technique is said to approach one part in 1000. However, this value pertains only to the measurement operation.

Considerable discussion has centered on the optimum concentration of solution required for maximum reliability of the photometric measurement. Assuming certain instrumental conditions, it has been calculated [7] that the transmittance reading should be 36.8% for minimum photometric error. Stearns [23] calculated this value to be 28.2% for the optics of the General Electric instrument. He recommends, as a general practical working range, adjusting conditions to keep the transmittance between 20 and 60%.

More recently Reilley and Crawford [19] have made an extensive analysis of the precision theoretically achievable by four different methods of measurement with spectrophotometers. In the recommended "trace-analysis" method "the instrument is set to read 100 (transmittance) when exposed to light that has passed through pure solvent and to read 0 when exposed to light that has passed through a reference solution somewhat more concentrated than the sample."

One should take advantage, of course, of optimum conditions of measurement. However, in practical analysis more error is likely to be involved in the operations and processes of sampling, separations, and color formation than in the final operation of measurement.

DISCUSSION

Discussion Leader
M. L. Moss
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The talk we have just heard brings out a number of points of interest. As Professor Mellon mentioned in the beginning of his chapter, colorimetry is one of the oldest types of methods used in chemical analysis. Many of these colorimetric methods are characterized by

extreme simplicity, making them highly useful for large-scale repetitive testing in industry, medicine, water treatment, industrial hygiene, and other important fields. An analyst confronted with the necessity of devising an original method for a particular situation usually considers possible colorimetric methods because their evaluation is relatively straightforward and conclusive. Although colorimetric methods are highly sensitive as a rule, they are applied over comparatively wide ranges of concentration and are by no means limited to "trace analysis."

The general field of photometric chemical analysis may be said to include colorimetry, turbidimetry, nephelometry, and fluorometry. Of the photometric methods, those involving colorimetry are the most widely used, and the words *photometric* and *colorimetric* are often used interchangeably, probably for this reason. Problems of chemistry and instrumentation are similar among the several types of photometric analysis. Colorimetric methods may be designated direct or indirect according to whether the color is in proportion to the desired constituent or is an inverse function of the concentration as in those methods based on fading reactions where the color intensity is less, the higher the concentration of desired constituent.

Professor Mellon discussed the three main aspects of colorimetry: theory, instrumentation, and chemistry. Although more work has been done on the chemical problems in colorimetry, the theory and instrumentation appear to be on a more satisfactory basis. The absorption laws of Lambert, Beer, and Bouguer quantitatively express the relations of color intensity, concentration, and cell length to one another. Depending on the instrument used, maximum precision is said to be achieved for transmittance measurements of the order of 37%.

Precision of colorimetric measurements has attracted considerable attention for some time. Mehlig advocated taking transmittance measurements at 10 or 12 wavelengths and averaging the final results. More recently, differential measurement of the sample against a standard instead of a blank so as to extend the photometer scale has come into popularity and has been shown to offer advantages of high precision, particularly at high concentrations. Lothe has published an interesting paper providing certain theoretical information concerning indirect colorimetry that has heretofore been lacking. According to this, the most favorable situation for high precision and accuracy is to work in the low-absorbance end of the photometer scale and read against a partially faded standard. These recommendations on

indirect colorimetric measurements should be very useful inasmuch as sensitivity of the indirect methods in differentiating between various concentrations leaves something to be desired.

As Professor Mellon pointed out, there is no shortage of instruments for colorimetric analysis. There has been a trend in the past few years toward inexpensive spectrophotometers. These now compete with the filter photometers which in the past had been the cheapest type of instrument on the market. For most determinations, a filter instrument is adequate although spectrophotometers are generally preferred.

As regards filter photometers, perhaps it should be mentioned that there is more to selection of a filter than a comparison of the wavelengths of maximum transmission or absorption for the filter and colored solution. This is all that is needed, provided the two curves are symmetrical. If the curves are not symmetrical, highest sensitivity will not necessarily be obtained with a filter having maximum transmission at the same wavelength as the absorption band in the colored solution. For example, the absorption peak for ferrous *o*-phenanthroline occurs at about 510 mμ, and the most favorable slope for the calibration using monochromatic light is obtained at 510mμ. However, with glass filters, increased sensitivity is obtained, the more nearly blue the filter is, and a 400-mμ filter is appreciably better than a 510-mμ filter.

Most of the difficulties in colorimetry are of a chemical nature, and in general there is no serious limitation attributable to the present state of the theory or instruments available. The most difficult problems have to do with separation or concentration of the desired constituent in order to obtain sufficient material to measure or to remove interfering constituents occurring in the sample. Conditions for controlling the color reaction have to be established, but these are usually not difficult to achieve.

Although I would not take exception to anything brought out in the previous paper, I should like to add a few comments concerning the effect of temperature. Once the color is developed, variations in temperature ordinarily do not affect the color intensity or the color measurement for practical purposes. The error encountered is no more than that corresponding to volume changes with temperature. Temperature is a critical factor, however, in certain cases where development of the color is a slow reaction. Reactions that are ionic in nature usually take place instantaneously, and there is no problem of time or

temperature. Color reactions that involve condensation or other slower chemical reactions may be affected considerably by both time and temperature. These conditions must be carefully controlled. Whenever development of the color requires more than a few minutes, one should be prepared for temperature effects. It may then be necessary to employ temperature control and a definite reaction time before reading the color.

Twenty years ago Lundell and Hoffman in their *Outlines of Methods of Chemical Analysis* brought out that the analyst had little cause for complaint concerning the physical tools at his command. These were believed to be more than satisfactory for his needs at the time, and this is probably more nearly true today than it was then. The authors went on to point out that the chemistry on which the methods were based caused the greatest concern since the chemical reactions had not kept the pace with the other tools. Discoveries of new and useful reactions were becoming few and far between. This was the most serious obstacle then and still is.

In order to take full advantage of the available theory and instrumentation, more work will have to be done on the problem of separation and concentration of trace constituents. Otherwise we must have more selective chemical reactions on which to base our measurements. Until we have these better chemical reactions, we will have to live with difficult problems such as the one mentioned by Dr. Mellon involving simultaneous polycomponent analysis of unstable systems not conforming to Beer's law.

QUESTIONS AND ANSWERS

WILLIAM RIEMAN (*Rutgers University*): You mentioned that a spectrophotometric determination is most accurate if the transmittancy has a certain value, 37 or so per cent. I would like to point out that this statement is based on the assumption that the error in transmittance is the same throughout the whole transmittance range. Recent work done in the Beckman laboratories indicates that this assumption is not valid.

FRITZ WILL (*Aluminum Co. of America*): In recent years ultraviolet spectra have been used in inorganic analysis. Would Dr. Mellon comment on the future of ultraviolet spectrometry?

M. G. MELLON: You are an ultraviolet enthusiast, I am certain. I do object to saying ultraviolet light or talking about black light. Those are definitions I would insist upon. As far as the applicability of absorption in the ultraviolet region is concerned, it is becoming increasingly important.

M. L. Moss: Speaking of definitions, what do you think of considering

photometry as the general field, with four subdivisions: colorimetry, turbidimetry, nephelometry, and fluorometry?

M. G. MELLON: Professor Yoe should answer that question.

JOHN H. YOE (*University of Virginia*): I think that would be quite satisfactory. I wonder whether Dr. Mellon would like to comment on the possibility of making multiple analyses by the simple expediency of shifting wavelength?

M. G. MELLON: I should have mentioned that application. It seems to me that the most important applications of spectrophotometers consist in making simultaneous spectrophotometric examinations. In the infrared region, for example, one finds much greater opportunity. Workers in the infrared region have a tremendous wavelength range, such as 40 microns. In the ultraviolet we have two tenths of a micron. I know of at least one case in which eleven components are measured without making as many separations. The two chlorophylls are an example in the visible region. One extracts the mixture of blue and green from the leaf, and, having previously determined the appropriate wavelengths and the absorptive constants of the pure compounds, one is ready to make measurements of those two without making a separation. In the organic field, some of these separations become very difficult. I believe this is perhaps the most important kind of application of spectrophotometry.

EDWARD C. DUNLOP (*E. I. duPont de Nemours & Co.*): Can you give us any applications of turbidimetric methods to determinations other than chloride or sulfate ions?

M. L. MOSS: Calcium is determined turbidimetrically with fatty acids and carbonate with barium. Of course, there are reactions in what we call colorimetry where the colored products are actually insoluble. Colloids are sometimes used to keep them in suspension, such as agar or gum arabic with copper dithiocarbamate.

T. S. MA (*Brooklyn College*): I have two comments to make. One is about temperature effects, and the other about ultraviolet colorimetry.

Temperature sometimes is very important in the development of color. I have had some experience in the determination of phosphorus using the molybdenum blue method. When we have milligram quantities, we can develop the color at room temperature, but, when the amount of material is only microgram quantities of phosphorus, say in phosphoproteins, we have to develop the color at high temperatures. We do it at the boiling point of water and get very good results.

However, if other people use a temperature of, say, only 85°, the intensity of color produced will be quite different. This applies to many of the colorimetric methods.

With respect to ultraviolet colorimetry, some at New York University have been working on it for some time.

M. G. MELLON: As far as temperature is concerned, I wish at times I could make mixtures highly temperature-sensitive. Then perhaps we might acquire air-conditioned surroundings, which we do not now have.

N. D. CHERONIS (*Brooklyn College*): In reference to the reactions that are produced by carbonyl compounds with 2,4-dinitrophenylhydrazine, the effect of the radical that is attached to the carbonyl group has to be taken into consideration because many of the lower aldehydes or ketones usually react

at rapid rates. The rate of reaction drops off as soon as the radical attached to the carbonyl group becomes large or complex.

For example, formaldehyde and acetylaldehyde react 70 to 100 times faster than, say, C_7 aldehyde. In general, when we consider the so-called spot-test reactions which produce color, the effect of the radical attached to the compound has to be taken into consideration. This is particularly true when we claim that a reaction is specific.

ANDRÉ JULIARD (*Houdry Process Corp.*): I would like to point out the danger of using turbidimetric methods, for accurate determination of the trace amounts of substances in an unknown solution, on account of the tremendous effect of traces of various substances on the precipitation of the substances to be determined. For instance, the amount of hexametaphosphate at the level of a few parts a million is enough to disturb profoundly the precipitation of barium sulfate, which is used for the determination of sulfate by turbidimetry.

M. L. Moss: This is correct. We must be very careful in photometric determinations involving formation of a solid phase.

O. I. MILLNER (*Socony Mobil Laboratories*): On the subject of errors, one that I have never seen listed pertains to refraction effects. This is possible because in most instances we are concerned with aqueous solution, but in some work we did with nonaqueous media we did observe a rather critical effect due to a change in the index of refraction, when the reactant was added. This is not important in cells with parallel windows because it cancels out, but many of the commercial instruments use test tubes, and there one gets a lens effect. If the reference solution that is normally used to zero the instrument is different from the solvent in the sample, one can run into a serious error.

M. G. MELLON: I believe this is mentioned in a short paragraph in the chapter Dr. Gibson wrote in the book I edited some years ago.

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— 7 —

Fluorometry *

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ABSTRACT Fluorometric methods provide the best means of detection and determination of a number of inorganic ions. The reagents employed in these procedures are often quite specific and are extremely sensitive. Good quantitative and qualitative fluorometric methods for Al, Be, B, Cd, In, Li, F, Ga, U, Zn, Zr, and other elements have been described. The quantities determined are in the order of 1 μg in 10 ml of solution. In most cases organic reagents are employed which form fluorescent chelate compounds with the metal ions. Exceptions are the analysis of uranium, in which a fluoride carbonate melt is used, and some of the lanthanides are determined by direct measurement of the fluorescence of solutions of their salts. Fluorescent spectra of these complexes may be determined qualitatively with a spectroscope or quantitatively with a spectrophotometer or spectrograph. The spectra in the above instances range from 400 to 700 mμ. Quantitative measurements of fluorescent intensity in the blue or yellow range may be made with instruments employing normal phototubes or barrier layer cells. For measurement of weak intensities in the longer wavelengths of the red, a photomultiplier tube is necessary. Changes in molecular structure of the reagents may be made to alter the spectral position of the emission without interfering with the chelating properties of the molecule.

* The excitation and fluorescence spectra mentioned in this chapter were determined with the aid of a Frederick Gardner Cottrell grant of the Research Corporation, New York.

Fluorescent spot-test reagents have been developed for a number of ions. Recently developed apparatus and new reagents are expected rapidly to increase interest and application of fluorometric procedures.

INTRODUCTION

Fluorescence is a type of radiant energy emission which is produced when a substance returns to a normal state after having been raised to an excited condition by the absorption of energy. One of the first substances observed to produce a visual radiation after it has been excited by a high-energy source was the mineral fluorspar, and hence the term "fluorescence." If the emission persists over 10^{-9} second after the exciting source is removed, the phenomenon is called phosphorescence. The exciting source and the emission may be in any part of the spectrum. In the X-ray fluorescence spectrograph, both the exciting source and the emission are in the X-ray region.

Since its discovery fluorescence has played an important part in the development of chemistry. Probably the first application of fluorescence to analysis occurred in 1868 when the German chemist Goppelschroeder [20] observed that the natural dye morin would give an intense fluorescence with the aluminum ion. In 1895 Roentgen discovered X rays because of the fluorescence of barium tetracyanoplatinate (II). The experiments of Becquerel with the fluorescence of a uranium mineral led to the analysis of the ore which resulted in the discovery of radium, polonium, and radioactivity in general. About 1913 Moseley, through the use of fluorescent X rays which were produced by excitation of metals with cathode rays, discovered atomic numbers. Within the past few years, with the advent of sensitive Geiger counters and scintillation tubes, fluorescent X rays have become an important analytical tool.

GENERAL REFERENCES

Radley and Grant [31] have compiled an extensive review of the literature on fluorescence analysis in their book called *Fluorescence Analysis in Ultraviolet Light.* Bowen and Wokes [6] in a small book called *Fluorescence in Solution* discuss the essentials of the theory of fluorescence as applied to analysis. Practical techniques used in fluorometric analysis are given by White in a chapter that is contained in a book on *Modern Instrumental Analysis* edited by Boltz.[4] The general topics of fluorescence and phosphorescence are very ably dis-

cussed by Pringsheim.[30] Applications of fluorescence to organic analysis will be found in a book on color and fluorescence reactions in organic analysis by Pesez and Poirier.[28] Additional books in French, German, and Russian on fluorescence and fluorescence analysis, the title of which are self-explanatory, are given in references (10, 3, 15, 25). Further literature references are found in the reviews that have appeared in *Analytical Chemistry*.[37,38]

APPARATUS

The radiant energy involved in ordinary fluorometric analysis is between 2000 and 7800 A. This is illustrated in Fig. 1. Since wave-

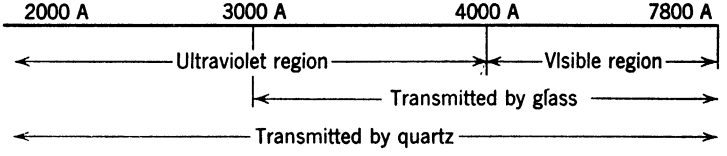

FIG. 1. Radiant energy involved in fluorometric analysis.

lengths over 3000 A are transmitted by glass and are not injurious to the eyes or skin, this is a very desirable range of the spectrum for practical work. In some applications shorter wavelengths, between 2000 and 3000 A, are desirable for excitation. In this case the eyes must be protected and the rays brought in direct contact with the sample or quartz containers used. For visual observation it is desirable to choose an exciting source in the near ultraviolet which will produce an emission in the visible range. A comparison of the relative

TABLE I

COMPARISON OF SOURCES OF ULTRAVIOLET LIGHT EXPRESSED IN ARBITRARY UNITS

Source	Relative Intensities at Emission Wavelength		
	2540 A	3650 A	4050 A
H100 A4 (General Electric)	0	820	350
H100 B4 (General Electric)	0	440	2
Low-pressure Hg lamp of B&L monochromator	280	638	100
Sylvania 360, 15-watt clear tube	0	26	3.5
Tungsten ribbon-filament lamp of B&L monochromator	0	52	130
Mineralight 41A	40	4.0	2.0
Xenon arc lamp	5	20	22

intensities of the excitation sources used in our laboratory are listed in Table I.

The xenon arc provides a reasonably constant source of ultraviolet radiation between 3000 and 4000 A. The high-pressure (H100) mercury-vapor lamps are very good sources for the 3650-A radiation. For qualitative purposes the 360 BL lamps are quite satisfactory and are extremely convenient.

Fluorometers. A number of fluorometers are advertised in the literature which have been developed essentially for vitamin deter-

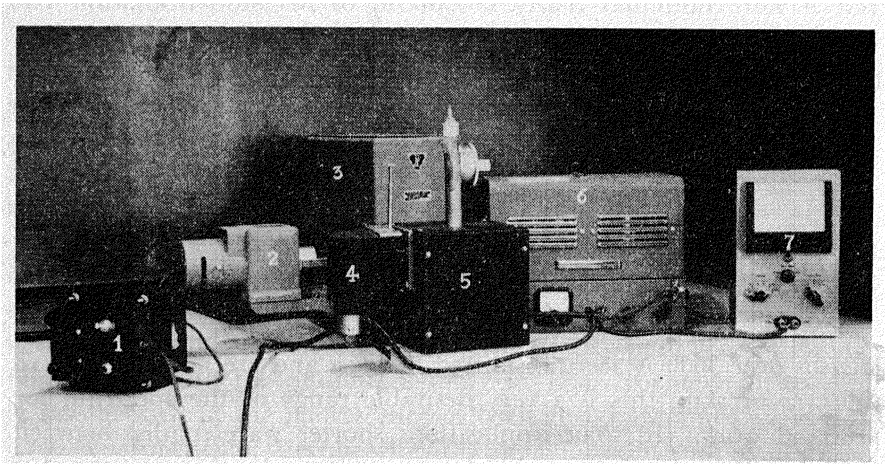

FIG. 2. Fluorometer with Bausch & Lomb monochromator as a light source: (1) Transformer, (2) low-pressure mercury-vapor lamp, (3) Bausch & Lomb grating monochromator, (4) photomultiplier tube with housing, (5) sample compartment, (6) power supply for photomultiplier tube, (7) RCA ultramicroammeter WV-84A.

minations and are equipped with barrier layer cells or ordinary phototubes. These instruments are quite satisfactory for measuring a relatively intense blue or green fluorescence but are generally not adapted for a weak intensity in the red region. Since phototube surfaces are not very sensitive to wavelengths above 6000 A, it is necessary to use an electron-multiplier phototube to achieve the response desired in this region. For this reason most research laboratories build their own instruments. The one used in our laboratory incorporates ideas of Fletcher et al.[13] and was built by graduate students;[16] this is illustrated in Fig. 2. A monochromator is pictured as the source of the exciting radiation; in routine work this is replaced by an H100 B4 lamp with an appropriate filter. This fluorometer consists essentially of an electron-multiplier phototube housing in which the 1P21, 1P28, and 931A tubes are interchangeable, a power source for the tube, an

RCA ultramicroammeter, and a cuvette holder with an exciting light source. The American Instrument Company, Silver Spring, Md.; the Farrand Optical Company, New York; and the Photovolt Corporation, New York, have announced the production of fluorometers equipped with electronmultiplier phototubes. One of these instruments [2] is designed to measure the fluorescence of either solids or liquids.

An essential piece of apparatus for the analyst is a view box in which a fluorescence may be observed in the laboratory. This has been described in detail elsewhere [39] and consists of a black box equipped with a filter like Corning no. 5874, a viewing aperture, and appropriate openings for the insertion of samples. A good light source for this view box is a 15-watt 360 BL lamp with an aluminum reflector. Sunlight or strong daylight can also be used as a source of ultraviolet light. This view box can be used for approximate and even accurate quantitative determinations.[14] It is also useful to check a series of fluorescent solutions if results on a photoelectric instrument are in question.

Fluorescence Intensity as Related to Concentration. The fluorescence intensity of dilute solutions is a straight-line function of concentration. Some authors have mistakenly assumed that this was a logarithmic relationship such as in absorbancy, and one of the early fluorometers was equipped with a logarithmic scale. The application of the Lambert–Bouguer–Beer law to fluorescence may be formulated as follows:

From the absorption law:

$$\frac{I}{I_0} = 10^{-ecd} = \text{fraction of light transmitted}$$

where I = the intensity transmitted
I_0 = the incident intensity
e = the molar extinction coefficient
c = the concentration
d = the cell thickness

$$1 - \frac{I}{I_0} = 1 - 10^{-ecd} = \text{fraction of light absorbed}$$

$$I_0 - I = I_0 (1 - 10^{-ecd}) = \text{amount of light absorbed}$$

The intensity of the fluorescence F is proportional to the amount of light absorbed; hence

$$F = K_1 I_0 (1 - 10^{-ecd})$$

By expansion of the term in the parentheses

$$F = K_1 I_0 \left[2.3ecd - \frac{(-2.3ecd)^2}{2} - \frac{(-2.3ecd)^3}{3} - \cdots - \frac{(-2.3ecd)^n}{n} \right]$$

If *ecd* is small, the latter terms can be dropped and

$$F = K_1 I_0 2.3ecd$$

or, since *c* and *d* are constants for one cell, and one compound and the light source may be kept constant,

$$F = Kc$$

With proper treatment as shown by Lothian [27] and Braunsberg,[7] a number of practical considerations can be derived from this equation. Not over 2% of the exciting radiation should be absorbed, and a large area of fluorescing molecules should be measured. Where the term *ecd* is greater than 0.05, the latter terms of the expansion cannot be neglected, and the plot of concentration versus intensity will produce a curved line. The straight-line relationship is the one commonly used and is illustrated in many published fluorometric procedures.

TYPICAL REAGENTS

Reagents generally employed for fluorescence analysis of inorganic ions are organic dyes or compounds which possess satisfactory groups for chelation. The determinations listed in Table II have been selected for brief discussion to illustrate typical conditions and re-

TABLE II

Typical Reagents for Fluorometric Analysis

Element	Reagent	Sensitivity, μg	Fluorescence Wavelength, mμ
Al	Pontachrome BBR	0.2	635–700
Al	Morin	0.0005	525–570
Al	Oxine	0.1	450–610
Be	Quinizarin	1.0	570–640
Be	Morin	0.004	510–615
Zr	Flavonol	0.1	420–520
F^-	Al–Alizarin Garnet R	0.1	550–600
U	NaF melt	0.0001	540–550
B	Benzoin	0.2	440–630
Tb	$TbCl_3$ (no addition)	0.005%	525–555
Li	Oxine	3.0	490–570

agents. The initials CI will refer to the color index of the British Society of Dyers and Colourists. The three *o,o'*-dihydroxyazo dyes given in Fig. 3 form excellent reagents for the determination of aluminum.

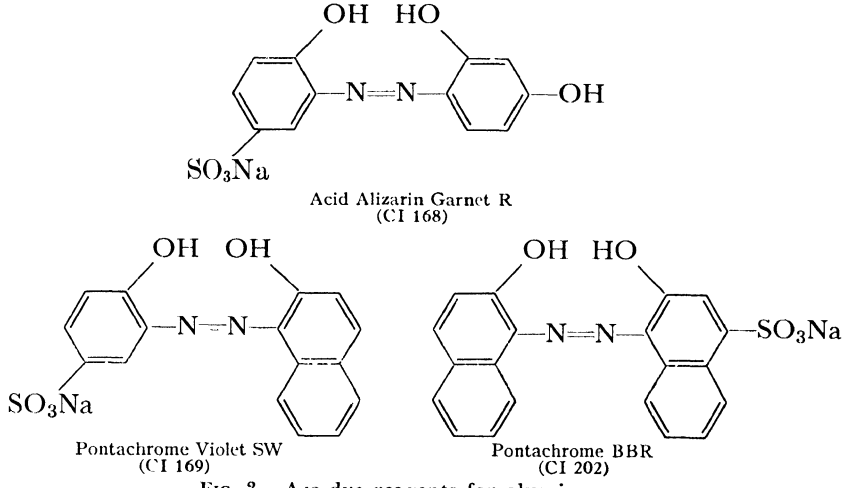

FIG. 3. Azo-dye reagents for aluminum.

These dyes are used as 0.1% solution dissolved in ethanol. The reactions are performed in water solution buffered at a *p*H of 4.6. Pontachrome BBR [40,35] was the first of these dyes developed as a highly selective and sensitive reagent for aluminum. The color change of the fluorescence with the increasing complexity of the molecule is interesting; Alizarin Garnet R produces a yellow fluorescence with Al^{+3} ions, with Violet SW the fluorescence is orange, and with Pontachrome BBR it is red. Alizarin Garnet R was developed as a reagent [32] in a search for a compound that had the desirable characteristics of Pontachrome BBR but with a fluorescence more easily measured with commercial fluorometers.

Alizarin Garnet R is a more sensitive reagent for Al^{+3} than Pontachrome BBR but the latter is less subject to interferences by traces of Fe^{+3}. These dyes have been subjected to a thorough study [17] and experiments indicate that the aluminum replaces one of the hydroxyl hydrogen atoms with a primary bond and coordinates with the opposite nitrogen and oxygen with secondary valence bonds. A one-to-one ratio of aluminum to dye is apparent in all three cases. The aluminum–Alizarin Garnet R complex has been shown by Powell and Saylor [29] to serve as an excellent quantitative reagent for fluoride. The decrease in the fluorescence of a standard solution of the complex on the addition of fluoride provides a quantitative measurement

of the amount of fluoride present. The aluminum oxine [23] and the aluminum morin [43] reactions have also been used in the analysis for fluoride. Alizarin Garnet R produces a beautiful spot test with aluminum on filter paper.

The natural dyes morin and quercetin and the related compound flavonol form fluorescent complexes with a number of ions. The structures of these compounds are shown in Fig. 4.

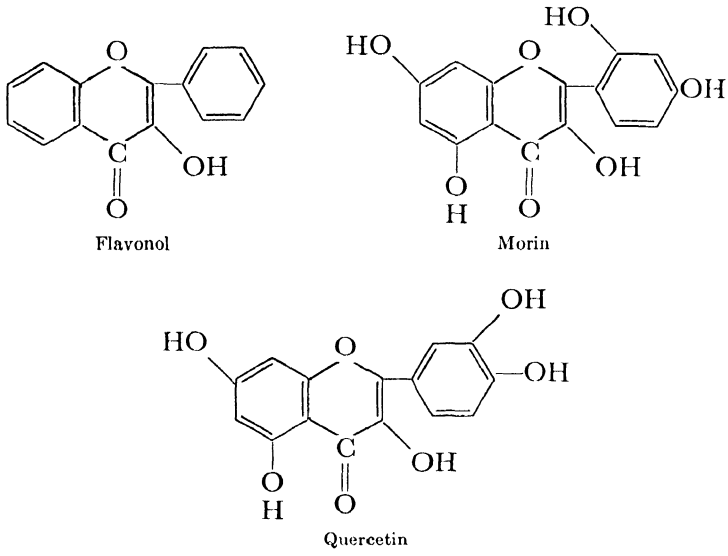

FIG. 4. Hydroxyflavone fluorescent reagents.

Morin and quercetin under proper conditions form fluorescent complexes with Al^{+3}, Th^{+4}, Zr^{+4}, Be^{+2}, borate and many other ions. These compounds may be purchased in highly purified forms and in this condition serve as excellent reagents. The ratio of metal to the organic compound seems to be 1 to 1 in each of these chelates; [5,17,33,36] however, the boron ratio may vary from this value. Evidence that the metal replaces the hydrogen of the hydroxyl group in the 3-position seems quite definite. Morin provides an extremely sensitive test for Al^{+3} in acid solution; this reaction was shown to be quantitative [41] some years ago. Morin is also a sensitive reagent for beryllium, but in contrast to aluminum the reaction medium must be alkaline.[26]

Flavonol forms fluorescent chelates with aluminum and other ions in solutions near the neutral point. The zirconium–flavonol fluorescence persists even in 0.2 *N* sulfuric acid, and flavonol is recommended

as a highly selective reagent for zirconium.[1] Both morin and flavonol are highly fluorescent on filter paper, and hence neither serves as a good spot-test reagent on paper. Quercetin, however, is an excellent reagent for zirconium as a spot test on paper; [36] the complex is highly fluorescent, and the reagent is not fluorescent. Quercetin in solution forms a yellow complex with zirconium which is only weakly fluorescent and serves as a colorimetric reagent.

Metal complexes with 8-quinolinol (oxine) are not highly fluorescent in water but give an intense fluorescence in chloroform or on paper. Almost any metal ion that precipitates with oxine may be determined fluorometrically if the oxinate is extracted from water with chloroform. The fluorometric determination of aluminum with oxine is a highly favored procedure which has been reviewed by Goon et al.[19] With the proper adjustment of *p*H, good separations of metals may be obtained by this chloroform extraction.

Collat and Rogers [8] have shown that it is possible to determine both gallium and aluminum in the same chloroform extract of the oxinate. These authors measure the fluorescence of the solution at two different excitation wavelengths. With these data and predetermined curves they are able to calculate the concentration of both aluminum and gallium in the mixture. Some of the metal–oxine complexes are much more intensely fluorescent in alcohol than in water. In the fluorometric determination of lithium with oxine a 95% ethanol solution is used as the reaction medium.

Benzoin serves as an excellent reagent for the fluorometric determination of boron; one microgram of boron can easily be detected in 25 ml of solution. The medium for this reaction is an alkaline solution of 95% ethanol. This procedure for boron was difficult at one time because of the rapid decrease in fluorescence of the complex; however Hoffman [24] has found that the complex is easily stabilized, and the determination of boron by this method should now find extensive application. The use of a mixture of potassium hydroxide and glycine in place of the sodium hydroxide makes the fluorescence stable for a period of 10 minutes, and the use of isopropyl amine to obtain the alkalinity extends the time to 20 minutes. Also after an initial reaction period of 5 minutes, the fluorescence will remain stable for 40 minutes if the solution is exposed to ultraviolet light only for the intermittent period at which readings are taken; the decomposition of the boron–benzoin complex is accelerated by ultraviolet light. Dimethylformamide as a solvent in place of alcohol will also stabilize this fluorescence.

The fluorometric determination of cadmium as described by Evcim and Reber [11] illustrates a procedure that deserves more attention in fluorometric analysis. These authors use a highly fluorescent reagent, 2-*o*-hydroxybenzoxazole, to precipitate cadmium. The insoluble chelate formed is filtered, washed, and dissolved in acetic acid, in which the chelate is destroyed, and the fluorescence of the dye is then measured.

The standard method for the determination of uranium [22] is an excellent example of the use of a solid matrix from a fusion mixture as a source of fluorescence for an analytical determination. In this procedure a uranium salt is fused with a mixture of sodium fluoride and sodium carbonate. The fluorescence of the solid resulting from this fusion is very intense and may be measured by either the transmission or the reflectance technique. In the former the photocell is on the opposite side of the solid from the exciting source, and in the latter it is on the same side. A quantity of uranium as small as 10^{-11} gram may be detected by the fluorescence of this solid.

Water solutions of inorganic salts of the elements are generally not sufficiently fluorescent for analytical purposes. However, some of the lanthanide salts have distinct possibilities in this respect. Fassel and Heidel [12] have shown that solutions of terbium chloride irradiated slightly below 2500 A gave a fluorescence at 5450 A which was sufficient for the analytical determination of terbium.

Fluorescence Spectra. The determination of the portion of the spectrum covered by the fluorescence of a material is obviously necessary in order to select correct filters for the measurement of the fluorescence. From a qualitative standpoint fluorescent spectra are easily determined with a simple Bunsen three-arm spectroscope. With a little experience these visual values will be found to check closely with photoelectric determinations. Since the wavelengths covered by the fluorescent band is independent of the exciting source, the choice of any source of irradiation that produces an intense fluorescence is satisfactory. These measurements are quite easily made with an arrangement of the Beckman DU spectrophotometer containing a photomultiplier tube. Several descriptions of modifications of the Beckman have appeared in the literature.[12,21] Probably the simplest arrangement of the Beckman for the measurement of fluorescent spectra is as follows: Remove the housing of the light source, and replace it with a black box equipped with a Corning no. 5874 filter on top and a shelf to hold a 1-inch cube cuvette at the light-entrance slit. For

irradiation below 3000 A the filter is removed. The fluorescent light passes through the monochromator and is measured over various wavelength settings on the transmission scale with the photomultiplier tube. A correction can be made for the sensitivity of the tube at the different wavelengths if necessary. For organic compounds and metal chelates, band spectra are obtained which are considerably narrowed at very low temperatures. Inorganic salts produce narrow

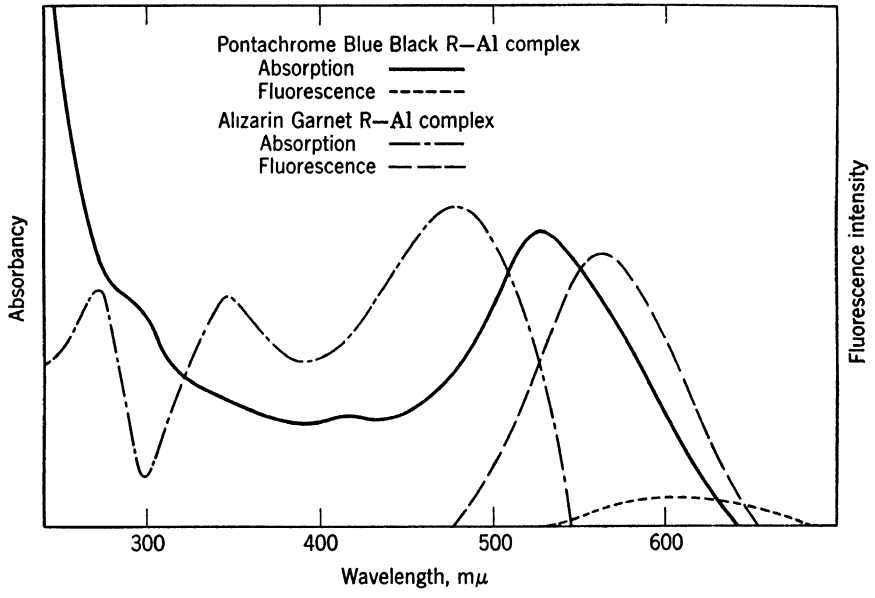

FIG. 5. Relation of absorption spectra of metal chelates to fluorescence spectra.[13]

bands which approach line spectra. For the organic compounds and metal chelates, the fluorescent band usually begins within the range of the longest absorption band of the compound. This relationship is illustrated in Fig. 5, and many examples are given by Cook et al.[9] Both the excitation radiation and fluorescence may be in the ultraviolet region of the spectrum, and Udenfriend et al.[34] have shown that fluorescence in the ultraviolet is extremely useful in the determination of some biological substances.

Wavelength of Maximum Excitation. The intensity of a fluorescence varies with the intensity and the wavelength of the exciting source. The wavelength for maximum excitation may be determined with a combination of a microphotometer and a monochromator. In our laboratory we use a Bausch & Lomb monochromator and a labo-

ratory-assembled microphotometer for this purpose. The fluorescent solution or solid is placed in front of the exit slit of the monochromator and its fluorescence measured with the microphotometer while the monochromator drum is rotated over the spectral range. The wavelength for greatest excitation seems to coincide with the strongest absorption band nearest the fluorescent spectrum band (Fig. 6). However, in some cases[5] the maximum excitation appears at a point of

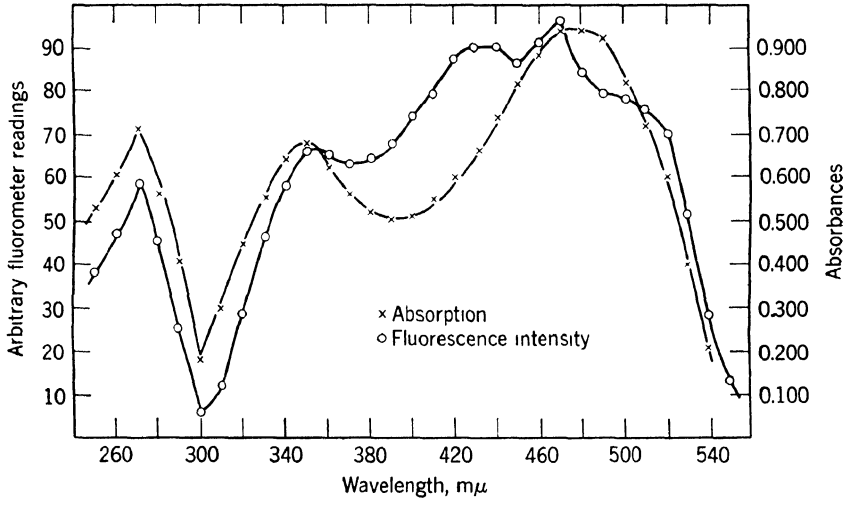

FIG. 6. Excitation and absorption spectra of aluminum acid–Alizarin Garnet R complex. Fluorometer readings and absorbances versus wavelength.

moderate absorption. It is obvious that the excitation wavelengths must be corrected for variations in intensity of the exciting source. For organic compounds or metal chelates where a visible fluorescence is to be measured, the high-pressure mercury-vapor lamp with an intense emission at 3650 A is a very satisfactory source of exciting radiation. If an irradiating wavelength is selected close to the fluorescent light, it is difficult to separate the two with filters.

Precautions. Fluorescent analysis is generally concerned with microquantities, and precautions must be exercised to avoid contamination from all possible sources. Filter paper contains a blue fluorescing dye which is easily extracted by water. Rubber stoppers, corks, the human skin, etc. contain materials that are extracted with alcohol or water solutions which may increase or quench a fluorescence. Commercial 95% alcohol contains a blue fluorescent impurity which accumulates

in the residue on distillation. Alkaline solutions may dissolve boron and aluminum from Pyrex glass. Containers may have an objectionable fluorescence.

Other Applications. The space allotted to this chapter does not permit the inclusion of the many applications of fluorescence analysis in organic and biological chemistry, mineralogy, and analysis under the microscope. The quantitative determination of many organic compounds by the intensity of the fluorescence of their solutions has been practiced for many years; examples of this are found in the analysis of riboflavin, quinine, fluorescein, and fluorescent dyes. In many analytical procedures, compounds are converted to fluorescent materials by simple reactions. For the identification of compounds the change of fluorescence with *p*H can often produce useful results, as has been illustrated by Goodwin and Kavanagh.[18] This discussion has covered only a few applications of fluorescent techniques, and the reader should refer to the references given above for additional material.

DISCUSSION

Discussion Leader
Sidney Udenfriend
National Heart Institute

I want to thank the speaker for a very excellent discussion of his applications of fluorescence and for the general discussion of the properties of fluorescence. He has asked me to make some remarks about fluorescence as applied to organic molecules of biological importance and to discuss features of its application to studies where fluorescence is activated and emitted in the ultraviolet zone. Since commercial instruments depend on activation with a mercury-arc fluorescence, applications have been limited to those compounds absorbing one of the few lines of the mercury spectrum. Actually fluorescence is very widespread and occurs over the entire spectrum; limiting it to the mercury arc is like limiting absorption spectrometry to a red lamp as a light source. Dr. Robert Bowman and I have devised an instrument which can estimate and measure emitted fluorescence continuously throughout the ultraviolet and visible range. Figures D1 and D2 show a diagrammatic sketch and a photograph of the spectrophoto-

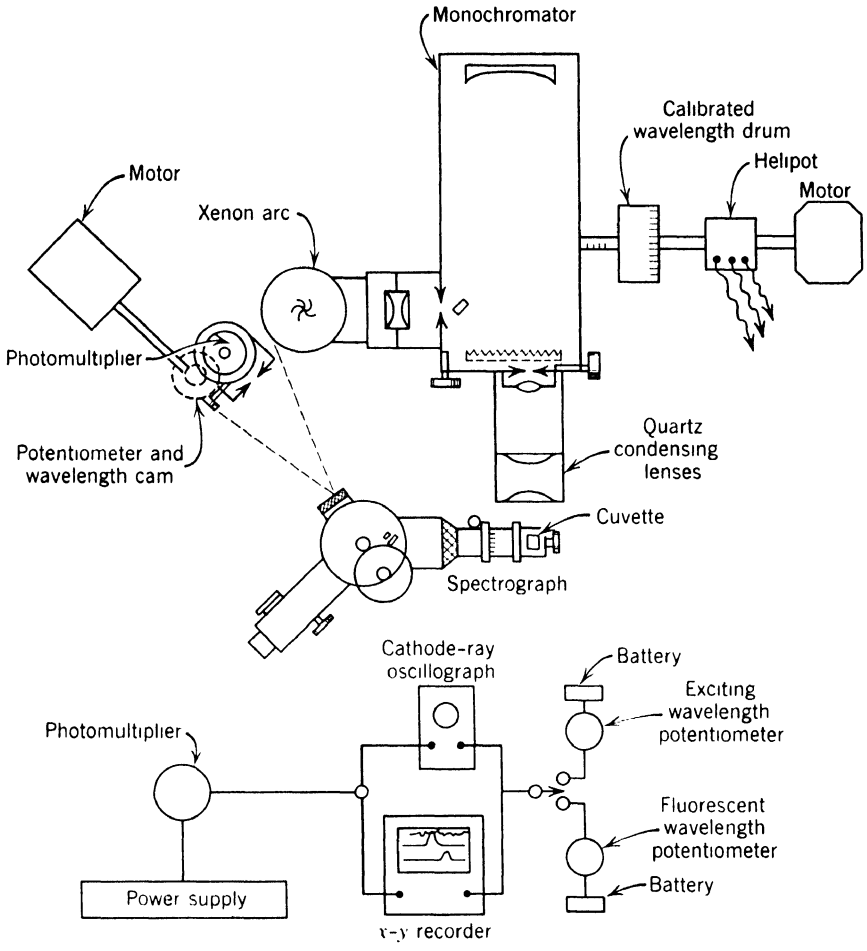

FIG. D1. Diagrammatic sketch of spectrophotofluorometer.

TABLE DI

Compound	Medium	Activation Maximum, mμ	Fluorescence Maximum, mμ
Epinephrine	0.01 *N* H_2SO_4	275	320
Norepinephrine	0.01 *N* H_2SO_4	275	320
Dihydroxyphenalalanine	0.01 *N* H_2SO_4	275	320
Tyrosine	0.01 *N* H_2SO_4	270	300–330
Morphine	0.01 *N* H_2SO_4	270–290	365
o-Aminophenol	4 *N* H_2SO_4	265	310
m-Aminophenol	4 *N* H_2SO_4	265	310
p-Aminophenol	*p*H 8	295	365–370

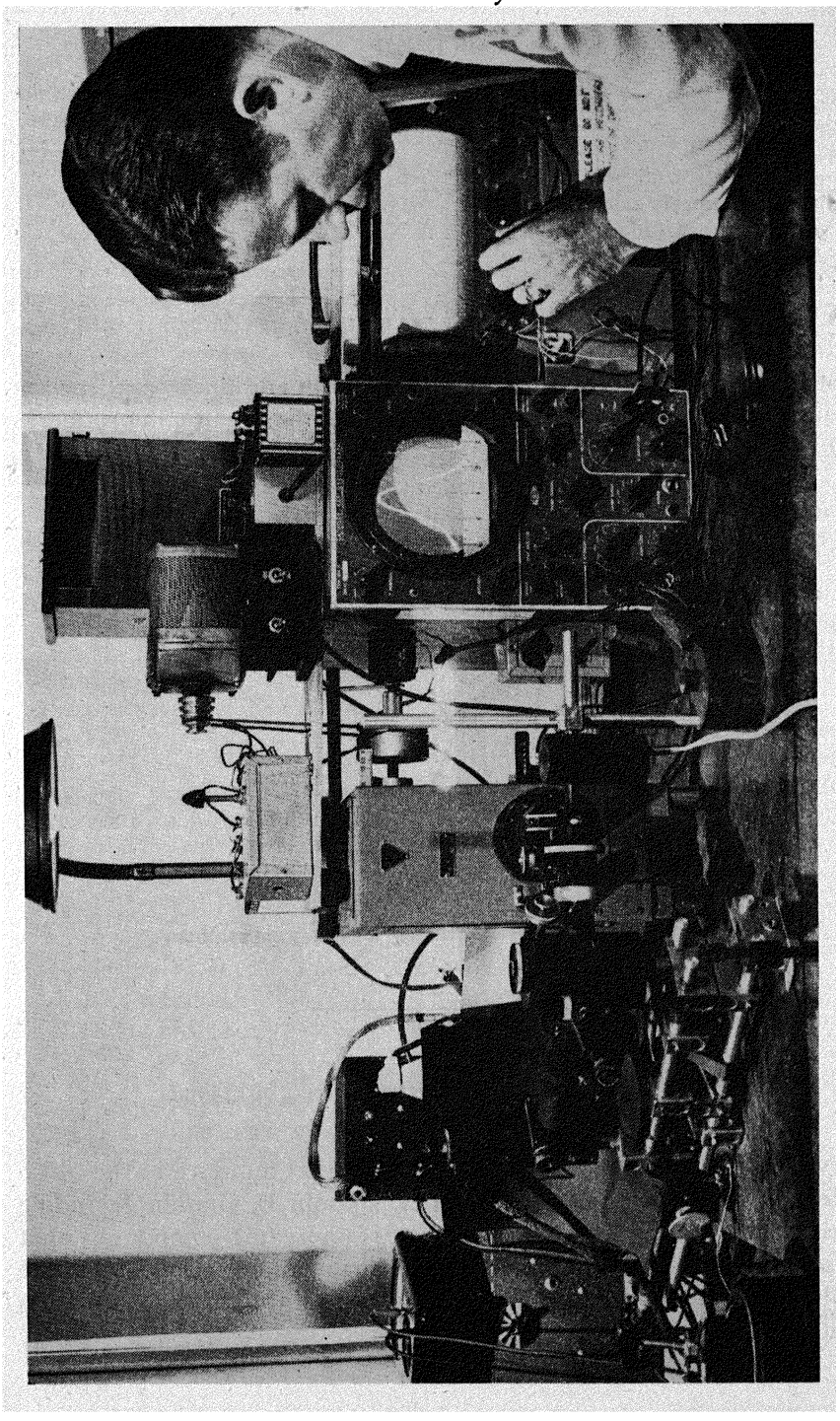

FIG. D2. Photograph of spectrophotofluorometer.

TABLE DII

Compound	Medium	Activation Maximum, mμ	Fluorescence Maximum, mμ
o-Hydroxybenzoic acid	*p*H 5–6	290	420
m-Hydrobenzoic acid	0.1 *N* NaOH	315	420
p-Hydroxybenzoic acid	0.1 *N* NaOH	280	330
Aniline	*p*H 9	290	360
Lysergic acid diethylamide	*p*H 9	315	440
5-Hydroxyindoles	*p*H 2–10	295	330

fluorometer used in our laboratory. Activation and fluorescent spectra of quinine are shown in Fig. D3. A list of some organic fluorophores and their fluorescent characteristics are given in Tables DI and DII.

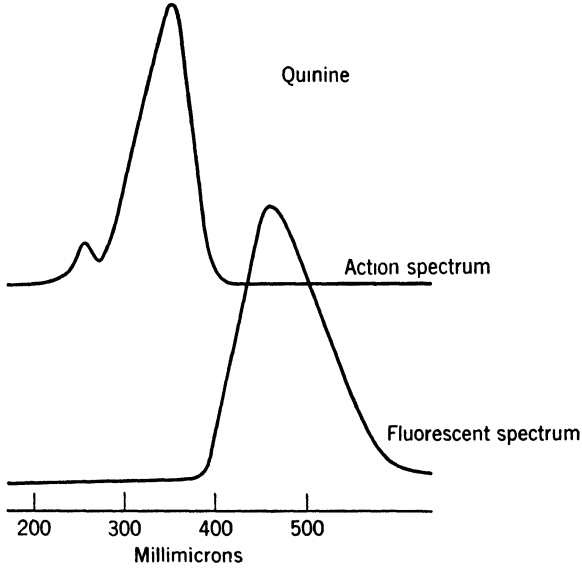

FIG. D3. Activation and fluorescent spectra of quinine.

I would like to point out that compounds that fluoresce in the ultraviolet are very numerous: i.e., purines, vitamin E, vitamin K, indole acetic acid. There are also a number of inorganic compounds that do so. Spectrophotofluorometer-type instruments should extend the applications of fluorescence to a degree comparable to those utilizing light absorption.

QUESTIONS AND ANSWERS

C. L. GEMMILL (*University of Virginia*): I would like to describe a modification of the Beckman DK-1 recording spectrophotometer for use as a spectrofluorometer.[44] Light is passed from a xenon arc light through a Bausch & Lomb monochromator. From this instrument the light passes through a hole in the back plate of the lamp house of the DK-1. The cell containing the solution under study is placed in the position formerly occupied by the tungsten light. The fluorescent light is picked up by the mirror on the back plate and is passed into the instrument. I have been interested in using this modification of the DK-1 to study the transfer of energy between molecules, particularly molecules of biological interest. Using a mixture of *p*-terphenyl and α-naphthylphenyloxazole (ANPO), it can be shown that the fluorescence of *p*-terphenyl decreases and ANPO increases when these two substances are in the same solution. The addition of thyroxine to the mixture causes the fluorescence of *p*-terphenyl to decrease further and of ANPO to increase. This effect is only obtained at high concentrations of ANPO.

SIDNEY UDENFRIEND: Two companies are developing spectrophotofluorometer-type instruments: the Farrand Optical Company and the American Instrument Company.

W. WAYNE MEINKE: What type of photomultiplier tube do you use?

CHARLES E. WHITE: 1P21 or 1P28. Some workers use end-window photomultiplier tubes and like them better. I am sure you will find the 1P21 or 1P28 tubes quite satisfactory.

DISCUSSANT: How does the metal chelate?

CHARLES E. WHITE: The chelating metal combines with the *cis* isomer of the azo dye and holds the molecule in a rigid planar position. Rotation around the azo group is prevented. The same thing is indicated if solutions of the azo dyes are cooled to rigidity; at a very low temperature a fluorescence is produced, whereas at a higher temperature the fluorescence is much less or nonexistent.

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— 8 —

Flame Spectrometry

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ABSTRACT This chapter reviews the general principles of flame excitation and its applicability for spectrochemical analysis. Work on a direct-reading flame spectrometer, which was constructed in the biophysics research laboratory, is considered, along with some studies on sources and mechanisms of excitation of radiating species. The phenomenon generally referred to as "interference" is reviewed.

INTRODUCTION

Their characteristic emission spectra have long served the qualitative identification of metals, and quantitative emission spectroscopy has become increasingly important as an analytical tool. Many workers have been intrigued with the opportunity for the simultaneous

* This work was supported by a grant-in-aid from the Kettering Foundation of Dayton, Ohio, and by a contract between the Office of Naval Research and Harvard University, Contract Nr5ori-07660.

and sensitive determination of many inorganic constituents of a sample, implied in the fundamental facts underlying spectrochemistry. Concerted effort has resulted in translation of the theoretical potential into practicable, though still highly empirical, analytical procedures. Throughout these pursuits, an inverse proportionality between simultaneity of detection on one hand and precision and sensitivity of analysis on the other has been apparent; and it has been the aim to increase the latter without sacrifice of the former, an aim that has been accomplished only in part.

In practice, studies toward this end have concerned themselves essentially with four parameters:

1. The source for excitation.
2. The elements for the dispersion of radiation.
3. Measurement of radiation intensity.
4. The preparation of the sample.

These factors have been discussed and reviewed.[17,18] Their implications for and applications to flame photometry and spectrometry have been discussed [19,30] and will not be stressed here. This chapter will be limited to some studies of flame spectrometry which have been carried out in our laboratory in collaboration with M. Margoshes and studies of the cyanogen–oxygen flame with Milton Baker and Anthony F. Bartholomay.

Principles. The flame, with less available energy of excitation than the arc or the spark, is nevertheless capable of exciting intense emission of elements with low excitation potential, chiefly the alkali metals and alkaline earths. Compared with the arc and spark, the flame is a stable source. When a proper burner is used under proper conditions, the flame is steady, and the intensity of radiation emitted is constant with time. The spectra excited in the flame, moreover, are relatively simple. For some elements, only two or three lines are observed. It is therefore easy to isolate emission lines or band heads for measurement from the continuum. High resolution will, however, increase the line-to-background ratio, improving both sensitivity and precision, and minimizing interference from other radiating species in the flame.[19,20,28,30] The initial temperature of the flame is controlled largely by the energy of the reaction between fuel and oxidant. However, the further decomposition of the products of combustion, and the heat capacity of the resultant fragments influences the final temperature. Therefore, not all of the energy made available by the reaction is reflected in the temperature of the flame. A proportion

of it is utilized to heat the fragments to the final flame temperature, which is thus a function of the fuels, oxidant, and sample employed. Table I lists the temperatures in degrees centigrade for various fuel–oxidant mixtures. The temperatures of most flames, conventionally

TABLE I

TEMPERATURES OF SOME FLAMES

Fuel–Oxidant Mixture	Temperature, °C	
	From Gaydon [13a]	From Mavrodineanu and Boiteux [20a]
Hydrogen–air	2100	2115
Hydrogen–oxygen	2810	2690
Acetylene–air	2250	2050
Acetylene–oxygen	——	3110
Methane–air	——	1955
Methane–oxygen	2737	2720
Ethylene–air	——	1895
Propane–air	——	1925
Propane–oxygen	2776	——
Manufactured gas–air	——	1840
Manufactured gas–oxygen	——	2800
Cyanogen–oxygen	4580	——

used in flame analysis, are of the order of 2800° C. They are adequate only to excite elements with low excitation potentials, such as the alkali metals and the alkaline earths; sufficiently strong emission may therefore be obtained for these elements, even with cool flames, in analytical work for which high sensitivity is required.

Such considerations led us to investigate the potential usefulness of the cyanogen–oxygen flame [1,29] as a spectroscopic source for elements requiring higher excitation energies than those discussed.

THE CYANOGEN–OXYGEN FLAME

The reactions of cyanogen with air and oxygen and their kinetics have been studied for many years.[12,13,15,25,26] In 1914, Reis [24] analyzed the products and the spectrum of this flame. On the basis of theoretical considerations, he suggested that the flame temperature was 4740° C. Following renewed interest in this source,[22,23] Thomas, Gaydon, and Brewer [27] measured the flame temperature experimentally by a determination of the vibrational intensity distribution of

the cyanogen bands. They found it to be 4800° K ± 200° K in stoichiometric C_2N_2–O_2 flames. Conway, Grosse and Wilson [10] measured the temperature of the cyanogen flame directly by means of the line-reversal method,[32] using the sun as a comparison radiator, and found it to be 4640° K ± 50° K when cyanogen and oxygen were equimolar. Their *calculated* value, 4835° K ± 50° K, is in good agreement with that of Thomas, Gaydon, and Brewer.[27] An increase in pressure to 10 atmospheres raised the flame temperature to 5050° K.[9]

Calculations of the theoretical temperature were based on the assumption that the reaction between cyanogen and oxygen is essentially

$$C_2N_2 + O_2 \rightarrow 2CO + N_2 + e$$

when equimolar quantities of both reactants are present. The high temperature of the flame is apparently due to the highly exothermic nature of the reaction coupled with the fact that only a very small fraction of the combustion products dissociate.

Theoretically, therefore, this flame should provide adequate energy to excite elements other than the alkali metals and the alkaline earths. No data on the excitation of spectra of elements in the cyanogen–oxygen flame have previously been published. Work was undertaken to test this hypothesis, and the feasibility of using the cyanogen–oxygen flame * as a spectrographic source.[1,29]

A burner system allowing the combustion of the two gases was constructed. It allowed the introduction of the sample solution into the burner as a fine fog suspended in the oxygen stream. The oxygen is first passed through a "Nebulizer," † an atomizer plus a trap, allowing for the return of large droplets. The sample fog and oxygen are premixed with equimolar amounts of cyanogen and flow out of the burner through a small hole (diameter = 1.1 mm) in its top, where the flame is formed. Figure 1 is a diagrammatic sketch of the appearance of the cyanogen–oxygen flame. It is narrow and has a very bright, bluish white inner cone and a distinctly blue outer cone. The flow rate for both C_2N_2 and O_2 was about 22 ml per second.

A 1.5-meter Wadsworth mounting spectrograph ‡ with a 15,000-line-per-inch grating was employed for this work and Eastman Kodak type 103-O film was used. The slit width was 65 mμ. The plate factor was 10.8 A per mm in the first order. The arrangement of the camera in

* The cyanogen was obtained through the courtesy of the New Products Division of the Cyanamide Company, New York.

† Parke Davis Company.

‡ Jarrell-Ash Company, Newtonville, Mass.

conjunction with the sensitivity of the film confined selection of lines for measurement to the region between 2800 and 5200 A. Film was developed for 5 minutes in D-19 developer and fixed in hypo for 20 minutes. Densitometry was performed with an ARL microdensitometer; the slit width was 10 mμ.

Figure 2 shows one of the spectra obtained. A vertical section of the flame was sampled in such a fashion that both portions of the white inner cone and blue outer cone were photographed. The inner cone of the flame corresponds to the cyanogen-rich part of the photographed spectrum. The outer cone of the flame, on the other hand, corresponds to the cyanogen-poor, blue, outer part of the spectrum. Lines of aluminum, barium, calcium, cobalt, chromium, copper, iron, lead, magnesium, manganese, nickel, silver, and strontium are detected. The exposure time of 4 minutes in conjunction with the known flow rate of the sample corresponds to a total of less than 1 μg of each element introduced into the flame. The spectral lines extend through both zones and are of nearly uniform intensity throughout. Thus, interference by cyanogen band heads, a potential source of difficulty, can be eliminated by sampling radiation of the outer cone only.

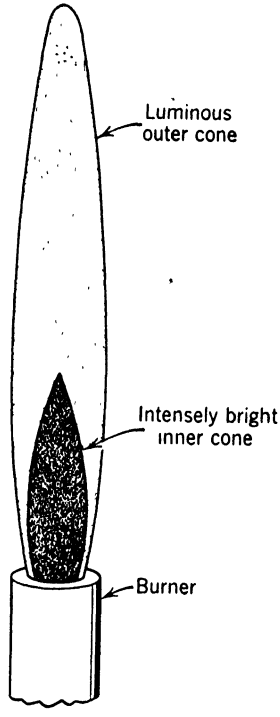

FIG. 1. Schematic diagram of the cyanogen–oxygen flame.

When aliquots of the solution used for this experiment were introduced into a hydrogen–oxygen flame under otherwise identical conditions, no spectral lines were observed. The cyanogen–oxygen flame thus permits sensitive spectrochemical determination of elements not readily excited by flames conventionally used.

Recent and unpublished experiments by Bartholomay and Vallee [2] have demonstrated that critical adjustments of the flow rate and the relative composition of cyanogen and oxygen, together with the rate of sample introduction, can further increase the analytical sensitivity. We are presently engaged in an evaluation of these parameters and a study of the analytical precision obtainable with this source, to be used in conjunction with the flame spectrometer to be described (see below).

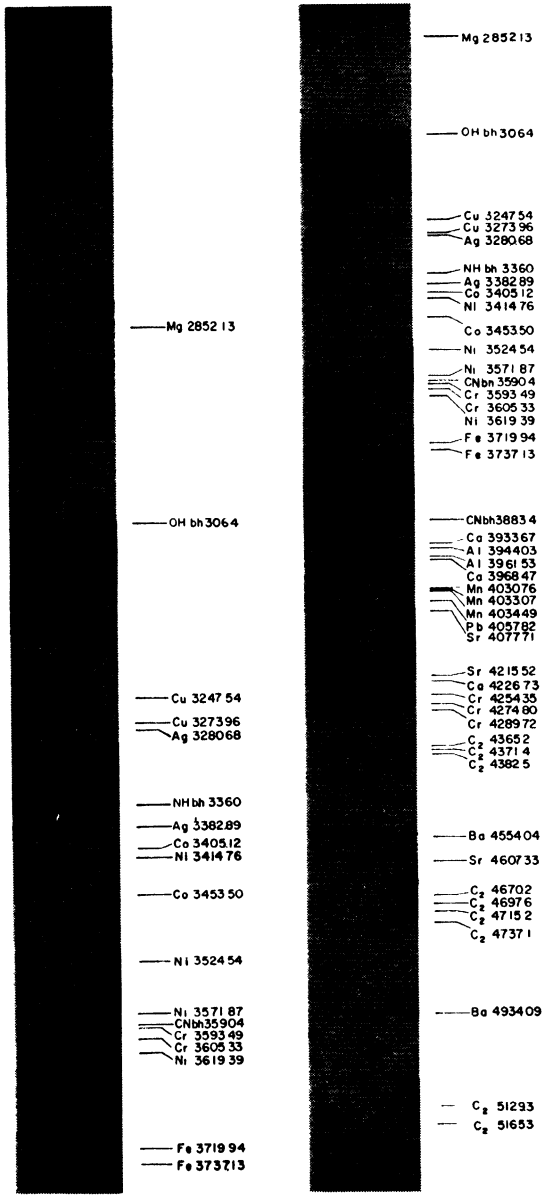

FIG. 2. Spectra obtained with the cyanogen flame source. The image of the flame was focused on the entrance slit of a stigmatic grating spectrograph. The bottom part of each spectrum corresponds to the lower part of the flame, and the top part of each spectrum corresponds to the upper part of the flame. Note that the emission lines of the elements persist in regions where the CN bands have disappeared. Thus, spectra *free* of CN bands can be obtained by proper diaphragming of the flame image. (From Baker and Vallee [1])

THE MULTICHANNEL FLAME SPECTROMETER

Principles. The instruments presently available for flame analysis are commonly and accurately described as *photometers.* They can be used only for the measurement of light at one wavelength at a time. The determination of different elements in a solution requires a separate set of adjustments of the prism or filter for each element to be determined. Thus, the prism is *employed as a monochromator* or else the filter isolates a given region of the spectrum. In addition, adjustments of the monochromator and in the electric circuit are necessary for the determination of each element, and separate samples for each are required.

Work in our laboratory has been directed toward the development of an instrument for the *simultaneous* determination of *several* elements at *several* wavelengths.[17,18] Since it utilizes the spectral distribution of radiation by the dispersing element, in this case the grating, to permit concurrent measurement at several wavelengths, we have chosen to call it a *flame spectrometer.* The principles underlying its design and use are referred to as *flame spectrometry,* as opposed to flame photometry performed at one wavelength.[19,28,30]

Such an instrument would be useful for general instrumental analysis for elements capable of being excited by flame sources. At present, and until the potential of the cyanogen–oxygen flame is fully realized, it is mainly applicable to the concurrent determination of the alkali metals and the alkaline earths. We have concerned ourselves especially with the simultaneous determination of sodium, potassium, calcium, magnesium, and strontium.[28] The *simultaneous* determination of these elements is capable of yielding more information, e.g., of possible physiological interactions between them than the *successive* assessment, and the use of successive samples may lead to errors due to a shifting base line. Moreover, calcium, magnesium, and strontium have been subject to study with some difficulty because of shortcomings of the existent techniques.[18] Limitations of sample size should be less problematic than they are found to be at times, since one sample can be employed for all five elements, and for more, if necessary.

It was intended to design an instrument with the following qualities:

1. Simultaneous detection of radiation emitted by different species.
2. High dispersion and resolution to minimize interference.
3. High sensitivity in the detection of radiation.
4. Simplicity and ease of maintenance.

The instrument now in existence in the laboratory achieves these

postulates. An oxyhydrogen flame is used as the source. A grating spectrograph provides high dispersion and resolution. Photomultipliers, chosen because of their marked response to low light levels, provide for sensitivities—in terms of limits of detection—to as low as 10 parts of sodium per billion parts of water. Five elements—sodium, potassium, calcium, magnesium, and strontium—are being determined simultaneously. Integrating circuits are not required, the sensitivity being adequate without them, and a considerable simplification of the electronics is consequently possible. Therefore, this instrument is more nearly "direct reading" than others so designated, since the elapse of time between the emission of light and its observance is minimized. The amplifiers are of the "plug-in" type to permit rapid interchanges when repairs are needed. Internal standardization is not employed; adequate precision is obtained without it.

The Multichannel Flame Spectrometer. The instrument has been described previously [28,30] and is shown in Fig. 3. Only a brief description will be given here. As presently constituted, it combines an oxy-

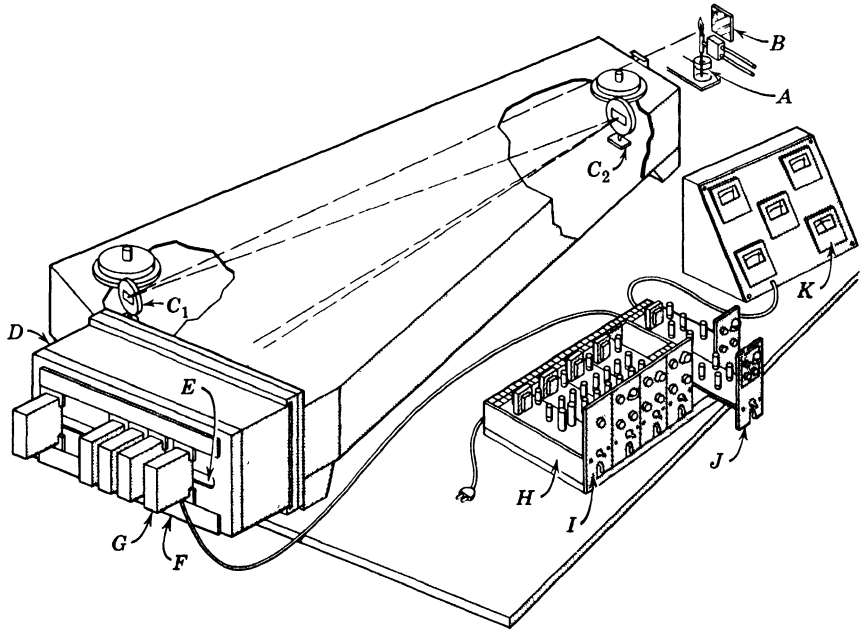

FIG. 3. Multichannel flame spectrometer. *A*, Beckman atomizer–burner; *B*, plane front-surface mirror; C_1, collimating mirror; C_2, grating; *D*, detector mount; *E*, aperture in detector mount; *F*, rails; *G*, detector chassis; *H*, bank of amplifiers; *I*, low-voltage power supply; *J*, high-voltage power supplies and amplifiers; *K*, microammeters. (From Vallee and Margoshes [19])

hydrogen burner (Beckman no. 4020) with a Wadsworth stigmatic mounting grating spectrograph (Jarrell-Ash). The grating has an effective surface of $3\frac{1}{2} \times 1\frac{1}{2}$ inches, 15,000 grooves to the inch, a radius of curvature of 1.5 meters and a reciprocal linear dispersion

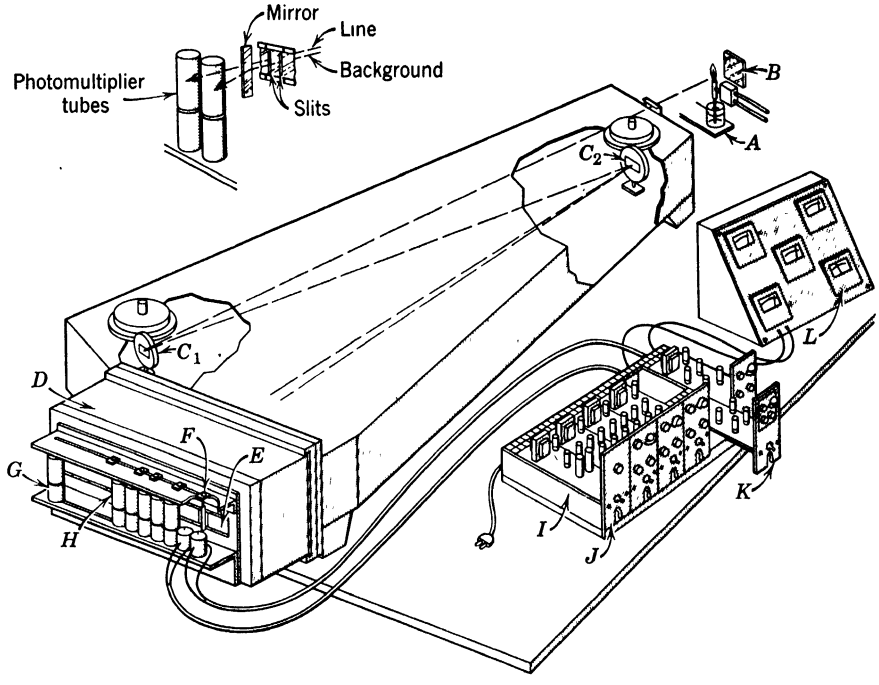

FIG. 4. Multichannel flame spectrometer with automatic background correction. *A*, Beckman atomizer–burner; *B*, plane front-surface mirror; C_1, collimating mirror; C_2, grating; *D*, detector mount; *E*, aperture in detector mount provided with exit slits; *F*, plane front-surface mirrors; *G*, photomultiplier mounts; *H*, photomultipliers; *I*, bank of amplifiers; *J*, low-voltage power supply; *K*, high-voltage power supplies and amplifiers; *L*, microammeters. Inset shows details of the exit slit–detector arrangement. (From Margoshes and Vallee [17])

of 10.8 A per mm in the first order. Five photomultiplier tubes provided with power supplies for low and high voltage and d-c amplifiers, are placed at the focal plane of the grating. IP-28 photomultiplier tubes (RCA) are placed on the magnesium oxide band at 3705 A, the calcium oxide band head at 5495 A, the sodium line at 5889 A, and the strontium line at 4607 A. One IP-22 photomultiplier tube (RCA) is placed on the potassium line at 7664 A. The entrance and exit slits are 0.4 mm wide. Microammeters, calibrated to read from 0 to 200 μa, serve at present as recorders for each channel. Control for

null and sensitivity adjustments are provided for. The amplifier incorporates an attenuator equipped with precision resistors, so that the sensitivity of the amplifier may be varied by a factor of 10 for each step of the attenuator switch. This device effectively expands the scale of the microammeter used to record the output signal.

In view of our previously reported [17,19,28] findings on the background due to extraneous cations in samples and their effect on analytical precision, it has been desirable to correct radiation received in any one channel for background. This correction can be performed manually.[17-19] It is, however, relatively simple to arrange matters in the instrument such that the background signal is automatically subtracted from the line-plus-background signal. For this purpose [17,18,20] the mounting for the photocells and exit slits have been modified so that each exit slit has two auxiliary slits, ¼ inch to either side, with a mirror mounted directly behind each auxiliary slit to reflect the light slightly to one side, as shown in Fig. 4. The light from one or both auxiliary slits is reflected into a photomultiplier mounted alongside the one being used to determine the line-plus-background intensity. The signals from the two photomultipliers are amplified separately, and the outputs from the amplifiers are connected so that the two signals are subtracted from one another. The difference between the two signals is the line intensity, corrected for the background. By use of this arrangement, 1 ppm of calcium can be determined in the presence of 1000 ppm of sodium. Under these conditions, the signal from calcium is 40 μa, the background is 360 μa, and the error in the background correction is 2 μa. This method of background correction has been incorporated permanently into the instrument (Fig. 4), is undergoing further tests, and is being extended to include all elements determined.

STUDIES ON THE EFFECT OF CATIONS

Effects of one cation on the emission intensity of another have been reported by various investigators.[4-6,8,31] Since the instrument described in this report is intended for samples in which the concentrations of sodium and potassium may be large compared to the concentrations of calcium, magnesium, and strontium, any interference caused by the alkali metals may be serious sources of error. The effect of large excesses of one cation on the emission intensity of another has been investigated by the direct observation of line and back-

ground intensities by means of readings taken at points to either side of the line or band as well as at their peaks.

Figure 5 shows a plot of emission intensities obtained between 5360 and 5635 A, including the CaO band head at 5495 A when solutions containing 1 and 10 ppm of calcium alone and in the presence of considerable excesses of sodium, potassium, and magnesium were

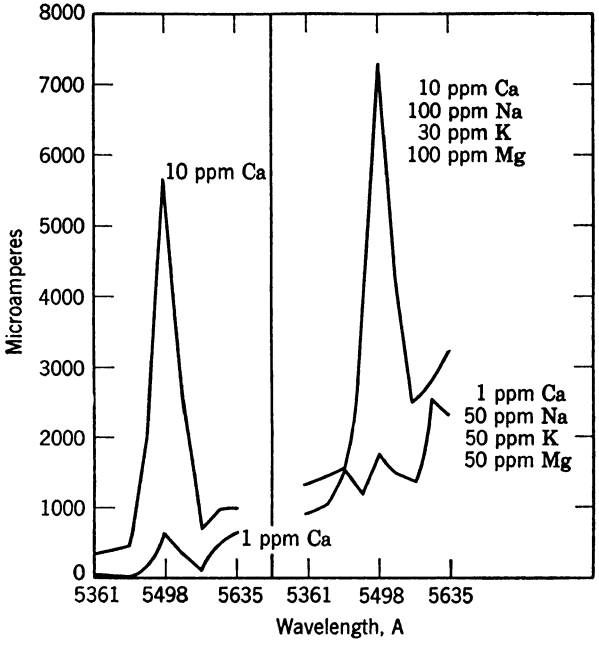

FIG. 5. Tracings of flame-emission intensities from 5361 A to 5635 A, including the CaO band head at 5498 A. Solutions containing 1 and 10 ppm of Ca alone and solutions containing the indicated concentrations of Na, K, and Mg—as chlorides—in addition to Ca were used. (From Vallee [28])

atomized in the burner. Emission intensity increases over the entire wavelength region when extraneous elements are present; this general increase in intensity of radiation is termed background. The intensity of the calcium oxide band head, superimposed on the background, is, however, not affected by the presence of the extraneous elements. As shown in Fig. 5 the relationship holds true even for 200-fold excesses of sodium or potassium (5 ppm of calcium plus 1000 ppm of sodium or potassium). Similar data have been obtained for magnesium in the presence of excesses of sodium, potassium, and calcium. In no case have changes in line intensity above background been found larger than the experimental error in the determination.

The background produced by any one element is proportional to the concentration of that element in the solution. Figure 6 shows readings obtained at the 5498-A calcium oxide band head when various concentrations of calcium, sodium, potassium, and magnesium are flushed through the burner. The top line is the analytical curve for calcium; the others show the contribution of each of the other ele-

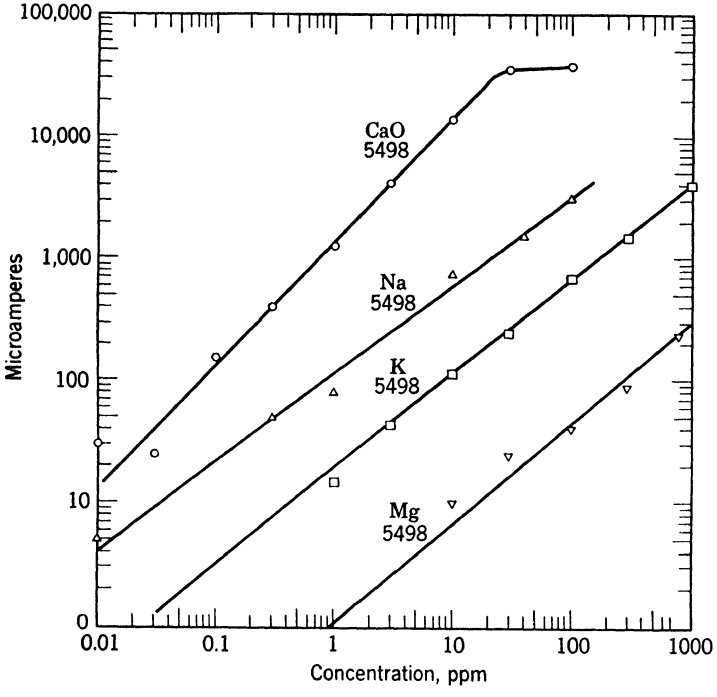

FIG. 6. Meter readings at the 5498-A CaO band head when solutions of Ca, Na, K, and Mg were atomized into the flame. (From Vallee [28])

ments to background at the calcium oxide band head. The analytical and background curves show similar proportionalities of meter readings to concentration. Such groups of curves may be obtained for each of the other channels. They are readily obtained by reading all of the channels when aspirating the calibration solutions.

The absence of effects of an excess of one cation on the emission intensity of another is of considerable significance for the analysis of biological samples. Normal urine and serum contain approximately 20 to 50 times more sodium than calcium, so that any effect of sodium on the emission intensity of calcium would lead to considerable difficulty in the determination of the latter in these fluids. It would be

necessary either to separate the calcium before analysis or to apply a correction factor for the effect of sodium, both procedures being cumbersome and leading to errors in analysis. Since these data illustrate that the effect of excess cations is due to the production of background, the problem is simplified materially. The concentrations of the excess cations can be measured, and their contributions to background can be estimated and subtracted from the meter reading. Automatic background correction permits determination of one cation in the presence of a considerable excess of one or several others.

STUDIES ON THE EFFECT OF ANIONS

Several workers have reported changes in light emission of an element measured and caused by the presence of similar concentrations of various anions.[3,11,14,21] Thus, for example, the emission of light

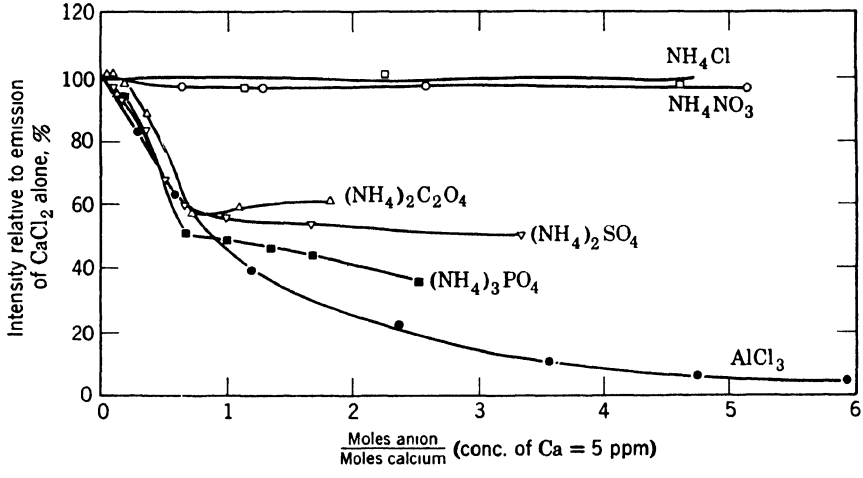

FIG. 7. Effects of added aluminum, oxalate, sulfate, phosphate, nitrate, and chloride on the emission intensity of the 5489-A CaO band head. (From Margoshes and Vallee[17])

by calcium has been found to be reduced by phosphate, sulfate, arsenate, and chromate,[3] as well as by aluminum,[21] presumably present as aluminate. All these anions are effective at concentrations comparable to the calcium concentration. The effect of various anions and of aluminum on the emission of light by potassium, magnesium, calcium, and strontium has been re-investigated in this laboratory.

Figure 7 shows the variation of calcium emission with increasing concentrations of aluminum, oxalate, sulfate, phosphate, nitrate, and

chloride. Phosphate, sulfate, oxalate, and aluminum, at concentrations below or approximately equal to the molar calcium concentration, sharply decrease the calcium emission. No further change in emission takes place with increasing anion concentration, once a certain critical concentration is exceeded.

Similar data have been obtained for strontium in the presence of aluminum, phosphate, oxalate, and sulfate,[19] and also for magnesium in the presence of phosphate and sulfate, though the lower level of light emission by magnesium, as compared to calcium and strontium, makes it difficult to work at sufficiently low concentrations to insure that no precipitation of insoluble salts occurs before atomization of the solution. The effects encountered with magnesium and strontium, however, are similar to those found with calcium. The effects of phosphate, nitrate, chloride, oxalate, sulfate, and aluminum on potassium have also been examined. None of these substances were found to alter the emission of potassium, even when their concentrations were 100 times the potassium concentrations.

The effect of various anions on the emission of light by the alkaline earths has been known for many years,[3,7,11,14,21] and several explanations for the effect have been given.[3,11,16] In certain cases, diatomic molecules are apparently formed in the flame, the concentration of such molecules following the mass-action law.[16] It is apparent that the curves in Fig. 7 do not follow this law. Another explanation [11,21] attributes the effect to cooling of the flame by the anions or compounds formed from the anions. No measurements of the flame temperatures have been published to substantiate this explanation, nor does it appear likely that such cooling effects are possible at the low concentration of anions employed. It has been proposed more recently [3] that compounds are formed in the flame in the presence of certain anions, which dissociate with difficulty, but the mechanism of formation of such compounds has not been described.

It would appear that the following events take place in rapid succession when the solution is sprayed into the flame:

1. The water, or other solvent, evaporates, leaving minute particles of the salt or mixtures of salts.
2. At the elevated temperature of the flame, decomposition or other chemical reactions may occur.
3. Salts, or their decomposition products, evaporate.

Only subsequent to these events can the metal atom be excited or form molecules such as CaO. If the salts in the sprays or their reaction

or decomposition products have extremely high boiling points, the particles may not evaporate completely during their passage through the flame, so that the atoms of the metal will not be available for and subject to excitation. The formation of such refractory compounds appears to be the cause of the anion interferences shown in Fig. 7.

This explanation may be illustrated by comparison of the melting points and, where known, the boiling points of the various calcium salts. Calcium chloride melts at a temperature of 772° C and boils at a temperature greater than 1600° C. Calcium nitrate melts at 561° C. Both these compounds give the same reading on the calcium channel when the solution contains the same calcium concentration, and neither excess chloride nor nitrate affect the light emission when present at concentrations within the range covered.

The presence of either phosphate or sulfate, however, causes a marked decrease in emission of light by calcium. Tertiary calcium orthophosphate melts at 1670° C; the other orthophosphate salts decompose at lower temperatures with the loss of water, forming calcium metaphosphate (m.p. 975° C) and calcium pyrophosphate (m.p. 1230° C). Calcium sulfate melts at 1450° C. All these compounds have high melting points. *Therefore, it seems that the anions causing a reduction in light emission of calcium form compounds with that metal that have comparatively high melting points and presumably high boiling points, so that calcium cannot be excited.*

Oxalate also depresses the light emission of calcium. This constitutes an example of suppression of radiation emission by formation of a *decomposition product* with a high boiling point. Calcium oxalate decomposes at the temperature of the flame with the loss of CO and CO_2, forming calcium oxide. The melting point of calcium oxide is 2572° C; the boiling point 2850° C.

Calcium oxide forms a series of compounds with aluminum oxide which may also be considered to be calcium aluminates, and which have high melting points. $CaAl_2O_4$ melts at 1660° C; $Ca_3Al_2O_6$ melts at 1535° C. It appears that these compounds with high melting points —and presumably high boiling points—are responsible for the effect of aluminum on the emission intensity of calcium.

With these considerations in mind, it is possible to understand the effects of these anions at such low concentrations. If their action depended upon collision processes, one would not expect to observe it at such low concentrations. With the mechanism described, however, the degree of dilution of the original solution is of little consequence, since evaporation of the solvent produces solid particles in any case.

ANALYTICAL RESULTS

Figure 8, a typical set of analytical curves for this instrument, shows the present limits of detection and the measurable range of concentrations. Although the exact lower limit of sensitivity depends on

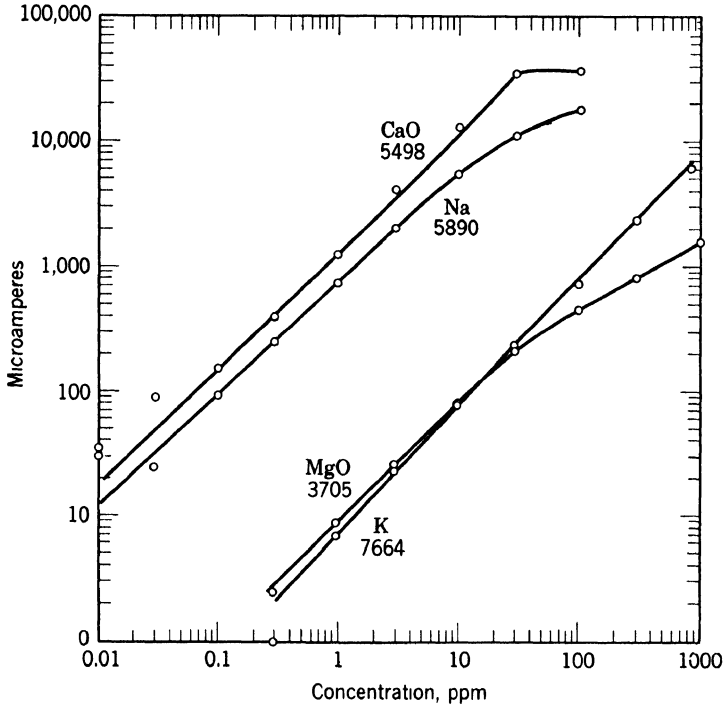

FIG. 8. Typical calibration curves for the multichannel flame spectrometer. (From Vallee [28])

such factors as the particular burner used, gas pressures employed, slit widths, quality of the grating, and the particular photomultipliers used (photomultipliers' sensitivities vary by a factor of three or more for different tubes), the lower limits of sensitivity for quantitative determination are normally 0.01 ppm for sodium, 0.1 ppm for calcium and strontium, 1 ppm for potassium, and 1 ppm for magnesium. One milliliter of solution is adequate for the determination of four or five elements; readings have been obtained with half of this volume of solution, and this volume could be reduced materially by readily feasible alterations in instrumentation, not undertaken so far for economic reasons.

The precision of analyses with the instrument is apparently determined in large measure by the errors involved in setting the amplifier balance controls and in reading the meters. If the amplifier balance can be set to ± 1 μa and a similar error is involved in reading the meter, then the total probable error in making a reading is 0 to 2 μa. Two solutions, one containing 3 ppm of sodium as NaCl, the other containing 3 ppm of sodium, 3 ppm of calcium, and 10 ppm of potassium, all as the chlorides, were used to test repeatability. The standard deviations of the microammeter readings varied from 1 to 3 μa in all cases, as would be expected if the errors in setting the amplifier balance and reading the meter are the controlling factors in determining the precision.

The average ammeter readings for the same element in the same solution agreed within 1% on different days when the experiment was repeated. It appears that it is not necessary to run frequent calibration curves for this instrument, provided that no changes are made in the instrumental variables such as burner position, gas pressure, slit widths, dynode voltages.

Standard deviation on replicates in terms of percentages are in the range of 1 to 2%. Similar precision may be expected routinely, provided that the meter readings are not too small. If the signal read on the meter is 10 μa, an error of only 1 μa in reading the meter would be a 10% error. This limitation of precision is, of course, not peculiar to this particular instrument, but is common to all instruments presenting the data in the form of a meter reading.

The range of concentrations covered by this instrument on a single calibration curve is much greater than is usual for a flame photometer. This may be ascribed to a combination of several factors:

1. The wide range of intensity response of photomultiplier tubes.
2. Use of precision resistors in the attenuator, making it possible to compare readings on one sensitivity range with readings made on another sensitivity range.
3. The linearity of the amplifier—a change of the input signal by means of the attenuator does not alter the amplification factor.

Because of the wide range of concentrations covered, it is possible to perform analyses of unknown solutions in less time than is possible with other flame instruments. The correct dilution factor is found with less trouble than with instruments having narrower concentration ranges when concentrated solutions are employed.

The "short-term" (hour-to-hour) precision of the instrument (1 to 2%) is about the same as for other instruments using the flame

source. Some of the variability in the readings presumably is due to noise generated either in the flame or in the photomultipliers, or both. Inclusion of a time constant of one or two seconds should reduce the effect of this noise considerably, increasing the precision of the instrument. Such a time constant has not yet been added to the instrument because it might conceal certain important observations of the characteristics of the instrument during its period of development.

The "long-term" (day-to-day) stability of the instrument appears to be exceptional, particularly for one in which d-c amplifiers are employed. Some difficulty has been found in setting the gas pressure to the same values each time the flame is turned on. This factor seems to be a most important one in determining whether or not the same calibration curve can be used from day to day. The flame conditions can be reset most exactly by using the meter reading obtained with one of the standard solutions as an indicator, adjusting the pressure to give the same reading each time.

This long-term stability of the instrument is especially valuable for analyses of samples in small groups. Calibration for each group of samples is not necessary, with a resultant saving of time.

The marked analytical sensitivity of the instrument is an additional important advantage. The effect of such biological materials as urea and protein on the readings obtained may be minimized by dilution of the sample. With this instrument, it is possible to determine sodium and potassium on a sample of normal urine diluted as much as 1000-fold. With such a high dilution factor, it is unlikely that the organic substances in the sample will have any noticeable effect on the results.

Flame spectrometry, it appears, represents a considerable improvement over previous methods of analysis with a flame source. Previous direct-reading instruments, flame photometers, could be used for the determination of only one element at a time, and several were restricted to the determination of sodium and potassium. The usefulness of the present instrument, now employed for the simultaneous determination of five elements, can further be extended with additional detectors and amplifiers. The cyanogen–oxygen flame, moreover, would permit the direct-reading analysis of many elements excited by this flame.

Precision obtained with this instrument is of the same order of magnitude as, and sensitivity is better than, is possible with flame photometers. The long-term stability is superior to that of previous instruments. The use of a grating monochromator provides better

spectral isolation of the analytical lines than is possible with a filter or with the small-prism monochromators commonly used in flame photometers. This superior spectral isolation reduces the effect of one cation on the intensity readings obtained at the analytical wavelength of another, making possible the determination of a given element in the presence of a considerable excess of other elements.

Any instrument should be simple to operate, relatively free from breakdowns, and easy to repair to be useful for routine work. Operation of the instrument described can be acquired in a relatively short period of time. The readily replaceable "plug-in" amplifiers make the instrument particularly suitable for persons without training or experience in the use of complex instruments. If there is a breakdown, the defective unit may be quickly removed and replaced by another without the necessity of keeping the instrument out of operation while repairs are being made. The burner may also be replaced quickly, if necessary. The optical section of the instrument should require no attention while in normal use.

The work here described has served to demonstrate the advantages of a grating monochromator for the simultaneous determination of several elements using the flame as a source, and has resulted in the development of an instrument of considerable utility, both actual and potential.

TRENDS IN FLAME PHOTOMETRY AND SPECTROMETRY

Predictions of the direction or speed of development of any field are hazardous at best. Some trends, however, are apparent.

The inability of flames now available to excite more than just a few elements effectively, enough to permit quantitative work in the parts-per-million range of concentration, is one of the severe present limitations of flame photometry. Flames of higher temperature will make possible the determination of many more elements at low concentrations. The lessening of interference from various anions in the sample is another advantage to be expected from hotter flames. Phosphate and sulfate, for example, suppress the emission of alkaline earths by formation of compounds which do not evaporate in the flame. If the flame temperature can be increased sufficiently, even the most refractory salts can be brought to their boiling points.

The use of better monochromators may be expected in the future. Filter monochromators and instruments of low dispersion are reasonably satisfactory for the determination of sodium and potassium in urine or serum, though even in such applications better mono-

chromators are preferable. For the determination of other elements, particularly when present as minor constituents, monochromators of high dispersion are essential. Monochromators of higher dispersion than are currently used in flame instruments would seem to solve the problem. Excellent plane replica gratings are now available at prices much lower than that of original ones, making use of gratings economically feasible.

The high sensitivity of photomultiplier detectors to low light levels should bring about their increased use in flame photometry and spectrometry. The greater sensitivity thus achieved permits the determination of smaller quantities of material and will also reduce errors caused by the presence of organic material in the sample. In the extreme red end of the spectrum, where the sensitivity of photomultipliers is rather poor, lead sulfide cells may be used until appropriate photomultipliers for this region are available at reasonable cost. The sensitive surfaces of lead sulfide cells may be made into almost any shape. They can therefore be fashioned into the form of spectral lines at the focal plane, eliminating exit slits.

Flame spectrometers permitting the simultaneous determination of ten or more elements may be expected. Some of the more recent developments in direct-reading spectroscopy may also be used with flame sources.

With such instrumental improvements, the stable characteristics of the flame can be fully utilized. Some instruments, now available, are permanently calibrated but at the expense of less than maximal sensitivity. Increased stability of detectors and amplifiers will allow the development of instruments giving nearly identical responses to the same light levels at all times, permitting the use of precalibrated meters while operating at maximum sensitivity.

With instruments thus improved, the flame may again become the most desirable source for emission spectroscopy.

DISCUSSION

Discussion Leader
V. A. Fassel
Iowa State College

Before discussing the technical aspects of Dr. Vallee's chapter, I should like to contribute another version of the earliest application of a spectrum. In GENESIS 9:14–16, God advised Noah: "When I bring

clouds over the earth and the bow is seen in the clouds, I will remember my covenant which is between me and you and every living creature of all flesh; and the waters shall never become a flood to destroy all flesh." This would appear to be a most practical application of a spectrum! Aside from the spectroscopic implications of the bow (rainbow) in the clouds, it has also been said that Noah had trouble with his arc (ark).

To an emission spectroscopist the results discussed by Dr. Vallee are very gratifying. Dr. Vallee and his associates recognized that "flame spectrometry" is really a form of emission spectroscopy and that the problems ought to be studied from a spectroscopic standpoint. The results that are being obtained by using this approach are rapidly bringing order out of chaos. This point can be illustrated by reviewing a bit of history. The technique of flame photometry "caught fire" in the United States when Barnes and his associates announced the very simple filter-type flame photometer for the determination of sodium and potassium. This simple instrument satisfied a great demand, and many thousands of rapid, accurate sodium and potassium determinations have been made and will continue to be made with simple, low-priced instruments of this type.

Subsequent to the development of these simple flame photometers, small, rather low-dispersion prism monochromators or spectrophotometers were adapted to observing the flame spectra. These instruments extended the scope of application of flame spectrometry to a larger group of elements. They have demonstrated their utility, and their usefulness in an analytical laboratory should not be underestimated.

Unfortunately, investigators from scientific disciplines other than spectroscopy, physics, or physical chemistry started playing with fire —both literally and figuratively. They took these instruments with their simple dials and meters and tried to extract from them more information than they were designed to furnish. This led to the so-called discovery of various anion and cation interference and interaction effects which tended to cloud considerably the potentials of this technique. For example, it was reported by some investigators that sodium determined in the presence of potassium would give high results, whereas others reported that low results would be obtained, and still others found no effect. In reality, as Dr. Vallee has just shown, many of these interferences disappear if one makes a simple correction for heterochromatic background contributed by the other ions present in the sample. Analytical emission spectroscopists have made these corrections for the past 20 years. It therefore does not seem inappropriate to suggest that instruments providing adequate

resolution and spectral purity be used when "interferences" are studied.

Before the advent of the CN flame, the investigators using flame excitation resigned themselves to being able to detect only about 40 elements. Moreover, the sensitivity of detecting many of these elements was not adequate for trace analyses. In a flame the ability to excite atomic systems to the degree required for high sensitivity of detection rests on one physical parameter—the excitation temperature. The ability to populate excited states increases exponentially with temperature. The CN flame goes a long way in reaching the excitation temperatures attained in arc or spark discharges, but the level attained in electrical sources is still several thousand degrees higher.

One should not lose sight of the fact that, as flame temperatures approach or equal those obtained in electrical discharges, the spectra will be equally as complex. The simple spectra as emitted by our oxyhydrogen or oxyacetylene burners will be lost, and large spectrographs will be required. Where a $1500 spectrometer provides adequate dispersion for an oxyhydrogen flame, a $9000 spectrograph may be required for a 6000° K flame.

One of the stated purposes of this symposium is to consider which of our analytical techniques are best suited for the determination of trace constituents in various fields of investigation. It is therefore appropriate to inquire when flame spectroscopy should be used in favor of conventional spectrographic analysis. A very important factor to consider is the large difference in facilities required for the two techniques. To be able to excite the spectra of all of the elements and to be able to observe all of the emitted lines in a wavelength region simultaneously under high resolution conditions greatly increases the cost of the instruments. There is not much logic in using a $25,000 spectrographic installation when a $3000 flame spectrometer does just as well. If the elements to be determined emit spectra sufficiently specific and with adequate sensitivity, and if the sample is readily brought into solution, the use of flame-spectrometric technique is certainly indicated. The determination of the alkali and alkaline-earth group elements plus perhaps a dozen other elements falls in this category, provided spectra of the matrices in which these elements are to be determined are not too complex.

For many elements, flame excitation does not provide adequate sensitivity for detecting the minute quantities which have biological significance. This point can be illustrated by comparing the absolute

detection sensitivities of various excitation techniques. Sensitivity comparisons are always somewhat hazardous, because the sensitivity values depend on specific operating conditions and on the criteria used in evaluating when the element signal has disappeared. Although these uncertainties impose some limitations on quantitative interpretations, the differences in sensitivity are so great that significant conclusions can be drawn. Because sample requirements vary for different excitation technique, it is appropriate to make these sensitivity comparisons on an absolute basis. The data in Table DI summarize the sample requirements for 5 different excitation techniques. Thus, a reported sensitivity of 1 μg means that this amount of the element is present in 2 ml of solution if excited by a flame, or in 5 mg of a solid sample if cathode-layer excitation is used in a d-c arc. The data summarized in Table DII show that the sensitivities attained in electrical discharges may exceed those attained in a flame by a factor of 1000. The sensitivity gap between flame and electrical sources for many elements may be expected to be decreased considerably when CN flames are used.

TABLE DI

SAMPLE REQUIREMENTS FOR DIFFERENT EXCITATION TECHNIQUES

Method of Excitation	Sample Requirements
Flame (Lundegardh)	2 ml solution
Flame (Beckman)	2 ml solution
Spark (porous cup)	1 ml solution
D-c arc (cathode excitation)	5 mg solids
D-c arc (anode excitation)	50 mg solids

TABLE DII

Excitation Source	Absolute Sensitivities, μg						
	Co	Zn	Be	Mn	B	Cu	Mo
Flame (Lundegardh)	2–20	6000	——	0.6	——	1	——
Flame (Beckman)	2	400	50	0.02	2	0.02	6
Spark (porous cup)	2	25	0.02	0.6	0.5	0.6	——
D-c arc (cathode)	0.01	0.5–1.5	0.01–0.05	0.05–0.3	0.05	——	0.05–0.005
D-c arc (anode)	0.1–0.3	0.3–5	0.1	0.02	0.05	0.005–0.02	0.3

It should be noted that the sensitivities of detection for the alkali and alkaline-earth elements in flames and electrical sources are probably comparable. It is very difficult to determine absolute sensitivities because of blank or residual impurities. The sensitivities of detection for the alkalies and alkaline earths in flame should be adequate for most purposes.

QUESTIONS AND ANSWERS

BERT L. VALLEE: I believe that the sensitivities of detection for the alkali metals and alkaline earths are actually *higher* in the hydrogen–oxygen flame than in the arc. It is impossible to predict the ultimate sensitivities that will be available with the cyanogen–oxygen flame, but I am inclined to believe that they will compare favorably with the d-c arc for many elements.

V. A. FASSEL: For elements that have low excitation potentials this situation will probably hold. But for those elements having higher excitation potentials my original statement still applies.

A. GOLDBLATT: Would Dr. Vallee elaborate on the circuitry of the flame spectrometer and compare it with other direct-reading spectrometers?

BERT L. VALLEE: These will be reported in several articles[18,19,30] to appear shortly in *Analytical Chemistry*.

W. D. COOKE: What is the general spectral characteristic of the spectrometer that you used in these studies?

BERT L. VALLEE: We employed a 1.5-meter Wadsworth spectrograph of the Jarrell-Ash Company. The plate factor was 10.8 A per mm in the first order.

W. D. COOKE: Would you estimate the relative matrix effect in an arc as compared to a flame? Consider a spectrographic analysis of a solid sample compared to the same sample put into solution and run in a flame. Would the matrix effect be less critical in the flame?

V. A. FASSEL: I see no reason why the flame should be less subject to extraneous element effects than electrical excitation sources. One cannot vary the identity and concentration of other elements in a sample without influencing the intensities of analysis lines, even though the concentration of the analysis elements remains constant. For example, the magnesium line intensities arising from 0.1% magnesium in pure copper and in brass would be expected to be different.

BERT L. VALLEE: Though such extraneous element effects do occur in flames as well as in arcs, I believe, on the basis of our studies so far, that it will be possible to control them more readily in the flame.

W. D. COOKE: What is the source of background radiation? Is it scattered light?

BERT L. VALLEE: No. We believe the background observed is a consequence of the molecular events when a solution is sprayed into a flame. First, there is dehydration and formation of crystals which must be vaporized before excitation of the atoms may take place. If these crystals have a high boiling point, vaporization does not occur, and a proportion of this material

will then serve as a source of "black-body" radiation, accounting for the observed background. Although we have no direct proof of this hypothesis, our data are best explained on this basis.

JAMES J. LINGANE: I should like to ask Dr. Vallee where he gets the cyanogen. Is there a commercial source?

BERT L. VALLEE: We are indebted to the American Cyanamid Company, New Products Division, New York.

JAMES J. LINGANE: Thank you very much.

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Potentiometry

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ABSTRACT Potentiometry has been applied in two major ways to the determination of minute amounts of substances: (*a*) by single electromotive force measurements after prior calibration of the process with known mixtures, and (*b*) by titration techniques.

In the titration field the potentiometric method lends itself well to the titration of moderate concentrations of substances in microvolumes of solvents. The titration of traces of substances at extreme dilutions, as for example from submicrogram amounts to a few micrograms in a macrovolume such as 5 ml, encounters as primary problems adsorption on the surface of the vessel and reaction of the titrant with the indicating electrode surface. It is only in situations where the equilibrium constants are favorable for a "complete" reaction and the kinetics of the titration reaction are favorable, that meaningful results may be obtained in the low-microgram range or even in the submicrogram range. A refinement of the compensation method of Erich Müller has proved to be very useful. The end point is taken as return to a preset equivalence-point potential upon adding reagent.

A brief review of typical specific applications to titrations in various fields of volumetric analysis are given.

INTRODUCTION

A potentiometric measurement, strictly defined, is one in which at the point of balance no current of electricity flows into or out of the cell that is measured; that is, the potential of the cell is compensated by an equal and opposite electromotive force that is measured. It seems probable, in the light of experience, that a slight input or drain of electric current near the point of balance may help to stabilize the electrode interfaces that give rise to the potentials. Subsequent to the advent of vacuum-tube voltmeters and *p*H meters with sensitive electronic circuits, it is probably that potentiometric measurements are more frequently made with such devices than by the classical potentiometric compensation method in many types of electrochemical work. Potentiometric measurements commonly include techniques that result in observations with currents of 10^{-6} down to 10^{-13} or 10^{-14} ampere flowing through the indicating cell and the vacuum-tube voltmeter.

In this chapter, major emphasis will be given to methods that rest on the classical compensation method, but it will be necessary to treat also certain methods in which a current of 2 μa or less is purposely passed through the pair of electrodes that are being used as indicators in a titration.

Two major techniques that have been developed through the classical compensation method are: (1) the concentration-cell method, and (2) potentiometric titration. These methods will be discussed before other techniques.

THE CONCENTRATION-CELL TECHNIQUE

A cell is constructed with two electrodes that are as nearly identical in properties as possible. Provision is made to keep the solutions in the two electrodes separate until preliminary adjustments have been made, including temperature equalization in a thermostat. A typical cell is shown in Fig. 1. Equal portions of the solution to be tested are brought to the same volume after addition of reagents such as acids and salts. To the "known" side of the cell is added a known amount of the substance that is being determined. These two solutions are placed around the electrodes and drawn into contact after temperature equilibrium is established. From the observed emf and

prior calibrations with pure salts and known additions, it is possible to calculate the concentration of the ion that produces the difference in potential. The essential condition for success in applying this method is that the ionic strengths be almost identical in the two halves of the cell and rather large compared with the contribution of the trace substance that is being estimated.

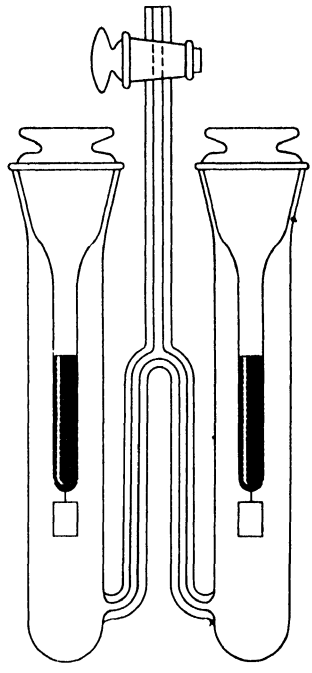


FIG. 1. Cell for emf measurement: stopper to base 17 cm; width 8 cm; base to capillary junction 8 cm; inner diameter of electrode vessels 1.5 cm. (From Furman and Low [1])

An illustration of the technique as applied to an equilibrium of a soluble redox system is the following: The two electrodes are of platinum, and the soluble redox system is the ferrous–ferric sulfate pair in dilute sulfuric acid. This system may be used for estimating traces of substances that either complex ferric ion or disturb the ferrous–ferric equilibrium by processes of reduction or oxidation. If the unknown mixture and suitable amounts of salt and acid plus ferric–ferrous mixture made up to a definite volume are placed in one half-cell and a like amount of ferrous–ferric mixture, salts, and acid in the other half-cell, then at 25° C the magnitudes of the reduction potentials of the two half-cells are, respectively,

$$E_1 = E^\circ - 0.0591 \log \frac{a'_{Fe^{+2}}}{a''_{Fe^{+3}}}$$

and

$$E_2 = E^\circ - 0.0591 \log \frac{a'''_{Fe^{+2}}}{a''''_{Fe^{+3}}}$$

where a', a'', a''', and a'''' are the respective ferrous and ferric ion activities in the two half-cells. It is apparent that, if the process of complexation, reduction, etc. has changed the ratio of ferric to ferrous ion on one side by a tenfold value, the cell will read 0.0591 volt. For a particular application it is necessary to choose one of the iron concentrations very carefully; for example, the ferric concentration is

critical if the process is one of complexing or reducing this ion. The concentration of the other ion, in this case ferrous, is not as critical.

An empirical calibration with known mixtures must be set up for a particular method. The method is most suitable for determining minor concentrations of a reactant in a background of high and constant ionic strength of salts and acid. Low and Pryde [2] developed this method for the estimation of fluoride in various substances. Their technique gave good results for relatively large amounts of fluoride and also was distinctly promising in the range of 1 to 5 μg of fluoride in 5 ml of solution. Obvious interferences are ions such as aluminum that complex fluoride very strongly and the presence of oxidants or reductants that alter the ferric–ferrous ratio. This method was found by investigators on the Manhattan Project [3] to be capable of determining submicrogram quantities of fluoride in 10 to 20 μl of solution with a sensitivity of 0.001 μg of fluoride. The liquid was in an air-conditioned space of controlled humidity. The change of the indicator wire relative to a reference half-cell was used rather than a concentration cell.

An application of a concentration-cell principle that is of biochemical interest depends on the reducing action of glucose upon a potassium ferricyanide–ferrocyanide mixture at controlled ionic strength.[4] The process has been used to study fundamentals of the kinetics of the process, and it is known to be adaptable to the estimation of glucose under various conditions.

If conditions are such that the only suitable indicating electrode is of the second class, that is, metal–insoluble salt such as the silver–silver chloride electrode, the calculation of the results from the emf readings is more involved, but the technique is suitable for trace testing. Furman and Low [5] developed this method for the estimation of traces of chloride in various types of nitrates. If the solution contained chloride at a concentration of x moles per liter at the salt concentration used in the test, the other half of the cell consisted of a like amount of the nitrate and reagents plus a known addition of chloride, e.g., 0.01 M, then the final effective concentrations of chloride in the two halves of the cell will be

"Unknown" side of cell
x M/liter from test subst.
y, concentration due to dissolved silver chloride

and

"Known" side of cell
x M/liter from test subst.
y', concentration due to dissolved silver chloride
0.01 M added chloride

where y and y' are substituted by expressions involving the solubility product of silver chloride, an activity coefficient, and the added chloride, 0.01 *M*. If second-order terms are neglected, the expression relating E_{obs} for the concentration cell, and x is

$$E_{obs} = 0.591 \log \frac{2(x + 0.01)}{x + (x^2 + 4S_0/f^2)^{1/2}} \quad \text{at } 25^\circ \text{ C}$$

where S_0 is the solubility product for silver chloride in pure water and the square of the activity coefficient at the ionic strength is f^2.

Significant results, accurate to 10%, were attained when the chloride concentration was 0.4 μg per ml, and at chloride concentrations 10- to 100-fold greater the accuracy was from 1% at the lower concentrations to a few tenths of a per cent at the 100-fold range. The concentrations of the nitrates were from 10^4 to 10^6 times as great as the chloride concentration.

Concentration-cell technique was shown by Johnson and Low [6] to be applicable to the precise adjustment of the silver and halide equivalence in the chemical method for estimating atomic weights.

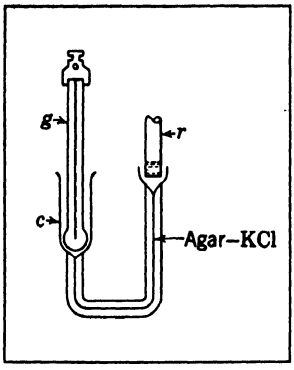
Blaedel et al.[7] applied essentially the same technique as was used for the estimation of chloride in nitrates to the problem of the routine estimation of minor amounts of chloride in sulfuric acid and sulfate solutions. Their precision and accuracy were comparable to those obtained in determining chloride in nitrates. They developed nomograms to simplify the calculation of the amounts of chloride from the emf readings.

The concentration-cell principle as applied to minor concentrations of substances in the electrode is illustrated by the fact that Hulett [8] found that 1 part per 100,000 of a more electromotively active metal in mercury could be estimated by making the suspected metal one electrode and pure mercury the other in a H-shaped cell, with a suitable conducting solution.

A number of other concentration-cell applications that have not been tested in the trace area are cited in accessible review papers.[9]

POTENTIOMETRIC TITRATION

For the past 30 years or more, there have been numerous papers on potentiometric titrations using conventional microburets capable of being read to the nearest 0.001 ml. The quantities of substances determined have usually been in the range from 0.2 to 2.0 mg dis-



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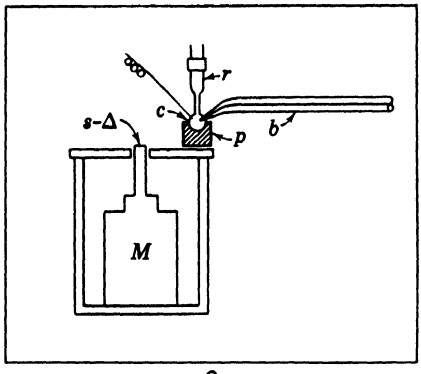

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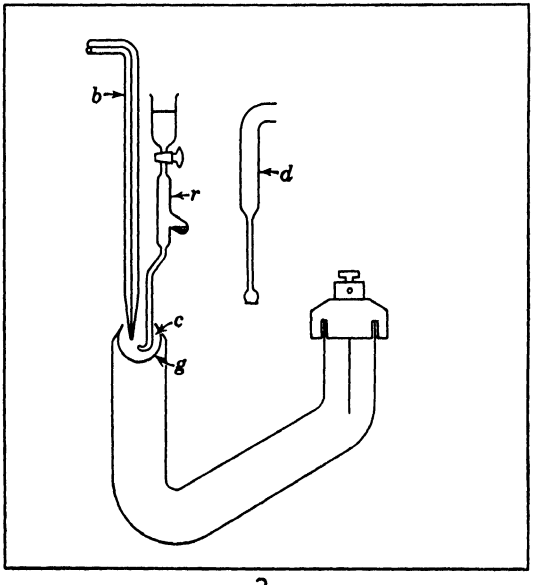

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FIG. 2. Apparatus for potentiometric microtitrations. (1) Apparatus of Catch et al. The glass microelectrode *g* makes contact with 0.1 to 0.2 ml of solution containing 2 to 5 mg of acid or base. *r* is the tip of a reference electrode. A conventional microburet is used. (2) Apparatus of Zürcher and Hoepe for agitating a single drop of solution. The shaft of the motor *M* has a triangular cross section and produces vibration by striking the metal frame. *p* is a paraffin block, *c* represents the solution, *r* is the tip of a reference electrode, and *b* is a buret tip. (3) Apparatus of Ingold for titration of acids. *g* is re-entrant glass electrode, *c* titration cell, *r* reference electrode. *d* is a tube for removing solutions and rinsings from the fragile cell. (From Furman [10])

solved in volumes from 0.05 to 5 or 10 ml of an appropriate solvent. For the smaller amounts of material the over-all accuracy has been generally between 1 and 5% of the substance determined. Some typical equipment is shown in Fig. 2.

A few typical papers are given in the accompanying table. In

Substance Titrated	Volume Titrated, ml	References
Organic acids	0.1–0.2	Catch, Cook and Kitchener, *J. Chem. Soc.*, 319 (1945).
Organic acids	1.0	Ingold, *Helv. Chim. Acta*, *29*, 1929 (1946).
Chloride	0.25	Schwarz and Schlösser, *Mikrochemie*, *13*, 18 (1933).
		Northrop, *J. Gen. Physiol.*, *31*, 213 (1948).
Silver	0.05	Zürcher and Hoepe, *Helv. Chim. Acta*, *21*, 1272 (1938).
Halides, acids, oxidants, reductants	0.05	Schwarz, *Mikrochemie*, *13*, 6 (1933).

Reference to other papers and reviews is given by Furman.[11]

general, the accuracy attained in titrating 100 μg of a substance in 4 to 5 ml of solution is of the order of 1 to 5%. The exact limit depends on the essential constants of the process such as solubility products, redox equilibria. The experimental uncertainties of buret readings and potentiometric determination of end points probably accounts for most of the error.

In a series of studies of the "coulometric titration" method, Furman and associates[12,13] have had occasion to inquire into the precision and accuracy of processes in which the reagent is generated electrolytically from a background medium of high ionic strength relative to the trace substance. There is no difficulty in generating reagents electrolytically with much greater precision than is possible in the addition of a reagent with any known form of microburet. With many methods of indication, fluctuations occur in the detection of the end point that are of the order of a substantial fraction of a microgram or more when the volume is 5 to 10 ml. In reviewing the potentiometric art, we have come to the conclusion[12] that the most sensitive method is that of opposing an emf equal and opposite to that preset as a suitable end point in the supporting solution to which the samples are added. Near the end point, the circuit may be left closed unless there is a considerable lag in the reaction. The current approaches zero linearly on a sensitive galvanometer as the equivalence point is

reached with decrease in amount of reactant and departs linearly, but with a different slope, from zero after the point is passed. The actual technique is thus amperometric if the circuit is closed, or potentiometric if the indicating circuit is opened and closed intermittently. With a galvanometer of high sensitivity there is ample sensitiveness to measure the change produced by much less than a thousandth of a microgram of reactant. This method will be referred to as the "sensitive end point." We believe that the accuracy of determinations made in this manner is limited primarily by such factors as incompleteness of reactions, unfavorable kinetic factors, and the amount of reagent or reactant necessary to form and remove electrode layers such as oxide layers on platinum or gold electrodes. The equilibria involved in removing adsorbed layers or in forming or removing these oxide layers seem to be promoted by allowing the indicating electrode to take up or deliver a minute amount of current near the equivalence point.

Using the "sensitive end point," it has been found possible, when a reversible process such as the ferrous–ferric equilibrium is involved, to determine certain materials with an error of 0.001 μg or slightly less. An example of the most favorable conditions is the following: A ferric solution is adjusted electrolytically to a solution potential slightly below that of the equivalence-point potential of the reaction of ferrous iron with an oxidant such as potassium permanganate. Now, if carefully weighed amounts of a very dilute permanganate solution are added, and the initial potential of the indicating system is restored by generating ferrous ion, it is found possible to get significant readings where the amount of oxidant ranges from 0.01 down to 0.003 μg in 5 to 10 ml. The average error was about 10%. This is the most favorable type of process thus far investigated.

It was shown that 100 μg of ferrous iron could be titrated with electrolytically generated ceric ion with an average accuracy of 3 parts per thousand.[12]

Theoretically an amperometric indication such as that proposed by Sease, Niemann, and Swift,[14] and used effectively by Swift and associates in an extensive series of subsequent papers on coulometric titration, may be used at fairly high currents and with less sensitive instruments, provided the changes produced at the indicating electrodes are opposite and equivalent.

In applying the "sensitive end point," it is important to set the potential of the background solution to a value close to but not necessarily at the theoretical equivalence point. The substance to be

titrated is then added, and the titration is carried to the restoration of the initial indicator potential. The "sensitive potentiometric end point" and the amperometric end point appear to be the best of the electrical indicators in the low-microgram and submicrogram ranges.

OTHER POTENTIOMETRIC TECHNIQUES

Potentiometry at Constant Current. A closer inquiry has been made into the interrelations of the end-point phenomena in various types of electrotitrations by Charlot and others,[15] by Reilley, Cooke, and Furman,[16] and by Kolthoff.[17] New methods of end-point detection have been based on the intentional passage of a current from a fraction of a microampere to about 2 μa through the indicating system. The indicating system may consist of two similar electrodes or of an indicator electrode and a reference electrode. The voltage between the electrodes is read on a suitable instrument, preferably a vacuum-tube voltmeter or a *p*H meter. An insight into the possibilities of the method in any given situation is gained by running polarization curves on the mixture in question at intervals during a titration.

This technique should be called potentiometric titrations at controlled-current input. With similar electrodes and reversible redox systems before and after the end point, the voltage shows a differential form rising to a sharp maximum at the end point. For this reason we have referred to it as a derivative polarographic titration.[18] Before the end point, if both oxidized and reduced form of the reactant are present, it will take only a small difference of potential to force 1 μa, for example, through the indicator electrodes. At the end point, if no redox system is present that is reversible at both electrodes, it will in general require a fairly high potential to force the same current through the indicator system. After the end point, if both reagent and its product form a reversible system, it will again require a very small voltage to force 1 μa through the indicator system.

Methods of this type may give a better indication than the classical potentiometric method if the polarization relationships are favorable.

Duychaerts,[19] Gauguin et al.,[20] and Adams[21] have studied the method as applied to an indicator and a reference electrode. It can happen that, with this electrode polarized either positively or negatively relative to the reference half-cell, the voltage will increase or fall more for a given addition of reagent than with potentiometric

indication. The reason for this is that the indicator electrode must move to a point where it finds a process that will enable it to allow the indicator current to pass. Adams [21] demonstrated that the voltage break at the end point in the titration of 10^{-4} *N* arsenite with 0.015 *N* potassium bromate was 9 times as great as by the potentiometric method. Even 10^{-5} *N* arsenite solutions showed favorable end-point breaks.

In the development of these methods, as well as in coulometric studies, we have found that polarization curves may be run very rapidly at solid electrodes by scanning with the current and reading the corresponding voltage on a *p*H meter.[22]

Chronopotentiometry. Delahay and associates [23] and Reilley et al.[24] have dealt with the theoretical and practical aspects of the potential time curves that result when a small constant current is passed through a quiet solution under diffusion-controlled conditions. The voltage of the mercury-pool electrode or a platinum electrode is read relative to a reference electrode. Either manual observations with a stopwatch or a suitable chart recorder may be used. The voltage–time plots or graphs show a series of waves, and the transition times from the beginning to the proper part of the curve of rising voltage are the essential observations. There is a linear relation between the square root of the transition time and concentration, at constant current. The theoretical relationships have been derived for multicomponent mixtures.[23] It is a characteristic of the method that the detection limit of a more difficultly reducible substance is somewhat enhanced by the presence of a more reducible substance. This technique has not been fully explored for trace work, but like mercury-pool polarography it is probably more sensitive than conventional polarography, owing to the large areas of electrodes that may be used.

Reilley and Scribner [25] have developed a new method of electrotitration which they call chronopotentiometric titration. The assembly for constant current is a series of dry batteries with appropriate high resistances for making a number of constant-current settings. At intervals during the part of the titration either prior to or just after the end point, the stirring is stopped, and the time in seconds to the transition voltage is read with the aid of a stopwatch and a *p*H meter. During the measurement the solution must be absolutely quiet. The small constant current is used in this fashion to get three points prior to or following the end point. The projection of a line through these three points cuts the zero time axis at the end point. A preliminary trial is made so that the transition time is about 60 seconds for a reactant that is disappearing, or 4 seconds for a reactant whose con-

centration is increasing. The points that are plotted are square roots of transition times versus milliliters of reagent. The method has not been tested as applied to traces of substances. It appears to have the same general sorts of applications as amperometric titrations.

SUMMARY

Potentiometric techniques are capable of giving reliable information on the amounts of traces of materials in the range from a few micrograms down to submicrogram quantities under favorable conditions. A concentration-cell procedure is tedious to establish, but when established it has many advantages for routine determinations.

Potentiometric titration has been demonstrated to be a successful technique for the estimation of many types of materials in the range from 10 to 100 μg in 5 to 10 ml or higher. The coulometric generation of reagents with amperometric indication generally gives uncertainties in the tenths or hundredths of a microgram range with a 5-ml volume. The compensated end point or "sensitive end point" may, under exceptionally favorable conditions, make it possible to estimate, within 10% or better, quantities of certain substances in the range from 0.01 to 0.003 μg in 4 to 5 ml.

The newer methods of potentiometry at constant current show, in selective situations, potential breaks at the end point that are more sensitive than classical potentiometric methods.

Chronopotentiometry and chronopotentiometric titrations have not been tested very extensively, but appear to offer possible advantages in various directions for the estimation of micro amounts of materials. These methods are related to classical polarography and to amperometric titrations, respectively, in their scope of application. Their sensitivity should be somewhat greater because of the increased electrode areas.

DISCUSSION

Discussion Leader
Harvey C. Diehl
Iowa State College

The three-dimensional method of correlating electrochemical processes [16] mentioned briefly above by Professor Furman merits close study by those interested in electrochemistry. In this approach, the fraction oxidized, potential, and current are plotted on the three axes, and

thus potentiometric titrations, polarography, and amperometric titrations are all brought into the same framework. This is a neat device and a very useful teaching tool.

The concentration-cell methods devised by Professor Furman and his students deserve more attention perhaps than they have received. We have used the concentration-cell method [2] for fluoride with good success for a number of years in our advanced course in analytical chemistry at Ames. Certain troubles we have experienced have been related not to the method itself but to the measurement of the small potentials involved. The potentials are of the order of millivolts and must be measured quite accurately. The Leeds and Northrup type K potentiometer is usually used. Even with the input lead short-circuited, we encountered serious, erratic fluctuations and occasionally steady drift in the galvanometer readings, and this behavior became progressively worse in the years following World War II. Strangely enough, the trouble frequently disappeared around 11 P.M. and began again in the early morning. Recently we traced the trouble to pickup of high-frequency radiation. The college TV, AM, and FM stations are located close to the chemistry building, an amateur operates at odd times in the electrical engineering building, and, even closer, our emission spectrographic people arc and spark at intervals with some kilowatts of power. Apparently corrosion has occurred at various electric contact within the potentiometer, hardly remarkable in a chemical laboratory, and the copper oxide film formed acts as a rectifier; a-c radiation picked up is converted to direct current and registered on the galvanometer. The trouble was finally eliminated by a combination of the following tricks: an electrical filter which bypasses high-frequency current was placed in parallel with the galvanometer, all the external wiring of the potentiometer was made with shielded wire and the shields brought to a common ground without forming closed loops, and the entire apparatus was placed on a sheet of aluminum foil which was also well grounded.

In connection with the determination of small amounts of materials by potentiometric methods, we should not overlook the electrical methods for the determination of hydrogen-ion concentration. These are certainly trace-analysis methods of the most effective sort. The glass electrode, in particular, is a device that is highly specific in action. Similar indicating electrodes which would be specific for other metal ions would be extremely useful. Certain clay membranes have been shown to respond to differences in calcium-ion concentrations from one side of the membrane to the other, but such membranes have

not been put to practical use. Some of our own work at Ames has been directed to finding such metal-ion indicator electrodes. Our approach has been the use of organic oxidation–reduction couples of the quinhydrone type which combine in one molecule the oxidation–reduction characteristics of the quinone–hydroquinone couple and the property of forming chelate rings with metal ions. Most of the work has centered about the couple 5,8-quinolinedione-5,8-quinolinediol and the quinhydrone which the two form. The diol forms insoluble derivatives with bivalent metals. The electrode functions in much the same manner as the quinhydrone electrode. Thus, a solution containing nickel ions is saturated with 5,8-quinolinhydrone and with the nickel derivative of 5,8-quinolinediol; the potential of a platinum electrode in the solution is then measured against a reference electrode. The potential turns out to be a function of the nickel-ion concentration, and thus the electrode becomes a *p*Ni meter. The theory has been worked out as an extension of the quinhydrone electrode theory, and the experimental work has shown the theory to be essentially correct. The electrode works for nickel in the presence of the alkali and alkaline-earth metals but is not specific for nickel as other bivalent ions respond also. It may ultimately be possible to build some specificity into the electrode by altering the organic molecule.

There are in the literature a number of what might be termed "screwball" indicating electrodes. These have been reported to do some astonishing things in the line of responding to metal ions and changes in their concentration. There has been renewed activity along this line in recent months, and more work may turn up some surprising applications. Dr. Fritz of Iowa State College has reported, for example, that a silver wire made the anode briefly in a solution containing bis(2-hydroxyethyl)dithiocarbamate subsequently responds to changes in mercuric-ion concentration.

* * *

CHARLES N. REILLEY (*comment added by mail*): A potentiometric method for estimating small (e.g., 6 μg per ml) quantities of metal ions by titration with ethylenediaminetetraacetic acid (EDTA) has been devised. Metal ions which may be titrated in this manner include calcium, magnesium, strontium, barium, manganese, cobalt, nickel, copper, zinc, cadmium, mercury, lead, bismuth, and the rare earths. The titration cell is illustrated in Fig. D1. The mercury pool at the bottom of the cell serves as the indicator electrode while a small calomel cell serves as the reference electrode. In practice, approximately 1 ml of the sample solution is added to the titration cell. This

solution is made alkaline with ammonia buffer (0.1 *M*) and then one drop of 4×10^{-5} *M* Hg(II)–EDTA indicator solution is added. The solution is then titrated with standard 0.00500 *M* EDTA solution made by dissolving

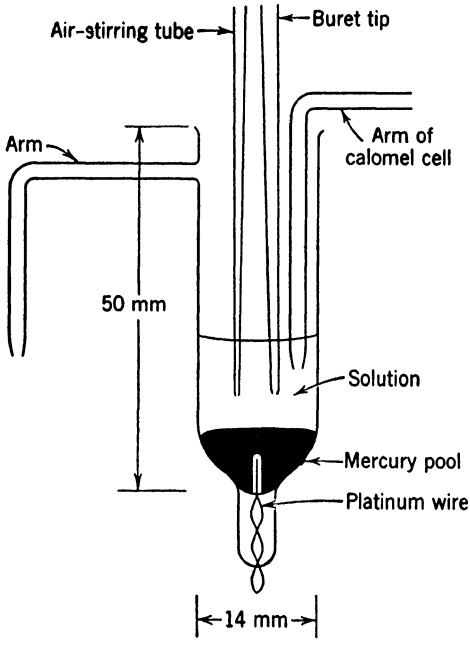

FIG. D1. Titration cell.

1.8619 grams of disodium dihydrogen ethylenediaminetetraacetate (previously dried at 66° C for 48 hours) in 1000 ml distilled water. A Gilmont ultramicroburet (Emil Greiner Co.) of 0.1 ml total capacity was employed to issue the EDTA titrant.

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Coulometry

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ABSTRACT Coulometric techniques are based on Faraday's law and, in essence, employ electrons as the primary reagent. Thus, by measuring carefully both the current and the duration of the electrolysis, one can calculate how much material has reacted. At the present time, one can detect $10-12$ equivalent and can determine $10-10$ equivalent within 1%. Extension of the technique to still smaller quantities will be extremely difficult for samples of large volume, because impurities in the reagents, including distilled water, are often much greater than the substance being determined. The technique, however, is eminently suited to analyses involving small volumes of sample. The limitation in such a case will probably be in obtaining a suitable means of indicating the equivalence point.

Hydronium and hydroxyl ions, as well as a number of oxidants, reductants, and precipitants, have already been generated in coulometric determinations. In addition, certain elements can be concentrated by deposition and then determined by coulometric measurement of the dissolution current. The versatility of the method, in terms of the variety of reagents that can be generated and its ability to handle samples of small volume, makes coulometry an attractive approach for many biochemical analyses.

Coulometry is the measurement, in terms of coulombs, of the number of electrons that have to be transferred across an electrode-solution interface to carry to completion the reaction of a particular substance in a sample. Though there are a number of variations of the technique, they all fall into two categories. In direct determinations, one measures the number of coulombs required to deposit onto an electrode or to dissolve from it an element such as copper. In indirect determinations, one measures the number of coulombs required for the production of a substance that is known to react stoichiometrically with the substance being determined. Thus one can produce Fe(II) from Fe(III) to react with an oxidant such as permanganate. It is also possible to start with a neutral solution of salt and to evolve either hydrogen or oxygen at an electrode, thereby producing hydroxide or hydrogen ions, respectively, for acid–base titrations. Finally, one can start with an anode of a metal such as silver and from it produce silver ions for the precipitation of halide [6,8,14] or for a complexation reaction with an ion such as cyanide. Thus, the indirect procedures offer the possibility of a wide variety of reactions and hence should be more widely applicable than direct ones. In the United States, Swift and his co-workers, followed closely by Furman and his associates, have been the outstanding contributors to this field.

Progress in coulometry through 1953 has been reviewed thoroughly by three different authors.[3,5,7] Because these reviews have been so recent and so thorough there appears to be little reason for the present contribution to repeat their efforts. Instead, it would appear to be more appropriate to present the principles and the salient points in a way that would be helpful to the nonspecialist contemplating the use of coulometry for trace analysis.

GENERAL BACKGROUND

The reviews reveal that, although the basic concepts and the practice of coulometry are very old, its inherently high sensitivity has not been exploited until the recent surge of interest in analysis at and below the microgram level. One can easily see why coulometry is a promising technique if one realizes that currents and times of the order of microamperes and seconds, respectively, can be measured with ease to the closest 1%. A simple calculation shows that one microampere-second is 10^{-11} equivalents, i.e. $(10^{-6} \times 1)10^{5}$, a magnitude corresponding to millimicrogram amounts of most simple substances.

It is important to note that in coulometry, where electrons are used

as the reagent, there is no restriction relating to the volume of the sample. Therefore, in principle, the method is independent of the size of the sample and of the concentration of the substance in the sample. It is also important to note that the method deals with a measurement of the number of coulombs required for a particular reaction, but does not include any provision for determining the point at which the reaction is complete. In other words, detection of the end point is not incorporated in the basic principle and must be performed independently. In one sense this is a great advantage because it means that all of the known methods of indicating equivalence points can be considered in devising a procedure. However, it also means that one's ability to use coulometry, particularly in the millimicrogram range, may be limited by the sensitivity with which one can detect the equivalence point.

The use of coulometry also implies that, in measuring the current, all of it—or at least a known fraction of it—is undergoing the desired reaction. This factor is called the "current efficiency." Ordinarily, one aims for a current efficiency of 100%, not because of the simplicity of calculations, but because that value is presumably more easily maintained than smaller values and should be more readily reproduced in going from one determination to the next. As indicated by Cooke,[3] the optimum conditions for such variables as the current, electrode size, concentration of the reagent, and *p*H can most rapidly be surveyed by polarographic techniques. From the practical standpoint, however, a sufficient number of examples involving different reagents have been worked out so that a new procedure can usually be evolved simply by patterning it after published procedures. This would most certainly be true if one were planning to adapt a particular reagent, which has already been studied, to the determination of a new substance.

It is unfortunate for trace analysis that current efficiency becomes an increasingly serious problem as one progresses from microgram to millimicrogram mounts of substances. The decreased efficiency is the result of simultaneous side reactions which probably take place to about the same extent under all conditions, but which, when smaller amounts of substances are being determined, consume a larger fraction of the total number of electrons. These can be simultaneous chemical reactions such as the evolution of hydrogen or oxygen at the generator electrode, or reactions involving impurities in the reagents, on the electrode surface, or in the distilled water. In addition, a physical phenomenon may contribute to the blank. In order to change the

potential on an electrode, a certain number of electrons must be consumed by the process of charging the electrode, just as they are when one charges the plate of a condenser. Though such a process can, in principle, be compensated for by the determination of an appropriate blank, the number of coulombs involved may be large compared to the number consumed in the desired reaction. One can decrease the size of the electrode, but this soon reaches a practical limit in direct procedures if the volume of the sample is large. Finally, there is always the possibility in trace analysis that the amount of reagent required to change the indicator will constitute a major portion of the total titration. As a group, these sources of error will constitute a formidable barrier in attempting to extend coulometry below the millimicrogram level.

In considering possible applications for coulometry in the field of biology, it is immediately apparent that, if one is going to perform reactions simply by adding or taking away electrons, the electrode reactions are going to be confined almost entirely to simple inorganic ions. It is true that some organic analyses can be performed coulometrically, but these are usually of the indirect type and involve reaction of an organic substance with an inorganic reagent which has been produced coulometrically. Comparatively few reactions with organic compounds have, as yet, been reported at the trace level, but published procedures for determinations of trace elements should be useful with little or no modification. However, no specific applications have been reported for biochemical systems.

EXPERIMENTAL DETAILS

Electrolytic Cell. Sizes of cells ranging from a volume of the order of 0.01 to 100 ml have been reported. In order to decrease the resistance of the electrolytic cell, it is advisable to add a certain amount of inert electrolyte. In coulometric titrations where a reagent is being generated, the electrolyte which is being used as the source of the reagent is ordinarily sufficient by itself because it is usually present in a concentration of 0.01 *M* or more.

To avoid complications, one ordinarily works with only one generator electrode in the system, its counterpart being separated from the cell by means of a salt bridge. It is also possible to generate the reagent in a stream that is flowing into the desired solution in much the same way as one titrates from a buret.[4] In coulometric titrations,

an indicator must be present either in the form of a substance that will change color or in the form of a supplementary electrode system.

TYPICAL ASSEMBLIES AND THEIR APPLICATIONS

Constant Current. The most widely applicable technique and the one used for most coulometric titrations involves the passage of a constant current. This has the advantage that one need only measure the time required to reach the equivalence point in order to make a simple calculation of the number of coulombs. A number of relatively expensive electronic instruments for supplying constant current have been reported in the literature, but, for most purposes, and certainly for exploratory studies, a simple battery in series with a resistor is sufficient. The resistor should have a value that is much larger than the resistance of the electrolytic cell plus the other components in the circuit, so that its value, together with the voltage of the battery, will be the chief factors that determine the magnitude of the current passing through the electrolytic cell. A student potentiometer or a vacuum-tube voltmeter can be used to measure the potential drop across a smaller resistor whose value is accurately known and was selected such that the current calculated from the potential drop can be determined with the desired precision. A little experience will show that the current will tend to change somewhat as the titration proceeds, but, after the first few minutes of use each day, the drift will slow down to such an extent that analyses with a precision of 1% or better can be obtained by making a single measurement of the current during the course of the titration.

As indicated earlier, coulometric titrations for which one ordinarily uses the constant-current technique have been used to titrate inorganic ions which can be determined with varied reagents such as Ti(III), Ce(IV), chlorine, hydroxyl, hydronium, and silver ions. Organic compounds have not been oxidized or reduced directly for use as reagents, but they can be titrated as acids or bases, oxidized or reduced by inorganic ions (thiodiglycol), brominated (aniline, 8-hydroxyquinoline), or precipitated with ions such as silver (mercaptans). Even though these reactions can be performed by conventional titrimetric means, the use of coulometry obviates the need for preparing and storing standardized solutions. As pointed out by Cooke,[3] it may sometimes be advantageous to consider the use of coulometrically generated reagents in place of standard solutions, i.e., those of hydroxide

or silver ions, which tend to change with time when stored in a very dilute form.

Variable Current. The use of a variable current obviously is more complicated because it involves integration of the current over a period of time. On the other hand, such an approach is a virtual necessity if one is measuring the current during the electrodeposition of an element such as copper or silver. When an electrolysis is carried out at a fixed voltage, the current decreases as the concentration of the element in the solution decreases with time. As pointed out in the published reviews, integration is not very difficult and may be done in a number of ways: by electronic instruments, by using a chemical coulometer in series with the analytical cell, and by measurement of the area under a curve on a current recorder. The variable-current technique can be used to advantage in working with very dilute solutions of elements where after collection and concentration of the element by a long electrolysis, without regard for the current efficiency, the amount of the deposit can be determined by coulometric dissolution. The amount of an element that can be determined by this procedure may be so small that, if one were to use a constant current of about a microampere, the time required to dissolve the deposit would be less than 1 second.[10] If, on the other hand, one were to use a smaller current, in order to increase the time interval, errors might arise from a low- or variable-current efficiency of the dissolution. Thus, it appears to be somewhat easier and possibly more precise to use variable current obtained by scanning the voltage in the direction of more noble potentials. Using this technique under ideal conditions, one should be able to determine in one run two or more elements in a given solution.

Working in the macrorange of concentrations, it is necessary to carry out electrolyses with rather complicated apparatus, a potentiostat being the most suitable device. However, when very small amounts of material are involved, an elaborate control of the potential is unnecessary, so that a very simple electrolysis setup comparable to that used in most elementary courses in quantitative analysis is often sufficient. In view of the fact that recording of the variable current over a shorter period of time can easily be carried out on a recording polarograph, it is advantageous to use this apparatus because the deposition can be followed. To analyze very dilute solutions which produce little or no deposition current, a polarograph can be used to adjust the potential of the electrode and, after a standard length of time, to record a current-time curve during the short interval re-

quired for dissolution of the deposit at more noble potentials. In such a case, a fast recorder (1 second or less for full scale) and possibly a tenfold faster motor on the slide wire of the polarograph will be needed.

Procedures based on coulometric dissolution can easily be visualized for elements such as copper, cadmium, lead, zinc, and silver. Unfortunately, many elements are not readily deposited, if at all, from aqueous solutions, and some that do deposit are not readily dissolved. In these classes are elements such as aluminum, zirconium, uranium, beryllium, magnesium, iron, manganese, and molybdenum. Reactions other than deposition or dissolution can also be carried out: i.e., determination of iron by reduction of Fe(III) to (II). However, when a choice is possible, such a reaction is faster to perform indirectly by coulometric titration with a suitable intermediate. On the other hand, for cases where the technique is feasible it offers the following advantages: First, because one need only deposit and dissolve millimicrogram amounts of material, very small samples can be taken for analysis. Thus, in many cases where the sample must be digested before making a determination, the digestion time can be shortened considerably. Second, for samples where microgram amounts of materials may be present, one gains little by depositing the element completely before making the dissolution. Hence, one need only electrolyze for a period of 5 to 15 minutes under carefully controlled conditions, in order to deposit an arbitrary but constant fraction of the element from both a standard and a series of unknown solutions. Third, in view of the fact that one can gain selectivity by proper selection of the electrode potential during the electrolysis and during stripping, one can, in principle, eliminate the necessity for independent separation steps by solvent-extraction, chromatography, or similar procedures. Fourth, many samples already contain sufficient electrolyte so that one need not run further risks of contamination by adding high concentrations of additional electrolyte. Hence, time-consuming decontamination procedures for additional solutions need not be carried out, as they must be for procedures based on extraction using dithizone. Direct procedures offer the attractive possibility of simplifying existing procedures, as well as that of exploring smaller, more dilute samples.

Though, strictly speaking, an amperometric method rather than coulometric, an interesting application of the variable-current technique has recently been reported by Baker and Morrison [1] for the indirect determination of small amounts of fluoride and cyanide.

Many other ions should be susceptible to the same approach. In essence, these workers eliminated the integration step by carrying out the dissolution procedure under standard conditions, so that a single measurement of the dissolution current after a fixed time was a direct measure of the concentration. Fluoride was determined in an acetic acid medium using aluminum as the sacrificial anode; cyanide, in a medium of 0.1 *M* sodium hydroxide using a silver anode. The rate of dissolution of the anode as measured by the instantaneous current was proportional to the concentration of the fluoride or cyanide. No external source of electrons was used, when, because the reactions proceeded spontaneously, the anode was connected to the platinum cathode through a microammeter. Though the authors did not specifically mention any control of the temperature, this factor could be an important variable.

Continuous Analyses. It is obvious that, in order to analyze either a flowing sample or a static sample to which material is being added at a variable rate, it will be necessary to use a variable current. In this case, however, the current is changed so as to maintain a fixed concentration of the reagent in the solution at all times. Hence, in principle it is different from that in the preceding section in which the current was assumed to change with time in a more or less regular way.

As pointed out in the review articles, commercial instruments are available for carrying out analyses of this type. In one instance a gas stream containing oxidizable organic sulfur compounds is passed through a solution in which bromine is being generated. A change in the concentration of the bromine from the present value causes the generation current to increase (or decrease) in order to maintain that value. Another company has devised a similar instrument for analyzing the chlorine content of water using coulometrically generated ferrous iron. Many other applications are easily visualized, including analyses of effluent phases from continuous countercurrent separation processes.

One can also imagine an interesting variation which might be of use in respiration studies. For example, in a closed, constant-volume gaseous system, a constant pressure of oxygen can be maintained by generating it electrolytically. A record of the current required to maintain the pressure constant provides a direct measure of the oxygen consumption at any time.[2] Though the greater solubilities of carbon dioxide and hydrogen cyanide may pose problems, one can visualize a similar setup involving the liberation of hydrogen ion in solutions

containing bicarbonate or alkaline cyanide, respectively. Other applications will undoubtedly present themselves to biologists and biochemists in search of suitable automatic techniques.

Miscellaneous. It may be helpful to know that coulometry may be used as a means of identifying products, including intermediates in certain reaction mechanisms. Thus, on page 294 of the review of Delahay [5] is a summary of a technique developed simultaneously by two other groups of investigators which will permit determining the number of electrons involved in a particular polarographic reaction. In principle, the electron transfer can be determined directly from a polarogram, but in practice, particularly when working with organic compounds whose reductions are fraught with many difficulties, the results may be ambiguous. Upon measuring the number of coulombs required for fractional completion of an intermediate reaction and the limiting currents on polarograms before and after carrying out the coulometric measurement, the equivalent weight of a substance can be determined.

Coulometry has also been used as a source of one or both of the measurements made in analyzing binary mixtures of chemically similar substances by indirect methods. In principle, a measurement is made on a mixture in its original state and a second one after carrying out a chemical reaction in which both substances react. For example, a mixture of sodium and potassium chlorides is weighed, and then, without separation, it is converted to the sulfates and weighed again. The amounts of sodium and potassium present can then be calculated by setting up simultaneous equations. In principle, any method for obtaining a measurement is suitable, and both need not be the same. However, it was only in 1953 that coulometry was first employed in this way.[9,11]

CONCLUSIONS

1. The inherently high sensitivity of coulometry makes it ideally suited for trace analysis at the millimicrogram level.

2. Direct procedures, involving the simultaneous selective separation and concentration of an element in very dilute solutions, offer a promising means for simplifying and speeding up analyses of certain trace elements for biological systems. The analyses for copper in serum [12] and for lead or mercury in urine [13] are obvious possibilities with a high probability of success.

3. Indirect procedures involving the electrolytic generation of a reagent provide tremendous versatility and make possible the per-

formance not only of oxidation–reduction reactions but also acid–base, precipitation, complex-formation, bromination, and other types of reactions. Determinations of amino acids by acid–base titration and the sulfur-containing amino acids by titration with silver or bromine should be feasible.

4. An important limitation of coulometry is shared with most methods of trace analysis: the introduction of impurities from reagents, solvents, and the walls of the apparatus must be minimized. At the same time, losses incurred while samples are being prepared for a determination and losses onto the walls of the vessels during the determination must be guarded against.

5. A second limitation, also shared with other methods, is imposed by the sensitivity of available indicators for detecting the equivalence point.

DISCUSSION

Discussion Leader
W. D. Cooke
Cornell University

Since so many of the discussions of the previous chapters have begun with historical aspects, I would like to make note of a fine paper on coulometric analysis, which appeared 40 years ago. In 1917, G. G. Grower published an article in the *Journal of the National Bureau of Standards,* which presented a method of measuring the thickness of tin coatings on copper wires. The sample was the anode of an electrolytic cell, and the tin was anodically oxidized by an externally supplied current. The potential of the sample, against a reference electrode, was followed, and an abrupt change was noted as the last of the tin plate was removed from the wire. The current–time curve was then integrated to find the number of coulombs consumed by the anodic process, and the thickness of the tin calculated.

It is odd that many papers employing the same principle are finally appearing after a lapse of 40 years. The value of this technique was not realized for a very long time. I believe that Grower was far ahead of his time and analytical chemists either did not understand the implications or were reluctant to accept such a radical departure from accepted methods.

I might mention some work we have been pursuing at Cornell along

the lines of stripped deposited metals as discussed by Professor Rogers. In working in this field, one is faced with the problem of choosing an appropriate electrode on which to plate the sample. If one works with a solid electrode, or a solid electrode with a thin coating of mercury, there are disadvantages involved with the conditioning of the electrode surface. The deposited metal, however, is concentrated at the surface so that high sensitivity is realized on the stripping process. If one avoids the conditioning problem by using a pure mercury electrode, the deposited metal diffuses into the mercury, decreasing the surface concentration and lowering the sensitivity. We have found that a mercury-pool electrode, about 1 mm in diameter, retains a high sensitivity without the problem of electrode conditioning. In this method, the solution above the electrode is rapidly stirred so that a relatively large amount of metal can be deposited in a short time. Because the mercury pool is so small, no stirring occurs in the mercury. Hence, the metal accumulates essentially at the surface, and is available for the stripping process. Further, in contrast to solid electrodes there is no interference of one deposited metal on the stripping process of another, unless the potentials are so close that the anodic waves overlap.

I might also comment on the procedure for using external generation of reagents.[4] One of the reasons for devising external generation procedures was that the oxidation of Cl^- ions proceeds at a lower potential than the oxidation of H_2O to H^+ and O_2. Therefore, acids could not be generated in the presence of chloride ion. One of my graduate students, not realizing this fact, was titrating bases with coulometrically generated acid in the presence of NaCl and obtaining correct results! After some speculation we decided that, even though chlorine was being formed at the anode, the stoichiometry of the titration was preserved by the reaction

$$Cl_2 + 2OH^- \rightarrow OCl^- + Cl^- + H_2O$$

in which the base was being titrated with chlorine.

It has been seen that acids, bases, oxidizing, reducing, and precipitating agents can be generated coulometrically. Professor Reilley of the University of North Carolina has made a significant contribution by generating a complexing reagent: namely, Versene. The electrochemical generation of Versene was accomplished by a rather ingenious method. Professor Reilley might tell us a bit about this.

QUESTIONS AND ANSWERS

ANDRÉ JULIARD (*Houdry Process Corp.*): The method for generating Versene electrolytically seems to be very interesting for trace determinations. I would like to know if the Versene is added as a solid material. May we obtain some information on that technique?

CHARLES N. REILLEY: The successful coulometric titration of metal ions, such as calcium, copper, zinc, and lead, was effected by the indirect generation of EDTA–(H_4Y) released upon reduction of Hg(II)–EDTA chelate at a mercury pool. Hg(II)–EDTA has a stability constant of approximately 10^{22} and consequently forms one of the most stable chelates with EDTA. The electrode process whereby EDTA is liberated is given by

$$HgY^{-2} + 2e \rightarrow Hg^{\circ} + Y^{-4}$$

The EDTA thus liberated is employed to titrate the metal ion of the sample:

$$Ca^{+2} + Y^{-4} \rightarrow CaY^{-2}$$

The procedure is, of course, also applicable to the titration of metal ions which forms chelates more stable than Hg(II)–EDTA. In this case, introducing the sample of metal ion causes a displacement of the Hg(II) ion:

$$M^{+2} + HgY^{-2} \rightarrow MY^{-2} + Hg^{+2}$$

The Hg(II) ion is consequently titrated by the EDTA released electrically by the reduction of Hg(II)–EDTA. By proper control of *p*H, selective titrations can be performed—with the more stable chelating metals (e.g., heavy metals) being selectively titrated at the low *p*H values and with subsequent titrations being effected at higher *p*H values for the titration of the less stable chelating metals (e.g., alkaline earths). The development of a sensitive and compatible end-point detection method was a major problem which had to be solved before the method could become generally useful. For this purpose a *p*M indicator electrode system of unique operation was employed (see Fig. D1). The electrode consists of a mercury drop suspended in a cup at the end of a J tube. This electrode will develop a potential in the vicinity of the end point directly proportional to the *p*M (log metal-ion concentration) as can be deduced from the half-cell

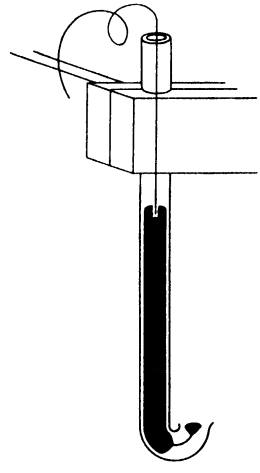

FIG. D1. A *p*M indicator electrode system.

$$Hg \mid HgY^{-2}, MY^{-2}, M^{+2}$$

and the corresponding Nernst equation

$$E = E^{\circ}_{Hg^{\circ}} + \frac{0.059}{2} \log \frac{[M^{+2}][HgY^{-2}]K_{MY}{}^{2}}{[MY^{-2}]K_{HgY^{-2}}}$$

where HgY^{-2}, MY^{-2}, and the stability constants (K's) of the Hg(II)–EDTA and metal–EDTA chelates are constants.

JAMES J. LINGANE: Professor Rogers pointed out that it is usually assumed that one needs 100% current efficiency for the generation. I think Professor Rogers really means 100% titration efficiency, which is not quite the same thing. For example, in the coulometric titration of ferrous ion with ceric ion, oxidation to ferric ion proceeds both by direct anodic reaction and via the electrogenerated ceric ion. If the generation of ceric ion is, say, only 90% efficient, but only one third of the ferrous ion is oxidized via ceric ion, then the net titration efficiency will be 96.7% in spite of only a 90% current efficiency for the titrant generation.

W. D. COOKE: Using your terminology, I would say that the generation of hydrogen ion is proceeding at zero efficiency, even though the titration efficiency is 100% efficient.

JAMES J. LINGANE: In your paper you mentioned that a few per cent chlorine generation caused no error. I wonder if this would still be true if, say, 50% of the current resulted from chlorine generation rather than oxidation of water.

W. D. COOKE: If one looks at the potentials, it appears as though one would get mostly chlorine. If the titration were proceeding at high current density, there is always the possibility of losing chlorine gas before it reacts with water.

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Polarography and Voltammetry

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ABSTRACT Polarographic and voltammetric methods are valuable in trace analysis because concentrations as low as 10^{-5} to 10^{-7} M can be determined with volumes of solution as small as 0.3 ml, and even with smaller volumes. The substance being analyzed is reduced or oxidized electrolytically, and its concentration is determined from current–potential–time relationships. Two main difficulties are encountered in the application to trace analysis. (1) An appreciable fraction of the current through the electrolytic cell is consumed in the charging or discharging of the double layer. (2) The current for reduction or oxidation of impurities and/or the solvent may be much larger than the current for the electrolysis of the substance being analyzed. These two causes of error and possible means of minimizing them are discussed in detail for the following methods of polarography and voltammetry: electrolysis at constant potential with a stationary working electrode, conventional polarography, electrolysis in unstirred solution with potential varying linearly with time, electrolysis with superimposed alternating voltage (sine and square waves), voltammetry at constant current, electrolysis with moving electrodes or stirred solutions. Methods based on catalyzed electrode reactions, suppression of polarographic maxima, and double-layer phenomena are also briefly discussed.

INTRODUCTION

Polarography is based on the interpretation of current-potential curves obtained with the dropping-mercury electrode. Since its invention in 1922 by Professor J. Heyrovsky of the Charles University, Prague, polarography has been progressively developed into the most important and versatile method of electroanalytical chemistry. Polarography and its modified forms can be grouped under the generic term *"voltammetry,"* which was coined by Laitinen and Kolthoff.[31]

The electrolytic cell in voltammetry and polarography is essentially composed of two electrodes which are connected to a power supply. The substance being analyzed is reduced or oxidized on the *working* electrode, whereas some other reaction occurs at the other electrode of the cell. Either the potential of the working electrode or the current through the cell is controlled, and voltammetric methods can be classified accordingly. The following classification will be adopted:

Methods with controlled potential:
- Electrolysis with a stationary working electrode at constant potential in unstirred solution.
- Electrolysis at constant potential with the dropping-mercury electrode (polarography).
- Electrolysis with a stationary working electrode at a potential varying linearly with time (oscillographic polarography).
- Electrolysis in unstirred solution with a stationary working electrode at a periodically varying potential (sine or square wave).
- Electrolysis at constant potential in stirred solution and/or with a moving working electrode.

Methods with controlled current:
- Electrolysis at constant current with a stationary working electrode in unstirred solution (voltammetry at constant current, chronopotentiometry).
- Electrolysis with periodically varying current in unstirred solution (oscillographic polarography).

Polarographic and voltammetric methods are valuable in trace analysis because concentrations as low as 10^{-5} to 10^{-7} M can be determined with volumes of solution as small as 0.3 ml or even smaller volumes. These methods have in common two main sources of difficulties in their application to trace analysis: (1) The current for the reduction or oxidation of the solvent and/or impurities may be much larger

than the current for the electrolysis of the substance being analyzed. (2) An appreciable fraction of the current through the electrolytic cell may be consumed in the charging or discharging of the double layer. These two main difficulties, which are encountered regardless of the nature of the substance being analyzed, limit the application of polarographic and voltammetric methods at extremely low concentrations. A discussion of these two limiting factors is therefore essential.

MAIN DIFFICULTIES IN APPLICATION OF VOLTAMMETRIC METHODS TO TRACE ANALYSIS

Electrolysis of Solvent and Oxidation of the Working Electrode. Interference resulting from electrolysis of the solvent will be first discussed for the important case of aqueous solutions. Organic solvents will be briefly considered afterwards.

Water is electrolyzed with the evolution of hydrogen on the cathode * and the evolution of oxygen on the anode according to the reactions

$$2H^+ + 2e \rightarrow H_2 \quad (1)$$

$$O_2 + 4H^+ + 4e \leftarrow 2H_2O \quad (2)$$

These reactions require an overvoltage, which varies with the nature of the electrode and electrolyte and with the current density. Sufficient kinetic data are available [4] to calculate the potentials at which reactions 1 and 2 occur at a given current density. Potential–current relationships for mercury and platinum, which are the electrode materials most frequently used in electroanalytical chemistry, are listed in Table I. In practice, it is preferable to determine experimentally potential–current relationships because kinetic data for electrode processes are markedly affected by the presence of impurities in solution. The data of Table I are solely given for the purpose of calculating approximate values of the potential at which hydrogen or oxygen evolution interferes with the electrolysis of the substance being analyzed. For instance, currents as low as 5×10^{-9} amperes are encountered in the polarographic determination of traces of metals (Fig. 2). Since the area of the mercury drop in a polarographic determination is of the order of 0.02 cm^2, the current density for the above current is 2.5×10^{-7} ampere per square centimeter. The potential at which hydrogen evolution interferes with a polarographic determina-

* The cathode is the electrode of a cell on which an over-all reduction occurs, and the anode is the electrode on which an over-all oxidation takes place.

TABLE I

POTENTIAL–CURRENT RELATIONSHIPS FOR THE EVOLUTION OF HYDROGEN AND OXYGEN *

Metal	Medium	Potential–Current Relationship †
	Evolution of Hydrogen	
Hg	0.1 *N* HCl	$E = -1.54 - 0.123 \log i_{CD}$
Hg	0.1 *N* NaOH	$E = -2.24 - 0.102 \log i_{CD}$
Pt	2 *N* H_2SO_4	$E = -0.45 - 0.118 \log i_{CD}$
	Evolution of Oxygen	
Pt	0.1 *N* H_2SO_4	$E = 2.26 + 0.098 \log i_{CD}$
Pt	0.1 *N* NaOH	$E = 1.37 + 0.073 \log i_{CD}$

* Calculated from kinetic data in ref. 4.

† Potentials in volts versus NHE (European convention); current densities in units (amperes per square centimeters); equations are written for 25° C.

tion under these conditions is of the order of −0.7 volt vs. NHE, i.e. −0.95 volt vs. SCE for 0.1 *N* hydrochloric acid and about −1.55 volts vs. NHE, i.e. −1.80 volts vs. SCE for 0.1 *N* sodium hydroxide. It can be concluded from these and similar calculations *that electrolysis of the water is not a source of serious difficulties in the application of polarographic and voltammetric methods to trace analysis.*†

When a mixture of an organic solvent and water is utilized, other reactions in addition to the evolution of oxygen and hydrogen may have to be considered. For instance, the anodic oxidation of ethyl alcohol to acetaldehyde may interfere with the analytical determination when a mixture of water and ethyl alcohol is used in a determination involving anodic oxidation of the analyzed substance. The possibility of interference can, in general, be readily established from current–potential curves for the solvent in the absence of the substance to be analyzed, but under otherwise identical conditions to those prevailing during actual analysis.

The anodic oxidation of the working electrode generally does not interfere with the analysis provided that this electrode is not made too anodic. The necessity of taking this precaution is obvious with mercury electrodes, but it should not be overlooked even with platinum electrodes. Platinum is remarkably inert even at very anodic potentials because it is passivated by a layer of oxide. The cathodic

† However, hydrogen evolution interferes with cathodic processes on a platinum working electrode when the potential is too negative (see Table I).

reduction of this oxide layer may interfere with the determination of traces of elements, as was pointed out by Kolthoff and Tanaka.[27] The reader is referred to the thorough investigation of these authors for details.

Electrolysis of Impurities in Solution. Electrolysis in polarography and voltammetry is carried out in the presence of an electrolyte—the *supporting electrolyte*—whose functions are (1) to decrease the ohmic drop in solution, and (2) to minimize migration of the electrolyzed species in the case of the reduction or oxidation of an ion (for details see books on polarography [9,16,25,38,41,48]). The concentration of the supporting electrolyte is quite large, perhaps 0.1 to 1 M in ordinary polarographic work and 0.01 M in trace analysis, and consequently impurities such as metal ions in this electrolyte may interfere seriously with the determination of traces of elements. Another impurity in the electrolyte is dissolved oxygen which is reduced to hydrogen peroxide and, at sufficiently cathodic potentials, to water. The concentration of oxygen in air-saturated aqueous solutions is approximately 8 ppm or 0.25 mM at room temperature. Finally, organic solvents often contain appreciable quantities of reducible or oxidizable substances. These various impurities must be removed as completely as possible, particularly when concentrations as low as 10^{-6} to 10^{-7} M have to be determined.

Oxygen is generally removed by bubbling nitrogen through the analyzed solution before electrolysis. Reduction of oxygen to water by sodium sulfite can also be utilized in aqueous solutions having a pH larger than 6 to 7. Details can be found in texts on polarography,[9,16,25,38,41,48] and only two essential points will be mentioned here: namely, the necessity of using very pure nitrogen and the usefulness of bubbling nitrogen with a gas disperser. If the nitrogen contains only 0.01% oxygen—and this corresponds to a very pure commercial product—the concentration of oxygen for the solution saturated with the sample of nitrogen is approximately 10^{-7} M at room temperature. In trace analysis this concentration may not be negligible in comparison with the concentration of the substance to be analyzed. Commercial nitrogen can be purified by passing it through a solution of vanadous sulfate according to a procedure recommended by Meites and Meites.[39] Another procedure, which can reduce the oxygen contents to 4×10^{-5}% is to pass the gas over finely divided copper [40] at 250° C. It is advantageous to speed up the removal of oxygen by increasing the area of contact between the

gaseous and liquid phases by bubbling nitrogen through a gas disperser.

Reducible or oxidizable impurities can generally be removed from the supporting electrolyte by *pre-electrolysis.* This method of purification is quite old since, according to Bockris,[4] it was first applied in electrochemical studies by Lewis and Jackson [34] about 50 years ago. A large mercury pool (perhaps 5 cm in diameter) is generally used as cathode in the reduction of impurities, whereas a platinum electrode is utilized for the anodic oxidation of impurities. The general technique is similar to that of electrolytic separations.[36]

Charging or Discharging of the Double Layer. An electrode-solution interphase behaves as a condenser having a rather large capacity. The existence of this capacity can be easily explained by considering an electrode that is, for example, positive with respect to the electrolyte with which it is in contact. The electrode attracts the anions and repels the cations, and a double layer is thus established. The electrode-solution interphase thus behaves as a condenser. This condenser can be characterized by its *integral capacity,* which is defined by the relationship

$$Q = c_i(E_m - E) \tag{3}$$

where Q is the electric charge on the electrode; E is the potential of the electrode with respect to some reference electrode, for example the normal hydrogen electrode; and E_m is the potential, as measured in the same scale as E, when the charge Q is equal to zero.‡ The potential E_m for a given metal varies with the nature of the electrolyte; E_m also depends on the nature of the electrode.

The integral capacity is a function of electrode potential (Fig. 1), and it is therefore useful to define the *differential* double-layer capacity by the relationship

$$c_d = \frac{dQ}{dE} \tag{4}$$

The current for the charging or discharging of the double layer as the potential is changed can be computed [37] from equation 4. By definition of a current one has

$$i_c = \frac{dQ}{dt} = \frac{dQ}{dE}\frac{dE}{dt} \tag{5}$$

‡ Equation 3 is written to make the capacity current, which is derived below, conform to the usual convention in polarography of regarding cathodic currents as positive.

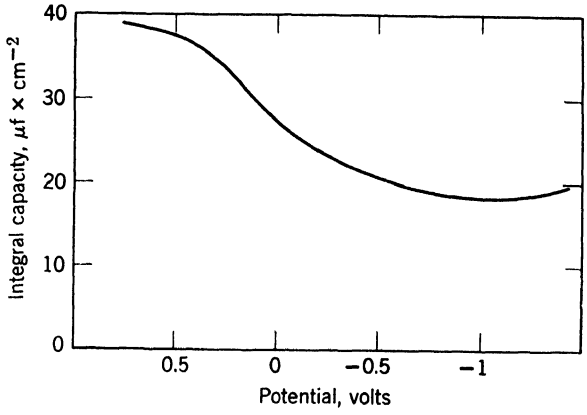

FIG. 1. Variations of integral capacity with potential for mercury in 0.5 *M* sodium sulfate according to Grahame.[13] Potentials are referred to the potential at zero charge, which is −0.20 volt vs. NHE or −0.45 volt vs. SCE (reproduced from *Chem. Rev.*; diagram slightly different from the original).

or, in view of equation 4,

$$i_c = c_d \frac{dE}{dt} \tag{6}$$

Conversely, the capacity current is readily computed from the integral capacity when the potential of the electrode is kept constant and the electrode area varies. Thus,

$$i_c = \frac{dc_i}{dt}(E_m - E) \tag{7}$$

If the potential of the electrode and its double-layer capacity remain constant, the capacity current is equal to zero, at least once the double layer has been charged.

The total current for an electrode process is the algebraic sum of two components: (1) the current corresponding to electrolytic reduction or oxidation (this is the *faradaic current* according to a term coined by Grahame),[13] and (2) the capacity current for the charging or discharging of the double layer. The faradaic current is related to the concentration of the analyzed substance (and also to the concentrations of reducible or oxidizable impurities), whereas the capacity current is practically not. It is therefore essential to keep the capacity current at a minimum value and/or to compensate for its effect. The manner in which either or both procedures are applied

in the various methods of polarography and voltammetry is discussed in the following sections.

ELECTROLYSIS WITH A STATIONARY WORKING ELECTRODE AT CONSTANT POTENTIAL IN UNSTIRRED SOLUTION

The experimental setup in this method is very simple: The cell composed of the working electrode and an *unpolarized electrode,* i.e., an electrode whose potential remains constant regardless of the current density, is connected in series with a galvanometer or a pen-and-ink recorder to a power supply (potentiometer) having an adjustable output voltage. The unpolarized electrode is, for instance, a calomel electrode working at a sufficiently low current density. The working electrode is, in general, *a platinum disk, a mercury pool, a platinum wire,* or *a hanging mercury drop.* The platinum-disk electrode devised by Laitinen and Kolthoff [30] duplicates very well conditions of linear semi-infinite diffusion and can be used with relatively small volumes of solution. The platinum-wire electrode is convenient when only a small volume of solution is available. This is not true of the mercury-pool electrode because pools having a diameter of at least 1 to 2 cm must be utilized if a reasonably flat electrode is desired. Pools of smaller diameter exhibit a marked curvature because of the meniscus effect, and the geometry of the electrode becomes uncertain. Furthermore, the wetting of the wall of the container below the surface of the mercury makes the area of the electrode quite uncertain. The hanging mercury-drop electrode, which has been sometimes utilized in electrochemical studies,[12] is very advantageous when a stationary mercury electrode must be used. Mercury flows at a constant rate through an ordinary polarographic capillary, and the mercury drops are caught in a groove of a small glass spoon which is attached to the capillary with such an inclination that the drops roll slowly down the groove. The rolling drop touches the tip of a small gold-plated platinum wire sealed in the end of a fine tube (about 0.1 mm of wire is left uncovered by the glass). The wire is coated with gold because mercury does not adhere to platinum. The drop is removed from the platinum tip by collecting additional drops until the limit of adhesion is exceeded and the drop is dislodged.

The theory of currents observed with a stationary electrode at constant potential in unstirred solution is well developed, and the reader

can find the details in books on polarography.[9,16,25,38,41,48] The following equation holds when the current is entirely controlled by diffusion of the reducible or oxidizable species toward a plane electrode

$$i = \frac{pAC^0}{t^{1/2}} \tag{8}$$

There A is the area of the electrode, C^0 the concentration of the analyzed substance, t the time elapsed since the beginning of electrolysis, and p a proportionality constant whose explicit form is not needed here. It can be seen from equation 8 that the current decreases continuously during electrolysis and is proportional to the concentration of analyzed substance. It is not advisable to utilize for quantitative determinations current readings obtained beyond 1 or 2 minutes after the closing of the electrolysis circuit: convection then interferes with diffusion, particularly with cylindric or spherical electrodes.

Equations comparable to 8 have been derived for spherical and cylindrical electrodes, and the reader can find the details in the specialized literature.[9,25]

The electrode area and the potential are constant in this method, and consequently the capacity current drops to zero as soon as the double layer is charged after the closing of the electrolysis circuit. The time required for the charging or discharging of the double layer is, in general, much shorter than one second since the time constant of the circuit is quite small. The capacity current does not interfere with the measurement of the faradaic current, and one of the main difficulties in the application of voltammetric methods to trace analysis is eliminated.

To my knowledge, application of this method to trace analysis, and particularly the use of the hanging mercury drop, has not been investigated although some useful results could possibly be obtained.

ELECTROLYSIS WITH THE DROPPING-MERCURY ELECTRODE AT CONSTANT POTENTIAL (POLAROGRAPHY)

The apparatus in polarography is the same as in the previous method, but a dropping-mercury electrode is substituted for the stationary working electrode. The potential is kept essentially constant during the life of a mercury drop, and either the average current during drop life or the maximum current at the end of the drop life is measured as a function of the potential of the dropping-mercury

electrode. A *current-potential curve* or *wave* is obtained in this manner.

The theory of polarographic waves is well developed, and the reader can find the details in books on polarography.[9,16,25,38,41,48] The theory of irreversible, kinetic, and catalytic waves has been discussed in detail.[9] The book of Meites [38] provides an excellent intro-

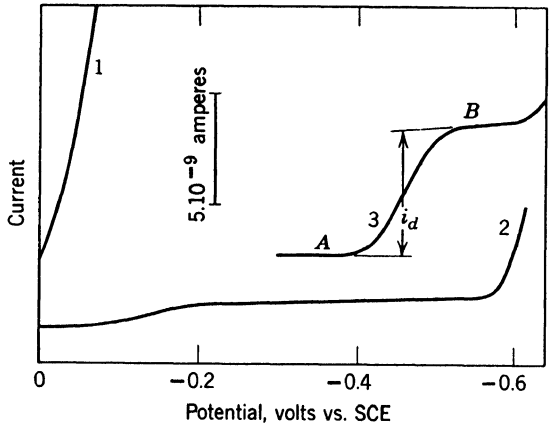

FIG. 2. Tracings of polarographic current–potential curves according to Kelley and Miller.[22] Curve 1—1 *M* potassium nitrate without compensation; curve 2—same as curve 1 but with compensation; curve 3—1.2×10^{-6} *M* plumbous ion in 1 *M* potassium nitrate with compensation (reproduced from *Anal. Chem.*; diagram slightly different from the original).

duction to the method, and so does the text of Müller.[41] The periodic reviews of Lingane [35] cover recent developments.

An example of polarographic wave which was recorded by a technique described below is shown in Fig. 2 (curve 3) for the reduction of 1.2×10^{-6} *M* plumbous ions. The wave height or *diffusion current* § i_d obeys the Ilkovic equation,[19]

$$i_d = 708nm^{2/3}t^{1/6}D^{1/2}C^0 \tag{9}$$

where i_d is the current during drop life in microamperes; 708 a constant that holds for 25° C; n the number of electrons involved in the electrode reaction; m the rate of flow of mercury for the dropping-mercury electrode in milligrams per second, t the time in seconds elapsed since the beginning of the drop life, D the diffusion coeffi-

§ We assume here that there is no complication resulting from partial control of the wave height by the kinetics of a chemical reaction preceding electron transfer (kinetic currents).

cient of the substance being reduced or oxidized at the dropping-mercury electrode in square centimeters per second, and C^0 the concentration of analyzed substance in millimoles per liter. The average current during drop life can readily be derived from equation 9 by integrating the current over the drop life and by dividing the result by the drop life τ. The average current is given by an equation similar to 9 except that the numerical coefficient is 607 and that $t^{1/6}$ is replaced by $\tau^{1/6}$.

The Ilkovic equation was derived for conditions that are simpler than those prevailing at an actual dropping-mercury electrode, and several attempts have been made to improve this equation by the introduction of additional terms. Details can be found in the recent review of Hans,[14] but we need not go into this matter since the corrections are relatively minor.

By taking the representative values, $n = 2$, $m = 1$ mg per second, $t = 4$ seconds, $D = 10^{-5}$ cm^2 per second, one deduces from equation 9 that easily measurable || currents (5×10^{-4} μa) would be obtained even with concentrations as low as 10^{-7} M. However, the application of polarography to trace analysis is limited by the interference resulting from the capacity current and by the necessity of removing from solution the last trace of oxygen and other reducible (or oxidizable) impurities. The effect of the capacity current lends itself to a simple quantitative analysis.

The potential of the dropping-mercury electrode is essentially constant during drop life, and the capacity current can thus be calculated from equation 7 where the term dc_i/dt is computed by taking into account the expansion of the mercury drop. Since the integral capacity is proportional to the area of the electrode, one has

$$c_i = c_i^0 A \tag{10}$$

where c_i^0 is the integral capacity per unit area. The area of the mercury drop can be expressed in terms of the parameters m and t. Thus,

$$A = 0.00851 m^{2/3} t^{2/3} \tag{11}$$

The combination of equations 7, 10, and 11 yields the capacity current,

$$i_c = \frac{2}{3} 0.00851 \frac{m^{2/3}(E_m - E)c_i^0}{t^{1/3}} \tag{12}$$

|| Pen-and-ink recorders having a sensitivity of 10^{-14} ampere per millimeter deflection and even higher sensitivities are commercially available.

where i_c is in amperes, m in milligrams per second, t in seconds, and $E - E_m$ in volts. By taking the representative values $m = 1$ mg per second, $t = 4$ seconds, and $c_i^0 = 30$ μf per cm^2, one obtains

$$i_c = (E_m - E)10^{-7} \tag{13}$$

where the current is in amperes.

Distortion of polarographic waves is very pronounced when the variation in capacity current Δi_c from a point A (Fig. 2, curve 3) at the bottom of the wave to a point in the upper plateau B of the wave is of the same order as the diffusion current. The difference Δi_c is, in view of equation 13,

$$\Delta i_c = (E_B - E_A)10^{-7} \tag{14}$$

where E_A and E_B are the potentials of the dropping-mercury electrode at A and B. The difference $E_B - E_A$, which can be set at 0.2 volt for a reversible wave, can be as large as 1 volt for a very drawn-out irreversible wave. By prescribing the condition $i_d \geqq i_c$ and setting $E_B - E_A = 0.2$ volt (the most favorable case), one deduces the condition

$$C^0 > 5 \times 10^{-6} \text{ mole per liter} \tag{15}$$

Actually the graphic determination of diffusion currents is quite uncertain when the concentration of analyzed substance is smaller than 10^{-5} M, and it is advantageous to compensate the capacity current in polarographic trace analysis. This compensation involves serious difficulties for two reasons. (1) The capacity current is compensated by an electrical device whose output is constant at a given potential whereas the capacity current varies during drop life (see equation 12). (2) The capacity current, which is proportional to the product $(E_m - E)c_i^0$ (see equation 12), is not a simple function of potential because the integral capacity varies with potential (Fig. 1).

Two methods for the compensation of the capacity current have been proposed. In the first method, the compensating current is a linear function of potential, whereas in the second method the compensating current is the actual capacity current observed with the supporting electrolyte in the absence of the substance being analyzed. The circuit of Fig. 3, which was devised by Ilkovic and Semerano,[20] is utilized for compensation of the capacity current by the first method. The output voltage of potentiometer P is divided by the resistances R_1 and R_2 in series. The voltage across R_1 is applied to the polarographic cell C, and the current through the cell is measured or re-

corded with instrument G. The voltage across R_2, which is much smaller than the voltage across R_1, is applied to a resistance R_3 in series with instrument G. Furthermore, resistances R_1 and R_2 are quite low (R_1 = 1000 ohms and R_2 = 10 ohms, for example), and the voltage distribution across them is virtually independent of the current drawn by the cell. The countercurrent through resistance R_3 then varies linearly with the output voltage of potentiometer P, and

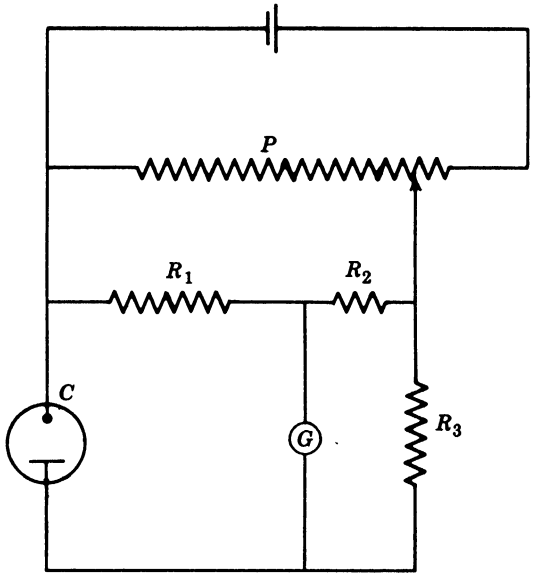

FIG. 3. Schematic diagram of circuit of Ilkovic and Semerano [20] for compensation of capacity current in conventional polarography.

compensation of the capacity current is achieved provided that resistance R_3 is properly selected. The selection of R_3 is made by trial and error with the cell filled with the supporting electrolyte alone. This compensating circuit is advantageous when concentrations lower than, say, $5 \times 10^{-5}\ M$ have to be determined, and the usefulness of the polarographic method can be extended with this compensating circuit to concentrations as low as, perhaps, $5 \times 10^{-6}\ M$. Commercial recording polarographs can readily be modified to provide for linear compensation of the capacity current. Since the capacity current varies continuously during drop life and the compensating current does not, it is advantageous to damp the current measuring or recording instrument.

The following method developed by Kelley and Miller [22] achieves

better compensation than the above circuit. A curve follower F is introduced in the circuit of a conventional pen-and-ink recording polarograph as shown in Fig. 4. The curve showing the variation of the capacity current with potential for the blank is first recorded with the curve follower. The operation of the curve follower during the recording of a wave is synchronized with the polarization unit P and the recorder R in such a manner that the capacity current is subtracted algebraically from the current through the polarographic cell C. Excellent compensation is obtained as is apparent from Fig. 2.¶

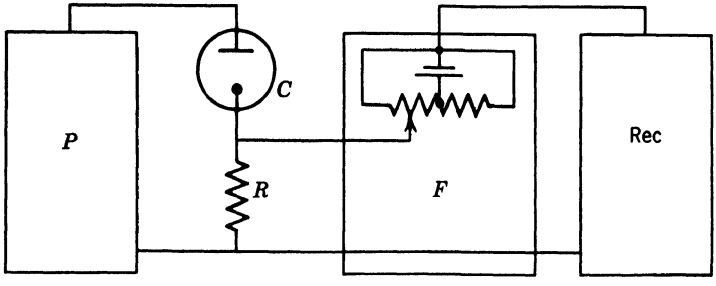

FIG. 4. Schematic diagram of recording polarograph with compensation of capacity current according to Kelley and Miller.[22]

Kelley and Miller reported that application of this method in the range of concentrations 10^{-6} to 10^{-7} M yielded results with a relative error smaller than 20%.

ELECTROLYSIS IN UNSTIRRED SOLUTION WITH A STATIONARY WORKING ELECTRODE AT A POTENTIAL VARYING LINEARLY WITH TIME (OSCILLOGRAPHIC POLAROGRAPHY)

The electrolysis, in this method, is conducted with the same apparatus as in voltammetry at constant potential except that the voltage applied to the cell varies linearly with time. The theory of this method for linear diffusion was developed by Sevcik [46] and Randles [43] for reversible processes involving soluble species. Others treated the case of cylindric electrodes,[42] and the reversible deposition of an in-

¶ It should be pointed out that curve 3 covers a range of potentials in the immediate vicinity of the potential of zero charge (E_m = −0.52 volt vs. SCE) where the capacity current is equal to zero; see equation 7.

soluble species.[3] A treatment for totally irreversible processes is also available [8] (for a general review see ref. 9).

Current–potential curves obtained by this method (Fig. 5) exhibit a maximum. The *peak current* i_p is proportional to the concentration of electrolyzed substance and, in the case of linear diffusion, to the square root of the rate of potential change. Thus,

$$i_p = pC^0v^{1/2} \qquad (16)$$

where C^0 is the concentration of analyzed substance, v is the rate of potential change, and p groups various terms whose explicit form is not needed here.

As in conventional polarography, it is not the absolute value of the faradaic current that determines the lower limit of determinable concentrations but rather the ratio of the capacity current to the peak current. The capacity current can readily be computed from equation 6 since the area of the electrode is constant. Thus,

$$i_c = c_d v \qquad (17)$$

where $v = dE/dt$. It follows from equations 16 and 17 that the ratio i_c/i_p is proportional to the square root of the rate potential change. The influence of the capacity current is minimized when current–potential curves are recorded at rate of potential change as low as possible. In practice, convection interferes with diffusion when the duration of recording exceeds 1 or 2 minutes, and the lowest possible rate of potential change is therefore of the order of magnitude of 0.01 volt per second (1 volt in 100 seconds).

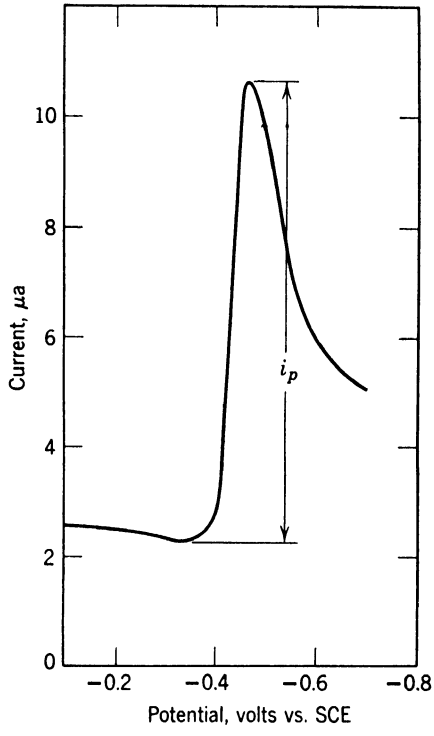

FIG. 5. Tracing of current–potential curve for the reduction of 10^{-5} *M* plumbous ions in 0.01 *M* potassium nitrate according to Strculi and Cooke.[47] Electrolysis in unstirred solution with a stationary working electrode at a potential varying linearly with time (reproduced from *Anal. Chem.*).

Even when the capacity current is not negligible in comparison

with the faradaic current, peak currents can still be determined with fairly good precision because of the favorable shape of current–potential curves. This is particularly so for reversible processes for which the current increases in the vicinity of the peak over a narrow range of potentials (Fig. 5). Irreversible processes yield rather drawn-out curves, and the precision is not so good as for reversible processes.

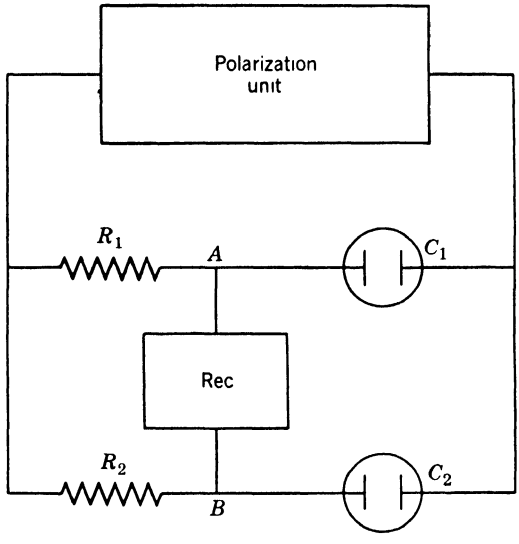

FIG. 6. Schematic diagram of circuit for compensation of capacity current in electrolysis in unstirred solution with a stationary working electrode at a potential varying linearly with time.

Compensation of the capacity current, which has not been made in this method to my knowledge, would be advantageous in the low concentration range. The circuit of Fig. 6 could be utilized to achieve this purpose.

The cells C_1 and C_2 are identical except that one contains the solution being analyzed and the other only the supporting electrolyte. Resistances R_1 and R_2 are identical if the working electrodes of the cells C_1 and C_2 are identical, or R_1 and R_2 are adjusted if this condition is not fulfilled. The value of R_1 or R_2 is selected in such a manner that the ohmic drop across the resistance in series with the cell containing the analyzed substance is of the order of 5 to 10 mv at the peak current. The voltage applied to the recorder *Rec* is, under these conditions, proportional to the faradaic current through the cell containing the analyzed solution.

Concentrations as low as $5 \times 10^{-6} M$, and even lower concentra-

tions, can be determined by this method as was conclusively shown by Streuli and Cooke.[17] These authors utilized a large mercury-pool electrode (about 5 cm^2) and a rather large volume of solution (perhaps 20 ml), but the method could be applied to smaller volumes of solution by the use of the hanging mercury-drop electrode and a polarographic microcell having a capacity of perhaps 0.3 ml. An ordinary polarographic cell could, of course, also be utilized with the latter electrode. The hanging mercury-drop electrode has a more reproducible area than the mercury-pool electrode. Currents observed with the hanging mercury drop would be much smaller (200 times) than with the mercury pool, but this would not cause any problem since pen-and-ink recorders having a sensitivity as high as 10^{-14} ampere per millimeter are commercially available.

ELECTROLYSIS WITH A STATIONARY WORKING ELECTRODE AT A PERIODICALLY VARYING POTENTIAL (SINE OR SQUARE WAVES)

The experimental conditions in this method are the same as in electrolysis at constant potential in unstirred solution except that the potential of the working electrode now includes a periodic component of small amplitude (not more than 5 mv). An alternating current whose magnitude depends, among various factors, on the concentration of analyzed substance flows through the cell. The theory of this method for sinusoidal currents was developed by several authors, among whom Ershler, Gerischer, Grahame, and Randles should be particularly noted. Details will not be given here because the method is primarily useful in electrochemical kinetics rather than in analytical chemistry, and the reader is referred to the original literature (see a general review in ref. 9).

Because of the rapid variations of potential, the capacity current is relatively large, and application to trace analysis is probably out of the question. However, the work of Franck [11] on the application of this method to the determination of the equivalence point of titrations should be mentioned. Rather conventional titrations were carried out sometimes at low concentrations—for example, the titration of $10^{-5}\,M$ potassium iodide with $10^{-5}\,M$ silver nitrate—and the equivalence point was determined from a plot of alternating current through the cell against volume of titrant. Frequencies as low as a few cycles per second were utilized at low concentrations to minimize the capacity current.

Barker and Jenkins [2] developed a method of electrolysis with superimposed square-wave voltage which can be applied to concentrations as low as $2 \times 10^{-7} M$ at least for the determination of reversibly reduced or oxidized substances. The effect of the capacity current is virtually eliminated by making the time constant of the measuring circuit much smaller than the period of the square wave. The capacity current is, under these conditions, practically equal to zero well before the reversal of voltage at the end of the period. The faradaic current is measured at the end of each period: i.e., at a time when the capacity current can be neglected. The method of Barker and Jenkins has the disadvantages of involving rather complicated instrumentation and of being essentially limited to reversible systems.

ELECTROLYSIS AT CONSTANT POTENTIAL WITH STIRRED SOLUTION OR A MOVING WORKING ELECTRODE

The limiting current i_d (Fig. 2) corresponding to the upper plateau of a polarographic wave is controlled by the rate of diffusion of the analyzed substance under the conditions generally encountered in polarographic analysis. Diffusion is rather a slow mass-transfer process, but the rate of transfer of reagent toward the working electrode can be increased either by stirring the solution or by moving the working electrode. The limiting current is increased accordingly, and the conditions are more favorable to trace analysis than in conventional polarography. Current–potential curves obtained under these conditions have the general shape of polarographic waves. The quantitative hydrodynamic interpretation of limiting currents is quite involved even for an electrode having a simple geometry, and it will only be mentioned here that limiting currents are proportional to the concentration of the substance being reduced or oxidized at the working electrode. Details on the theory of limiting currents in stirred solution can be found in several reviews.[9, 25, 49]

The geometry of the electrode-cell assembly can be greatly varied, and numerous types of rotated electrodes or systems of stirring are possible. The *rotated platinum electrode* devised by Laitinen and Kolthoff [32] has often been used particularly in determinations involving anodic oxidation of the analyzed substance. Platinum electrodes are not very useful at markedly cathodic potentials because of the low overvoltage for hydrogen evolution on this metal (see Table I), and this prompted Cooke [7] to develop a *rotated silver-amalgam electrode.* This electrode is identical to the one of Laitinen and Kolt-

hoff, except that an amalgamated silver wire is the working electrode instead of a platinum wire. The electrode can be kept over periods as long as several months, and the reproducibility of limiting currents is excellent (errors of perhaps 1 to 2%). The gain in sensitivity over the dropping-mercury electrode is very substantial, as can be seen from Fig. 7, and concentrations as low as $10^{-6}\,M$ can be determined. The limiting current fluctuates continuously about its average value, but this does not cause any serious difficulty since these fluctuations do

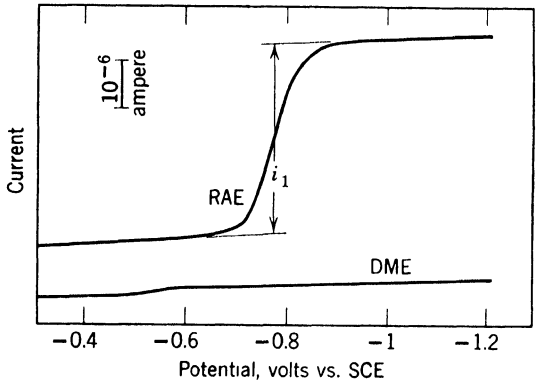

FIG. 7. Tracings of current–potential curves for the reduction of $10^{-5}\,M$ cadmium ion in $0.02\,M$ potassium chloride on the dropping-mercury electrode (DME) and the rotated amalgam electrode (RAE) after Cooke [7] (reproduced from *Anal. Chem.*).

not show on the current–potential curve if the recorder is properly damped. Appreciable hydrogen evolution begins at potentials less cathodic than for the dropping-mercury electrode, and the rotating silver-amalgam electrode fails in trace determination at potentials more cathodic than −1 volt versus the saturated calomel electrode.

A rotated mercury electrode of very ingenious design was described by Lee [33] who also gave a detailed treatment of the resulting current–potential curves. A small volume of mercury (0.1 ml) is placed in a plastic cup with an annular groove. The cup is rotated at a velocity that can be varied from 200 to 1800 rpm, and the mercury forms a ring which is used as working electrode. Current–potential curves similar to those obtained in polarography are observed, but limiting currents are much larger (200 times) than in the latter method, and concentrations as low as $10^{-7}\,M$ can be determined (Fig. 8).

Stationary mercury electrodes with external stirring such as the electrode devised by Arthur and co-workers [1] could possibly be applied to trace analysis. Finally, vigorous conditions of stirring such as those

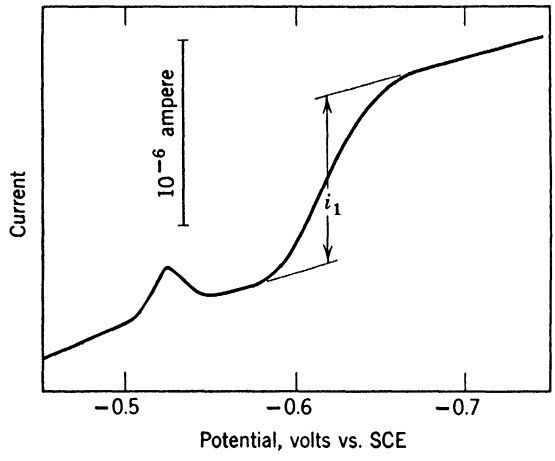

FIG. 8. Tracing of current–potential curve for the reduction of 2.5×10^{-7} *M* cadmium ion in 0.1 *M* potassium chloride on the rotated mercury electrode after Lee [33] (reproduced from the *J. Am. Chem. Soc.*). Note that the peak at −0.53 volt does not correspond to the reduction of cadmium ion.

prevailing in the *convection electrode* of Kolthoff and Jordan [24] might be advantageous in the voltammetric determination of low concentrations.

ELECTROLYSIS AT CONSTANT CURRENT WITH A STATIONARY WORKING ELECTRODE IN UNSTIRRED SOLUTION (VOLTAMMETRY AT CONSTANT CURRENT, CHRONOPOTENTIOMETRY)

The electrolytic cell in voltammetry at constant current is essentially the same as in electrolysis at constant potential. The cell is connected to a constant-current power supply, and the potential of the working electrode is recorded during electrolysis. Potential–time curves obtained in this manner are characterized by a *transition time* τ (Fig. 9).

The theory of this method, which was originally developed by Sand [45] and Karaoglanoff,[21] was further extended in more recent years. The reader can find details and references in a recent review.[10] A detailed treatment of the method is also available.[9]

The transition time, which is the counterpart of the diffusion current in polarography, is given by the following equation for a process entirely diffusion-controlled:

$$\tau^{1/2} = \frac{8.55 \times 10^4 n D^{1/2} C^0}{i_0} \tag{18}$$

There τ is the transition time in seconds, n the number of electrons involved in the electrode process, D the diffusion coefficient of the analyzed substance in square centimeters per second, C^0 the molar

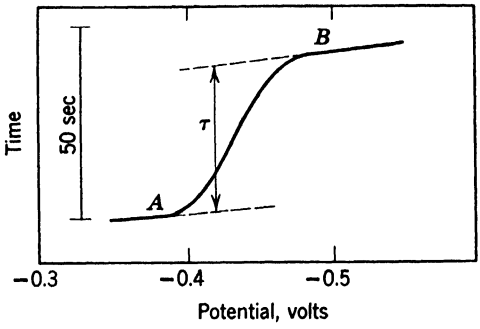

FIG. 9. Tracing of potential–time curve for the reduction of 1.54×10^{-5} *M* plumbous ions in 0.1 *M* potassium chloride according to Reilley et al.[44] Potentials are referred to the silver–silver chloride electrode in 0.1 *M* potassium chloride (reproduced from *Anal. Chem.*).

concentration of the analyzed substance, and i_0 the current density at the electrode in amperes per square centimeter.

Potential–time curves are recorded with a pen-and-ink recorder and transition times of the order of 10 to 50 seconds are generally measured.* The transition time can be adjusted to a suitable value by selection of the current density. It is seen from equation 18 that very dilute solutions could possibly be analyzed by selection of a sufficiently low current density. However, the effect of the charging or discharging of the double layer must be taken into account, and this can be done *approximately* in the following manner.

Assume that the constant current i_0 amperes per square centimeter is passed through the cell in the absence of any reduction or oxidation at the working electrode. The potential of this electrode varies progressively as the double-layer capacity is charged or discharged. We are interested in the distortion of the potential–time curve, and we consider the time interval τ_c elapsed as the potential of the working electrode varies from point *A* (Fig. 9) in the lower segment of the potential–time curve to point *B* in the upper plateau of this curve.

* Much shorter transition times can be measured with an oscilloscope, but this is not necessary in analytical applications.

The time interval τ_c is correlated to the quantity of electricity Q involved in the charging or discharging process by

$$Q = i_0\tau_c \tag{19}$$

The distortion of potential–time curves is negligible, provided that the time τ_c is negligible in comparison with the transition time for the same current density. Thus

$$\frac{\tau_c}{\tau} \ll 1 \tag{20}$$

or

$$\frac{i_0 Q}{(8.55)^2 \times 10^8 n^2 D(C^0)^2} \ll 1 \tag{21}$$

It follows from condition 21 that the effect of the double layer is minimized when the current density is as low as possible and the concentration of analyzed substance as high as possible. Very low current densities should thus be used in the application of this method to trace analysis. Such an application has not yet been investigated, but an experimental evaluation of the method as an analytical tool has been made by Reilley and co-workers.[44] The application of the hanging-mercury drop electrode in this method deserves an experimental study.

Voltammetry at constant current could possibly be applied to the determination of traces of metals which form amalgams. These metals would be reduced on a mercury electrode, and transition times for the anodic oxidation of the metals in the resulting amalgam would be subsequently determined.

ELECTROLYSIS WITH PERIODICALLY VARYING CURRENT IN UNSTIRRED SOLUTION (OSCILLOGRAPHIC POLAROGRAPHY)

This method is similar to voltammetry at constant current except that the current through the cell is a periodic function of time, generally a sine or square wave. This method was developed and studied in great detail by Heyrovsky. The reader is referred to a monograph [18] for an evaluation of the method as a possible tool in trace analysis.

MISCELLANEOUS METHODS

Catalytic Currents. Low concentrations of substances which catalyze electrolytic reductions or oxidations can sometimes be determined

by polarography. Applications, although not numerous, can be of interest in specific cases. A few examples will be given.

Hydrogen peroxide reacts with various substances to form peroxy compounds which are generally more easily reduced electrolytically than hydrogen peroxide. For example, molybdates, tungstates, and vanadates form peroxy derivatives and catalyze the reduction of hydrogen peroxide, as was shown by Kolthoff and Parry.[26] The reactions are for molybdates; for instance (molybdate ion is written MoO_4^{-2} for the sake of simplicity):

$$MoO_4^{-2} + H_2O_2 \rightarrow MnO_5^{-2} + H_2O \qquad (22)$$

$$MoO_5^{-2} + 2H^+ + 2e \rightarrow MoO_4^{-2} + H_2O \qquad (23)$$

The peroxy compound formed by reaction 22 is reduced by reaction 23 with the regeneration of molybdate ion. Since reaction 22 is rapid, a rather large cathodic current is observed at potentials at which the direct reduction of hydrogen peroxide yields very small currents. Concentrations as low as 5 gammas of molybdenum per liter can be determined by this method.

Another example of catalyzed reaction is the reduction of oxygen in presence of hydrogen peroxide. The *exaltation* of the oxygen-limiting current probably results [23] from the regeneration of oxygen by reaction of the ion O_2^-, initially formed in the reduction of oxygen, with hydrogen peroxide:

$$O_2 + e \rightarrow O_2^- \qquad (24)$$

$$O_2^- + H_2O_2 \rightarrow OH^- + O_2 + OH \qquad (25)$$

$$OH + e \rightarrow OH^- \qquad (26)$$

Concentrations of oxygen as low as 10^{-7} M can be determined by this method. A rotated gold electrode is particularly useful.[23]

Catalytic currents for the electrolytic evolution of hydrogen in presence of alkaloids or proteins containing sulfhydryl groups is also of practical significance.[25] Such catalytic currents can be explained in the case of proteins by assuming the following reactions: [5]

$$RSH + e \rightarrow RS^- + \tfrac{1}{2}H_2 \qquad (27)$$

$$RS^- + \text{proton donor} = RSH \qquad (28)$$

The theory of polarographic catalytic currents is well advanced and has been reviewed in detail.[9] Reference should be made in particular to the recent treatment of Koutecky.[28]

Suppression of Polarographic Maxima. Traces of substances such as gelatin and dyes suppress partially or completely the pronounced maximum which polarographic waves often exhibit. Calibration curves showing the relative decrease in the height of the maximum against concentration of maximum suppressor are quite reliable and useful in analytical applications. The method is not specific but may nevertheless be advantageous. Details can be found in texts on polarography.[16,25,38,41,48]

Application of Double-Layer Phenomena. The variation of the double-layer capacity which results from the adsorption of polar or-

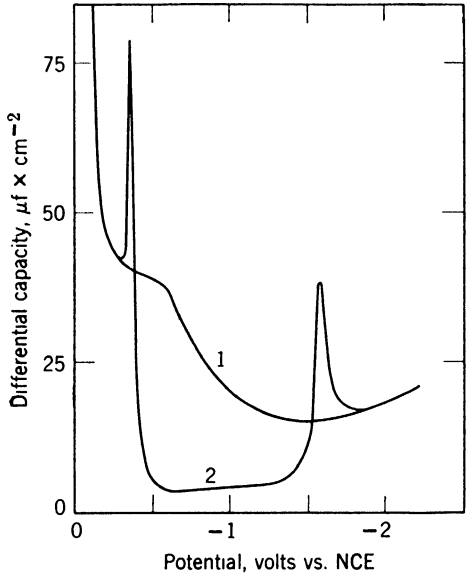

FIG. 10. Variations of differential capacity with potential for a mercury electrode in 0.5 *M* sodium sulfate (curve 1) and in the same solution saturated with heptyl alcohol (curve 2) after Grahame[13] (reproduced from *Chem. Revs.*).

ganic molecules on an electrode can be applied to analytical determinations. It is observed—and it can be accounted for theoretically[6]—that the amount of adsorbed substance depends on the electrode potential. The organic substance is completely desorbed at sufficiently cathodic and anodic potentials, and the double-layer capacity has then the same value as for the electrolyte in the absence of the organic sub-

stance. At intermediate potentials, however, the double-layer capacity is markedly affected (Fig. 10). Differential capacities are generally measured with an a-c bridge,[13] but the variations of the differential capacity with potential can be directly obtained, as was shown by Loveland and Elving,[37] by electrolysis with potential varying linearly with time. This follows directly from equation 6. Loveland and Elving made a detailed study of the adsorption of several alcohols on the dropping-mercury electrode by this method and deduced a correlation between the peak potentials (Fig. 10) and the concentration of alcohol. Such studies could possibly lead to useful analytical methods.

CONCLUSIONS

Conventional polarography can be applied without compensation of the capacity current at concentrations as low as $10^{-5}\,M$. However, compensation with a current varying linearly with potential is advantageous when concentrations below $5 \times 10^{-5}\,M$ have to be determined. The concentration range is then extended to $5 \times 10^{-6}\,M$ or even $10^{-6}\,M$ in favorable cases. The determination of concentrations as small as $10^{-7}\,M$ by conventional polarography is possible in some cases, provided that the capacity current is compensated by means of a curve follower. The application of the latter method requires relatively complicated instrumentation, and voltammetry with a stationary working electrode at a potential varying linearly with time is probably more advantageous. Concentrations as low as $10^{-6}\,M$ can be determined by the latter method without compensation of the capacity current. The limit of $10^{-6}\,M$ could possibly be decreased by perhaps one order of magnitude by the use of a simple circuit for the compensation of the capacity current.

Voltammetry with a rotated working electrode or with a stationary electrode in stirred solution is also a valuable tool in trace analysis especially because capacity currents are generally so low that they do not markedly distort current–potential curves.

Voltammetry at constant current could possibly be useful in trace analysis.

Volumes of solution of the order of 10 to 20 ml are most frequently used in polarography and voltammetry, but these methods can be applied even when only volumes of solution as small as 0.1 to 0.3 ml (and even smaller volumes) are available. Electrolysis with such small volumes of solutions is possible in conventional polarography and in voltammetric methods with stationary electrodes in unstirred solution.

Voltammetry in stirred solution or with rotated electrodes requires volumes of at least a few milliliters.†

DISCUSSION

Discussion Leader
Philip J. Elving
University of Michigan

Dr. Delahay has very ably defined the areas of polarography that may be of service in trace analysis. The present discussion is intended to emphasize several points involved in applying polarography to trace analysis, in particular, to consideration of means of circumventing or at least minimizing the two limitations on the use of polarographic techniques in trace analysis emphasized by Dr. Delahay.

It should be admitted that polarography as a technique for analyzing complex samples does not offer the same extent of general applicability and of discrimination that absorption spectrophotometry does, especially when applied to the analysis of multicomponent systems. On the other hand, this decreased applicability may enable the polarograph to be more immediately applicable in certain situations by reason of this compulsory selectivity.

One of the potential advantages in applying polarography to the determination of minor components in mixtures is the flexibility available in securing desirable separation of polarographic waves and of suppressing or shifting undesirable waves by varying the composition of the background electrolyte and the experimental conditions. Such possible means of increasing selectivity or even specificity of measurement should always be kept in mind. In this connection, mention should be made of the value of using in certain situations in trace analysis rather dilute, e.g., ca. 0.01 *M*, background electrolyte solutions to decrease the residual or capacity current.

Dilution by Background Agent. A problem that occasionally arises in trace analysis in connection with polarographic studies is the possible excessive dilution of the sample due to the addition of the background electrolyte. In most cases this is a trivial problem since one can usually add a saturated solution of the background material and the dilution need not exceed a few per cent. Nonconducting liquid

† The vibrating electrode of Lindsey [34a] might be more advantageous than the rotated electrode when small volumes of solution must be used.

samples may require greater dilution, owing to the necessity of having a final cell solution whose electric conductivity is appreciable. With certain organic samples, there may be a miscibility problem. Hydrocarbon samples can usually be diluted with an equal volume of methanol or 2-propanol, in which lithium chloride has been dissolved, to give a homogeneous cell solution of sufficiently low resistance that the resulting *IR* drop on polarographing the solution does not introduce appreciable difficulty.

Analysis of Air for Trace Components. One phase of trace analysis, to which polarography has been increasingly applied, is the determination of minute amounts of substances in gases, particularly in air as in industrial and civic hygiene and toxicological studies. The general technique applied has involved use of the polarographic background solution or part of this solution as the absorbing agent through which the sample gas is led; by increasing the period of absorption, it is often possible to build up the concentration of the desired substance to a conveniently measured range. In addition, it is possible to secure increased specificity in the sampling or trapping process by using as a component of the absorbent solution a reagent that reacts chemically with the desired species. For example, a solution of hydroxylamine or a suitable derivative may be used to remove carbonyl compounds from a stream of air.

The scope and sensitivity of applicability can often be increased by the use of the proper reactive absorbers. A number of papers have described the determination of minute amounts of benzene in air by aspirating the air stream through a solution of nitric and sulfuric acids, in which the benzene is converted to dinitrobenzene. Subsequent polarography of the absorber solution affords a rather high current flow per unit benzene concentration due to the reduction of both nitro groups, resulting in an effective 12-electron process per benzene molecule which is itself polarographically inactive.

Increase of the Ratio of Faradaic to Capacity Current. Dr. Delahay very justifiably stressed as one of the two major limitations on the application of polarography to trace analysis the difficulty encountered in the ratio of the faradaic current (usually considered as the diffusion current and originating in the electrolysis of the species being determined) to the capacity current (the so-called residual current, whose magnitude is due to the solution and electrode natures, the applied potential, and the experimental conditions). A value of this ratio exceeding unity is essential for valid trace analysis; Dr. Delahay pointed out two ways of correcting for the capacity current electrically

and indicated the inherent difficulties involved in the use of these methods. I would like to discuss some of the possibilities of altering solution composition, electrode nature, and experimental conditions which may result in decreasing the relative effect of the capacity current.

Specifically, I am going to discuss a solid indicating electrode, the graphite electrode, which was first proposed for use in polarography by Lord and Rogers [50] and which has been applied by Gaylor, Conrad, Landerl, and Elving.[51–53] In general, solid electrodes with their enhanced faradaic current to concentration ratios are preferable to the dropping-mercury electrode for trace analysis.

If one decreases the rate of polarization, i.e., the rate at which the potential applied to the solid electrode is varied, the capacity current is decreased whereas the faradaic current is unaltered. This is to be expected since the magnitude of the capacity current depends, among other factors, on the magnitude of the derivative dE/dt, where t represents time and E potential. Practically, this implies that as slow a scanning rate as possible should be used; e.g., if a conventional recording polarograph is being used, the minimum voltage span should be used for the standard scanning cycle. The Sargent Model-XXI Polarograph can be set to cover 0.5 to 3.0 volts in a period of 13 minutes. The Leeds & Northrup Electrochemograph uses a standard 2-volt span per 10-minute scanning cycle; however, it is possible to secure an accessory which permits use of a 0.5-volt or 0.2-volt span.

Another readily variable experimental factor, which can be used with solid electrodes, is stirring. Stirring will increase the faradaic current markedly while the capacity current will hardly vary. However, a word of caution is in order. Although stirring may increase the faradaic current manyfold, it may also decrease the reproducibility of the curve with the measurement error for the current increasing: e.g., from 2% to 10%. On the other hand, it is often true that the increased error in measuring a trace constituent is less important than the fact of being able to measure the trace at all.

Other possibilities for increasing the ratio of faradaic to capacity current can be found for different types of electrodes. For example, impregnating the pores of a graphite electrode with a wax or certain other types of material results in a decrease in both faradaic and capacity currents; however, the latter is decreased much more than the former. Typical results are those obtained in an unstirred solution which was $10^{-4}\,M$ in N,N′-di-*sec*-butyl-*p*-phenylenediamine in *p*H 5 acetate buffer in 1:1 water–2-propanol solution; the ratio of faradaic

current to capacity current was 0.1 for an unimpregnated graphite electrode, 7 for one treated with a silicone, and 20 for one impregnated with castor wax.[52]

It was also found possible to add an enhancing agent which would serve to increase the faradaic current without appreciably affecting the properties of the capacity current. In determining compounds of the phenylenediamine type, addition of a metal soap served to increase the faradaic current three- to fivefold.

Avoidance of Electrode Reactions Due to Impurities. The other major difficulty stressed as limiting polarographic investigation in trace analysis is that due to side electrode reactions which produce faradaic current; these usually involve the electrolysis of electroactive impurities in the background electrolyte system and the electroreduction of trace amounts of oxygen which were not removed from the test solution. The effect of electroactive impurities in the background system can usually be minimized, if not totally avoided, by a preliminary electrolysis of the background solution at the most advanced potential to be reached during the subsequent polarographic procedure; the sample is then added to the purified background solution.

As for the removal of the effect of residual dissolved oxygen, one perhaps obvious but nevertheless useful solution would be to avoid the difficulty entirely by switching, where possible, from the use of electroreduction to electrooxidation processes. Solid electrodes are generally better suited for anodic processes than mercury electrodes, owing to the ready oxidation of the mercury itself.

Determination of Inhibitors in Gasoline. A good example of the combination of the items mentioned in the previous paragraphs for a difficult type of trace analysis is in the polarographic determination of aminophenol- and phenylenediamine-type oxidation inhibitors in gasoline by their anodic wave.[53] The range of concentration of the inhibitor in gasoline is of the order of 1 to 30 ppm.

The experimental procedure is very simple. The gasoline sample is diluted with an equal volume of 2-propanol which is 0.2 *M* in lithium chloride. A small amount of barium naphthenate dissolved in isooctane is added as enhancing agent. The resulting test solution is transferred to a polarographic cell employing a simple electrode arrangement consisting of a wax-impregnated graphite rod (actually a spectroscopic carbon) as indicating electrode and an internal silver–silver chloride reference electrode. After pretreatment electrolysis to condition the electrodes, the solution is polarographed over the range of -0.25 to $+0.75$ volt, using a Sargent Model-XXI Polarograph.

In the range of 5 to 30 ppm of inhibitor in the gasoline (which would give half of this concentration in the polarographic cell), an accuracy and precision of 10% or better was obtained. One to two parts per million of inhibitor were determinable; this concentration corresponds to an inhibitor concentration in the polarographic cell of $3 \times 10^{-6} M$; the diffusion current for the specific arrangement and conditions used was about 0.2 μa.

QUESTIONS AND ANSWERS

LOCKHART B. ROGERS: I wonder whether by switching impregnating agents for the graphite electrode you really change the capacity or merely change the apparent or temporary capacity of the electrode which results from diffusion of the solution into the pores of the impregnated portion.

E. H. SWIFT: Paul, would you have any notion? I am not sure whether you are acquainted with the details.

PAUL DELAHAY: Adsorption of organic substances on an electrode affects the double-layer capacity but not to the point of greatly changing the ratio of capacity current to faradaic current. It is probable that the main effect of the addition of wax is to plug the pores. Thus, the true area of the electrode is greatly reduced, and the capacity current is decreased accordingly. The limiting current is not decreased so much as the capacity current because depletion of the electrolyzed substance in the pores of an untreated electrode, at any rate, makes the contribution of the current corresponding to the pore area quite small in comparison with the total limiting current.

PHILIP J. ELVING: I think that the effects due to changes in adsorption are relatively small; the phenomenon of decreasing the ratio of capacity to faradaic currents on impregnating a graphite electrode with a wax is very likely largely a mechanical effect. One possibility is that in the untreated graphite electrode the solution penetrates into the pores of the electrode where a normal capacity current is produced per unit area of internal pore surface; however, the supply of electroactive material is decreased during the course of the electrolysis, owing to the difficulty of supplying fresh electroactive material from the so-called bulk solution into the restricted and static minute volume of the individual pore. By impregnating the pores of the graphite, one decreases all of this semiavailable electrode area and returns to what may probably be considered to be a more nearly normal polarographic situation.

JAMES J. LINGANE: In a symposium devoted to polarographic trace analysis, two of the most sensitive techniques should be mentioned. These are catalytic currents and the effect of various substances on maxima. I do not think either Phil or Paul mentioned these. Would you like to say something about these possibilities?

PAUL DELAHAY: I did not have time to discuss catalytic currents and maximum suppressors in the lecture, but this material is covered in some detail in the manuscript. The number of reactions yielding catalytic currents is

very small in comparison with the infinite variety of substances giving ordinary limiting currents. I should think that catalytic currents are useful but limited to a few highly specific cases (oxygen, proteins, etc.; see chapter). Analytical applications of maximum suppressors are very limited in comparison with more conventional analytical applications of polarography for two reasons: (1) Most substances are not maximum suppressors, and (2) maximum suppression is not specific.

PHILIP J. ELVING: It might be emphasized that this very lack of specificity may be an advantage in certain cases. For example, in a solution obtained by dissolving a highly refined sugar, there is a repressive effect on the oxygen maximum which is proportional to the total content of nonsugar material; even though the exact nature of the latter is unknown, the effect observed can be correlated with certain properties and uses of the sugar.

Similarly, a paper published several years ago pointed out that certain types of impurities in petroleum materials had a definite effect on the oxygen maximum. These impurities were apparently nitrogen compounds, perhaps heterocyclics, and the polarographic phenomenon could be used as a very sensitive index for the determination of exceedingly minute amounts of this complex group of trace constituents.

A. A. SMALES: Would Professor Delahay give us his opinion of the potentialities of a-c polarography for trace element determinations?

PAUL DELAHAY: The application is quite limited with superimposed sine waves because the capacity current is preponderant. In applications to trace analysis one should work at very low frequencies—perhaps a few cycles per second. This was done by Franck who applied the a-c method to the detection of end points, but nevertheless did not titrate solutions below $10^{-5}\,M$. Barker and Jenkins used a superimposed square wave of small amplitude (a few millivolts) instead of a sine wave. If the time constant of the cell and its polarization unit are small in comparison with the half-period of the square wave, the capacity current drops practically to zero before the end of each half-period. Very low concentration—$2 \times 10^{-7}\,M$—can thus be determined (see chapter). I might add that applications of a-c methods are limited to so-called reversible or nearly reversible systems. The a-c response for irreversible systems is too small for all practical purposes. Another relative disadvantage in square-wave polarography is the complexity of equipment. One advantage—and a major one in some instances—is the possibility of determining a minor constituent in the presence of a large excess (1000 times) of a more easily reducible substance. This cannot be done in ordinary polarography or voltammetry without preliminary separation (or formation of a complex) of the major component. In many cases it may be simpler to have stirred solution or perhaps electrolysis at constant potential in unstirred solution. Analytical application of the latter method has not been made as far as I know.

A. A. SMALES: Is it not possible to use the stirred electrolyte in combination with the square-wave polarograph and get the advantages of both, in obtaining still better sensitivity?

PAUL DELAHAY: One does not gain much in sensitivity in a-c methods by stirring the solution because the alternating component of the diffusion layer is very thin.

W. D. COOKE: I might also mention that, since I have worked in both square-wave polarography and mercury-pool polarography, and you say there is no advantage in square-wave polarography, I will disagree with you. I think it does have some advantages, which are not apparent.

PAUL DELAHAY: Do you mean electrolysis with a sudden variation of potential or electrolysis with a superimposed square wave of small amplitude?

W. D. COOKE: We have ordinary square-wave polarography in the sense of Barker and Jenkins, but I think the advantage here is that very often a mercury pool does not work for specific applications so that we cannot use it for some things. Under these circumstances, square-wave polarography is useful.

ANDRÉ JULIARD (*Houdry Process Corp.*): Is the uncertainty of the value of the residual current not another factor that limits the application of polarography to the determination of trace amounts of depolarizing species? This uncertainty becomes greater in convection polarography, when the solution is vigorously stirred to increase the sensitivity of the method.

The extrapolation of the residual current by extending the slope of the polarographic curve at the foot of the curve is difficult when the wave is low. On the other hand, the residual current cannot be determined simply by running a blank with the supporting electrolyte, owing to the possible alteration of the residual current by the impurities present in the sample.

PAUL DELAHAY: Since the residual current is the sum of the capacity and faradaic currents for all the impurities, the problem is to purify the supporting electrolyte.

ANDRÉ JULIARD: Another source of residual current is the transfer of a small amount of mercury from the anode to the cathode. This transfer is catalyzed or inhibited by traces of impurities which are not always easily extracted from the sample.

PAUL DELAHAY: Quite naturally, but the shape of current–potential curve is a guide in the correction, although there is some uncertainty when the residual current is not negligible compared to the limiting current.

PAUL DELAHAY (*added in proof*): Since the preparation of this manuscript in the summer of 1955, application of the hanging mercury electrode to analytical determinations, as suggested in this chapter, was made by Shain and co-workers.[54,55] The anodic oxidation of metals after deposition on a stationary mercury electrode, which is also suggested in this chapter in a general way, was developed by Nikelly and Cooke [56] in a very elegant manner (anodic stripping voltammetry) for the analysis of very dilute solutions. These authors used a mercury pool for metal deposition, but it was shown by DeMars and Shain [57] that a hanging mercury electrode is very convenient in such studies. The latter authors were able to determine concentrations as low as 10^{-8} to 10^{-9} M. In addition to the method of anodic stripping voltammetry with continuously varying potential, as developed by Nikelly and Cooke,[56] two other methods have been studied, namely the potential-step and current-step methods.[58] A theoretical analysis of these two methods has been developed. Mention should also be made of recent work on electrolysis in stirred solutions [59] for trace determinations, and of the rotated mercury electrode of Stricks and Kolthoff.[60] Finally, reference is made to a review of electroanalytical methods in trace analysis.[61]

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Amperometry

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ABSTRACT Current may function either as a dependent or an independent variable in voltammetric procedures, and the resulting analytical methods may be classified on that basis. The usual measurement depends inherently on the mass-transfer processes of diffusion and/or convection and therefore has only a transitory existence whether long or short. The techniques employed not only allow direct calculation of concentration but may also serve as sensitive end-point detectors. Instrumentation and applications are described, with consideration given to limiting factors such as the double-layer capacitance and competitive reactions at the electrode surface.

The fundamental measurements in electroanalytical chemistry include *potential, current,* and *time.* The precise relationship among these three measurements and the composition of a sample depend in largest measure on the exact manner in which the measurement is made.

Consider an electrode at equilibrium with the solution in which it is immersed. The electrode will exhibit a potential, invariant with time and related thermodynamically to the composition of the solu-

tion. If by some external means the potential of the electrode is forced to assume a different value, current will then flow in the electrode circuit until the composition of the electrode and/or solution is altered sufficiently to exhibit this new potential under equilibrium conditions.

Although the direction of current and the number of coulombs (current × time) can be predicted a priori from thermodynamic considerations, the magnitude of the current at any particular time is a kinetic phenomenon and, in the last analysis, is determined experimentally. The factors that control the current may be outlined:

I. Mass Transfer of Chemical Species to the Electrode Surface. It is clear that a species can be oxidized or reduced at the electrode only if and when the species is brought to the electrode surface. Three methods for accomplishing this transport are through migration, diffusion, and convection.

1. Migration. In migration, the driving force which operates to move the species to (or away from) the electrode surface is the force exerted on a charged particle by a potential gradient existing in the body of the solution. Thus, for example, if the potential of the electrode is made more negative than its equilibrium potential, a potential gradient will be established in the body of the solution such that positively charged species will be attracted to the electrode, negatively charged species repelled, and no effect exerted on neutral species. In conductometric measurements, migration is the sole factor that limits current. In amperometry it is desirable to eliminate the contribution of migration. This end is accomplished by addition of an excess (about 100-fold) of inert electrolyte which in turn decreases the potential gradient proportionately.

2. Diffusion. This transport mechanism has its origin in a gradient of chemical potential, or more simply in a concentration gradient. Thus, if the concentration of the species in the bulk of the solution is greater than the concentration at the electrode surface (because of the electrode reaction), the species will tend to diffuse from the bulk of the solution toward the electrode surface. Under certain experimental conditions, useful equations relating the current and concentration of the species can be derived (on the assumption of diffusion as the sole means of transport) and applied to actual analysis.

3. Convection. This transport of species is accomplished whenever the solution bearing the species is stirred into the path of the electrode. This stirring action increases the rate of transport of species to the electrode and consequently increases the current.

II. Conversion of Species to "Active" Form. The electron transfer process may be considered to occur between the electrode and an "active" form of the species. The active form may be a particular one of several species in equilibrium with another, the equilibrium being shifted toward the active form as it becomes depleted through electrolysis. This shift may occur (*a*) in the diffusion boundary layer ("kinetic" currents), (*b*) at the electrode surface. If the shift in equilibrium occurs at a rate much slower than the rate of mass transfer, the current may depend entirely on this rate of conversion.

III. Electron-Transfer Process. After the species has been transported to the electrode surface and has been converted into active form, the electron-transfer process may be considered the next step. The rate of electron transfer depends on the nature of the active species and the potential of the electrode. Because this rate can be changed at will simply by altering the potential of the electrode, this process generally need not be the limiting step in determining the current.

The electron-transfer process also occurs in the reverse direction with the product at the electrode surface. The ratio of the forward and reverse rates of electron transfer depends on the potential.

At a given potential, the equilibrium potential, these two rates will be of equal magnitude and of opposite direction. The magnitude of the rates at the equilibrium potential, the "exchange" current, determines the reversibility or irreversibility of the over-all electrode process. If the rates of electron transfer are much greater than the rate processes I and II, the electrode process is reversible. If the electron-transfer rates are of the same magnitude or smaller than the rate processes I and II, the electrode process is irreversible, the degree of irreversibility increasing, the slower the rates of electron transfer compared to the rates of process I and II.

IV. Removal of Product. The accumulation of product may exert an effect on processes I to III, and rate of removal of product may consequently become a determining factor in the over-all rate processes which comprise the electrode reaction.

It is clear that one or more of processes I, II, III, and/or IV may constitute the slow step in the over-all rate process of which the current is a direct measure. By altering experimental techniques, emphasis on a particular step may be shifted to a different step. For this reason the mode of operation is of primary importance in determining the relationship between sample composition and current. The pattern of this chapter is consequently organized on this basis.

UNSTIRRED SOLUTIONS (usually diffusion-controlled current)

1. Dropping-Mercury Electrode (DME)
2. Stationary Electrodes

STIRRED ELECTRODES AND SOLUTIONS (usually convection- and diffusion-controlled current)

1. Rotating Platinum Electrode (RPE)
2. Rotating Mercury Electrode
3. Vibrating Electrodes
4. Convection-Controlled Electrode
5. Stirred Solutions
   (*a*) Sensitive End-Point Procedure with Platinum Electrode
   (*b*) Sensitive End-Point Procedure with Mercury Pool Electrode
   (*c*) Dual-Indicator Electrodes
      (1) Direct Current
      (2) Alternating Current

Scope of Amperometry

Amperometry in a generic sense implies the measurement of current, just as the field of *potentiometry* implies the measurement of

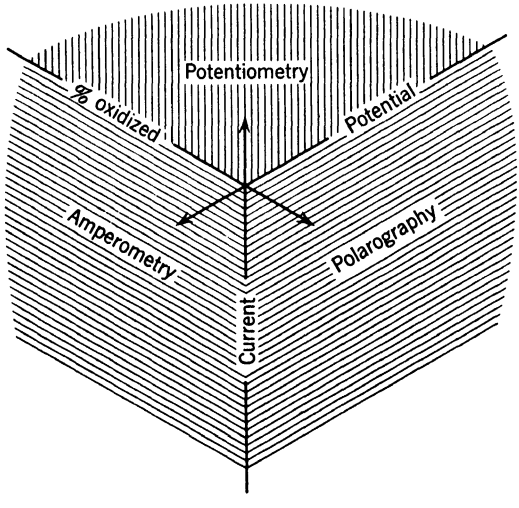

FIG. 1. Scope of amperometry.

potential. In *polarography* both potential and current are measured. The relationship between these three methods is illustrated in Fig. 1. Amperometric titrations are constant-potential planes (current versus volume of titrant), potentiometric titrations are constant, often zero,

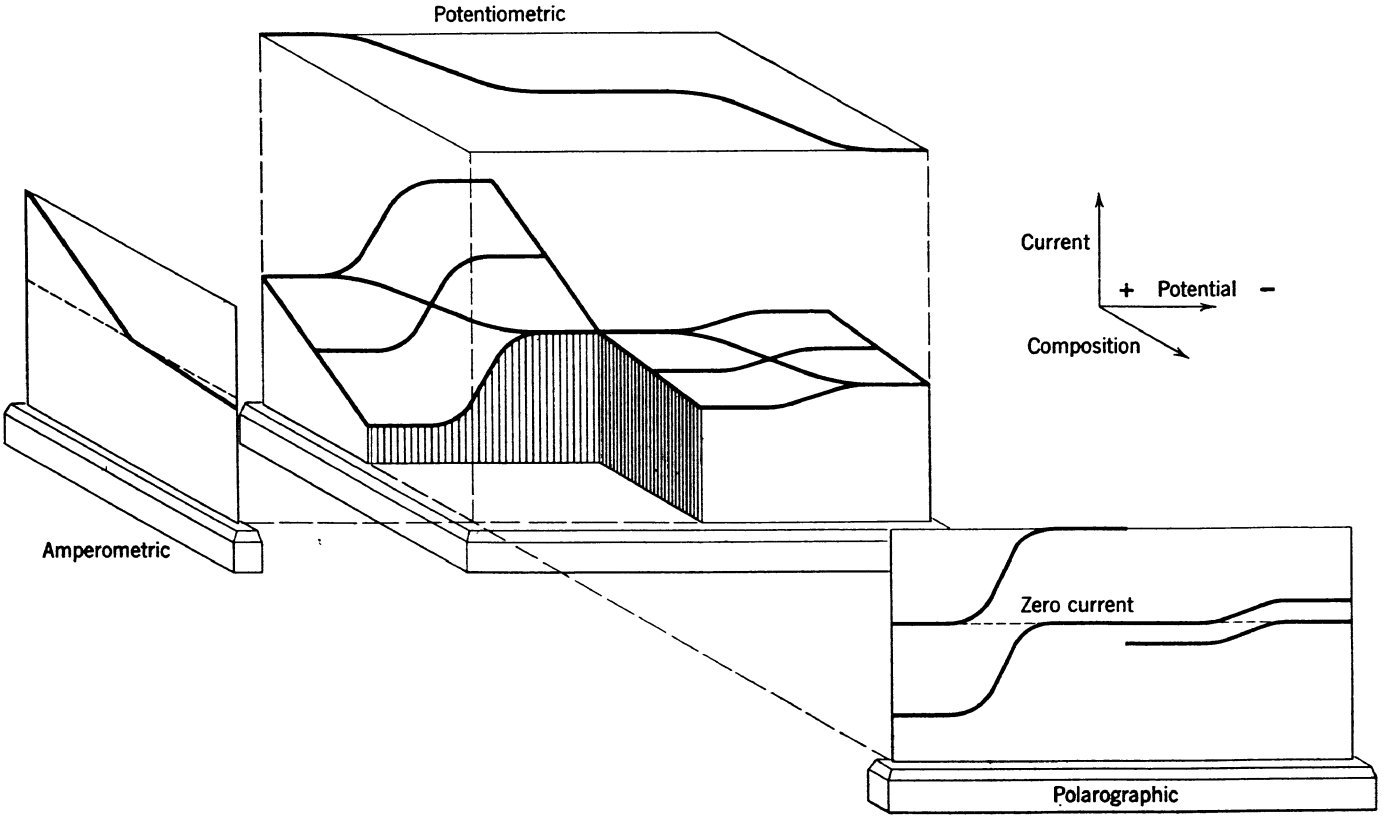

FIG. 2. Relationship among potentiometric, amperometric, and polarographic techniques for two reversible, diffusion-controlled redox systems.

current planes (potential versus volume of titrant), and polarography is represented by planes (current versus potential) for constant composition of solution. A knowledge of any one of the three methods is considerably enhanced by an understanding of the other two. Figure 2 illustrates the three methods for a diffusion-controlled process where one reversible redox system is titrated by a second reversible redox system. The surface, plotted in three dimensions, with current, per cent oxidized, and potential as coordinates, provides the fundamental picture. Equations for the diffusion-controlled reversible redox system and the consequent planes obtained by placing certain restrictions (e.g., constant potential) on the equation are described in further detail by Reilley, Cooke, and Furman.[50] Such surfaces are easily obtained experimentally, and the method can in this way be readily extended to the more complicated situations where the process is neither diffusion-controlled nor reversible. Kolthoff[21] has discussed these relationships from a similar standpoint.

General Methods of Amperometric Titrations

Shape of the Titration Curve. The course of a titration may be followed by employing amperometry as a technique for indicating the concentration of one or more of the species involved in the titration reaction:

$$\underset{\text{Sample}}{X} + \underset{\text{Titrant}}{T} \rightarrow \underset{\text{Product(s)}}{P_1} \qquad (1)$$

The shape of the titration curve depends on which of the species is indicated by the electrode process which in turn depends on the potential applied across the cell. Because current either will or will not flow for each of the three species, eight possibilities (2^3) exist and are summarized in Table I. Actually there is an infinite number if exact quantitative values are considered. In addition, the active species may give rise to anodic or cathodic currents. Furthermore the

TABLE I

POSSIBLE AMPEROMETRIC TITRATION CONDITIONS

× = active species				○ = nonactive species			
Type	*X*	*T*	P_1	*Type*	*X*	*T*	P_1
1	○	○	○	5	○	×	○
2	×	○	○	6	○	×	×
3	×	×	○	7	○	○	×
4	×	×	×	8	×	○	×

same line of reasoning can be extended to the successive titration of two-component mixtures where reaction 1 is followed by reaction 2:

$$\underset{\substack{\text{Sample}\\(\text{Second}\\\text{component})}}{Y} + \underset{\text{Titrant}}{T} \rightarrow \underset{\text{Product}}{P_2} \tag{2}$$

The actual shape of the titration curves is further governed by factors such as the equilibrium constants for reactions 1 and 2, rates of reaction, interferences and impurities, and linearity between the current and the concentration of the active species. This last factor is strongly dependent on the mode of operation employed.

If a linear relationship is assumed between the current and concentration

$$i = k_n C_n \tag{3}$$

certain factors concerning the accuracy of a method can be readily established. First, the actual slopes of the lines before and after the end point may be calculated from the values of k_n. The value of k_n may be positive or negative (cathodic or anodic currents, respectively). Before the end point the slope is given by

$$\text{Slope} = \frac{\Delta_i}{\Delta\text{titrant}} = \frac{k_P - k_V}{V} \tag{4}$$

where V corresponds to the volume of solution being titrated. The slope of the titration line after the end point is given by

$$\text{Slope} = \frac{\Delta_i}{\Delta\text{titrant}} = \frac{k_T}{V} \tag{5}$$

Least ambiguity in locating the end point occurs whenever the angle of intersection of the two titration lines is most acute: that is, for situation where the *difference* in slope of the titration lines before and after the end point has its largest absolute value.

$$\text{Difference in slope} = \frac{(k_X - k_P) - k_T}{V} \tag{6}$$

In practice one selects the applied potential and supporting electrolyte (and employs reasonably small volumes) such that the equation 6 has its largest absolute value.

No break occurs in the titration where $k_X + k_T - k_P = 0$. It is conceivable that this condition may be realized for types 1, 4, 6, 8 and

impossible for the other types. This does not constitute necessarily an "impossible titration" condition. In some instances,[41] such as titration of ferric ion with vanadous ion at a potential of −0.80 volt vs. SCE, the end point is taken at the point where the titration curve crosses the zero current line.

Another desirable situation is to operate under conditions where the current value at the end point is zero or nearly so. This condition ($k_P = 0$) allows the sensitivity of the current-measuring device to be increased considerably near the end point and consequently minimizes errors attributed to the measurement of current.

Methods for Indicating Species

Given the titration reaction represented in equation 1, how can one establish conditions so as to obtain a direct, and preferably linear, relationship between current and the concentration of a species (e.g., *X*, *P*, or *T*)?

The general answer is to devise an electrode reaction in which the species is involved as a *limiting* reactant. The species itself may be oxidized or reduced at the electrode.

$$X \pm ne \rightarrow \text{Products} \tag{7}$$

A linear relationship is then obtained if the reaction rate is controlled by the rate of diffusion and/or convection of *X* to the electrode surface.

In some cases, the species itself cannot be oxidized or reduced but may take part in the electrode reaction. For example, in complexometric titrations of metal ions using EDTA as titrant, the excess EDTA (H_4Y) appearing after the end point can be indicated by the following electrode reaction:

$$Hg + H_nY^{n-4} \rightarrow HgY^{-2} + nH^{+} + 2e \tag{8}$$

The amperometric technique can be adapted to acid–base titrations in a similar manner. By adding an excess of

$$HAc + HgY^{-2} + 2e \rightarrow Ac^{-} + Hg + HY^{-} \tag{9}$$

the Hg(II)–EDTA complex, the rate of the electrode reaction is limited by the concentration of the acid, HAc. The reverse reaction (right to left in equation 9), in presence of excess EDTA, may be employed to follow the concentration of the base, Ac^{-}, in a titration.

AMPEROMETRIC TECHNIQUES

Unstirred Solutions

Dropping-Mercury Electrode (DME). Laitinen,[32] Meites,[42] and Kolthoff and Lingane [28] have discussed in detail the advantages and disadvantages of amperometric titrations with the dropping-mercury electrode.

Advantages. 1. Renewed surface. This is especially desirable for precipitation reactions. As a result of this feature the current values are quite reproducible.

2. High hydrogen overvoltage of mercury. Because mercury exhibits a high overvoltage for evolution of hydrogen but low overvoltage for many other reactions, the scope of amperometric titrations is very wide. This feature makes possible the use of titrants (organic and inorganic) which are not potential-determining but which yield irreversible polarographic waves with well-defined diffusion currents.

3. Extrapolation to the end point. Because of the linear relationship between current and concentration, the end point may be determined by extrapolation of points obtained remote from the end-point region. This feature is useful in cases where equilibrium is attained slowly or where the titration reaction has an unfavorable equilibrium constant. Also only a few points need be taken on either side of the end point, which decreases the time required for a titration. The use of several points in effect "averages" several experimental current values and leads to a more reliable end-point determination. The absolute relationship between current and concentration is not necessary and therefore accurate calibration of the capillary, strict attention to the type and concentration of supporting electrolyte, and accurate temperature control are not necessary, unlike the situation for polarographic determinations. Simplified and less accurate equipment is required for amperometric titrations.

Disadvantages. 1. A practical disadvantage is that amperometric titrations are generally carried out at potentials more negative than -0.2 volt of SCE and consequently must usually be performed in the absence of oxygen. Considerable time is required to remove dissolved oxygen from the solution before titration and after each addition of titrant. One method of obviating this latter difficulty is to employ oxygen-free reagents, using the apparatus of Kolthoff and Langer.[26] A more convenient approach is to employ a titration cell such as that designed by Laitinen and Burdett.[33] A commercial form of this cell,

illustrated in Fig. 3, is available from E. H. Sargent Company, Chicago, Ill. The fritted-glass disk at the bottom of the cell causes the inert gas to issue through the body of the solution in very small bubbles so that time required to purge the solution of oxygen is decreased from about 10 minutes to only 30 seconds! Furthermore, the capillary is inserted into a shield which prevents disturbance of the diffusion cur-

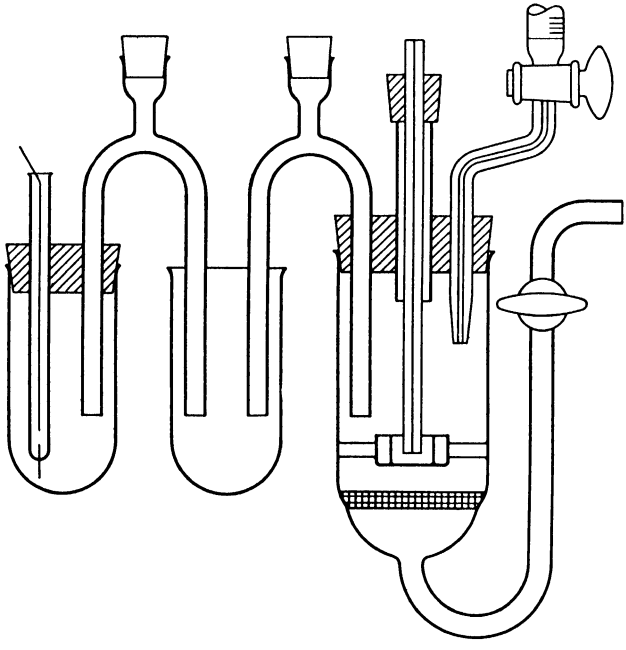

FIG. 3. Laitinen-Burdett-Sargent cell for amperometric titrations. (Courtesy E. H. Sargent Co.)

rent by the flow of gas. This feature allows continuous titrations with only a short time lag between addition of titrant and diffusion-current response.

2. *Residual current.* The diffusion current for the dropping-mercury electrode is given by the Ilkovic equation

$$i_d = 605nD^{1/2}m^{2/3}t^{1/6}C \qquad (10)$$

where the symbols have their usual meaning.[28a] Greatest weight sensitivity for a given species is obtained therefore by working in smaller volumes (e.g., higher concentrations), at potentials corresponding to large n values, and at high mercury pressures where the terms $m^{2/3}t^{1/6}$ would be larger. The residual current attributed to charging

of the electrical double layer is proportional to $m^{2/3}t^{2/3}$ and serves as one of the serious limitations to the use of the dropping-mercury electrode for microtitrations. The signal-to-noise (*diffusion current* to *charging current*) ratio is given by

$$\frac{i_d}{i_c} = k\frac{m^{2/3}t^{1/6}}{m^{2/3}t^{2/3}} = kt^{-1/2} \qquad (11)$$

Consequently more favorable response is obtained by working at high mercury heights. With the dropping-mercury electrode, the magnitude of the residual current becomes larger than the diffusion current of the species below 10^{-5} *M*. Kelley and Miller [19] describe a high-sensitivity polarograph with a linear compensator to minimize the effect of the residual current. The apparatus, however, is rather complicated and not entirely suitable for amperometric titrimetry.

3. Presence of interferences. The presence of large amounts of materials which yield polarographic diffusion currents at lower potentials than that employed for the titration cause considerable increase in the error in locating the end point. Changing the value of the applied potential, altering the supporting electrolyte, and/or addition of complexing agents may be helpful in some cases. Delahay [8] has discussed the methods for partially overcoming limitations imposed by side reactions attributed to electrolysis of solvent, presence of impurities such as O_2 and metal ions, as well as special electrode effects such as the formation of PtO_2 on platinum electrodes.

The addition of gelatin was found by Reilley, Scribner, and Temple [52] to reduce interference from the metal complexes in the complexon titration of lead–bismuth mixtures as illustrated in Fig. 4. By adding gelatin (0.033%) to the solution, the diffusion current attributed to the bismuth–EDTA complex is eliminated selectively, and the diffusion current of the lead ions could then be measured with greater sensitivity. This effect of gelatin furthermore appears to be a general phenomena in eliminating the diffusion current attributed to the metal–EDTA complexes. Complexometric titrations with EDTA promise to be useful for titration of microquantities of materials because of the great stability of their complexes and rapid attainment of equilibrium.

Applications. Amperometric titrations using the dropping-mercury electrode may be applied to the titration of solutions containing 100 μg per ml on a routine basis with an accuracy of about 1%. Under carefully controlled conditions this concentration or error level may be reduced about fivefold. Table II summarizes some typical appli-

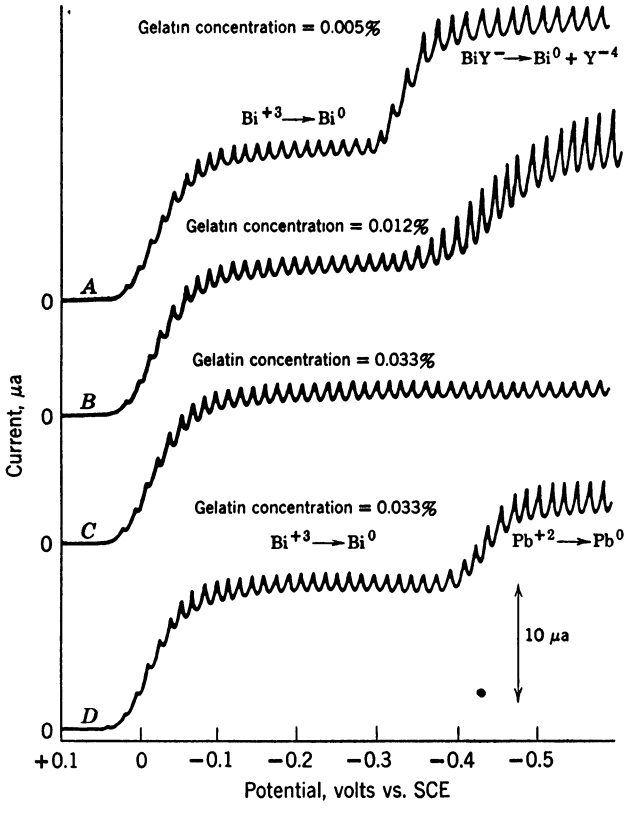

FIG. 4. Effect of gelatin on polarographic waves.[52]

TABLE II

TYPICAL AMPEROMETRIC TITRATIONS WITH DROPPING-MERCURY ELECTRODE

Substance Titrated	Titrant	Amount Present	Accuracy, %	Reference
Cobalt	α-Nitroso-β-naphthol	1–20 mg	0.5	27
Lead	Dichromate ion	0.001 *M*	0.3	29
Nickel	Dimethylglyoxime	0.01–0.0001 *M*	0.4	26
Titanium	Bromate, chlorate, chromate	0.0003 *M*		58
Aluminum	Sodium fluoride	10–40 mg	0.5–0.1	53
Cadmium	8-Hydroxyquinoline		1	60
Ferrocyanide	Hexamminecobalt (III) chloride	10^{-3} *M*	0.2–0.3	34
Fluoride	Thorium nitrate	0.005–1.0 mg	1	40*a*
Sulfate	Lead nitrate	0.005–0.2 g	0–3	61
Morphine	*p*-Diazobenzenesulfonic acid	4–20 mg	2.5	18

cations. The unusual sensitivity of the fluoride analysis is attributed to the sensitivity resulting from measurement of catalytic current.

Stationary Electrodes. In this technique, a potential (on the diffusion plateau of a normal polarogram) is suddenly applied to the electrode-solution interface of a quiet solution, and the resulting current is measured as a function of time. The great advantage of this procedure is that it minimizes (actually separates) the undesirable effect of the double-layer capacitance current. Immediately upon application of potential, the capacitance current is quite large but dies away rapidly at a speed dependent for the most part on the circuit resistance. The current that remains is then essentially the desired diffusion current (faradaic current) as its decay rate is much slower. If the diffusion process is linear (flat electrode surface and perpendicular to the cylinder containing the solution), the current varies with time as given by the following equation:

$$i = nFAD_0^{1/2}C^0/\pi^{1/2}t^{1/2}$$

where i is the current in amperes, F in coulombs, A in square centimeters, D_0 in square centimeters per second, C^0 in moles per cubic centimeter, and t in seconds. It is seen that the faradaic current decays inversely with the square root of the time and also that the current, at a given time after application of potential, is directly proportional to the concentration.

Stationary electrodes, despite some important advantages, have seldom been employed for amperometric titrations. Laitinen and Kolthoff [36] studied current–time behavior for electrolysis at a stationary platinum electrode under constant potential conditions. Although suitable amperometric titration curves could be obtained, Laitinen and Kolthoff adopted the procedure of waiting for at least 2 minutes after each application of emf in order that a fairly steady state of current would be reached. Under these conditions, the diffusion layer has time to develop extensively into the solution, and convection currents become troublesome. Laitinen and Kolthoff minimized the effect of convection currents by using platinum-foil electrodes sealed to a short section of glass tubing (which acts as a shield) or by using agar to stabilize the solution through increased viscosity. A similar technique was successfully applied more recently by von Stackelberg and co-workers [57] in the determination of diffusion coefficients.

With the high-speed recorders now available, one may readily measure the current–time response for electrolysis times *less* than one minute and in this way minimize the effect of establishing convection cur-

rents so that cells of more suitable design may be employed. In this way I have obtained quite satisfactory amperometric titration curves with systems analogous to those previously carried out by chronopotentiometry.[51]

Barker and Jenkins [1] employed a related technique which they term "square-wave polarography." The principle is illustrated in Fig. 5. The square-wave potential, I, of small magnitude is superimposed on the steady d-c applied potential. The resulting current would consist of a rapidly decaying current (capacitance current) illustrated in curve II taken on a solution containing no reducible species and a slowly decaying current (diffusion current) illustrated in curve III. Actually curve III contains both capacitance and diffusion currents, but, if current measurement is restricted to late times just shortly before each sudden change in the a-c potential, only the diffusion current is important. In this manner Barker and Jenkins could detect 2×10^{-7} N concentrations of ions that are reversibly reduced even in the presence of large concentrations of ions that ordinarily produce interfering waves. By preplating into a single mercury drop for 15 minutes, and then stripping the metal from the resulting amalgam anodically, detection of concentrations in the order of 10^{-9} N were possible. This technique was further employed by Ferrett and Milner [11] in a number of analytical problems with remarkable success. The high sensitivity of the method made possible the determination of copper and lead (5 to 0.1 ppm) in a number of polarographically important analytical reagents. Figure 6 illustrates a typical square-wave polarogram for very low concentrations of uranyl, copper, and lead ions in molar sulfuric acid. In view of the

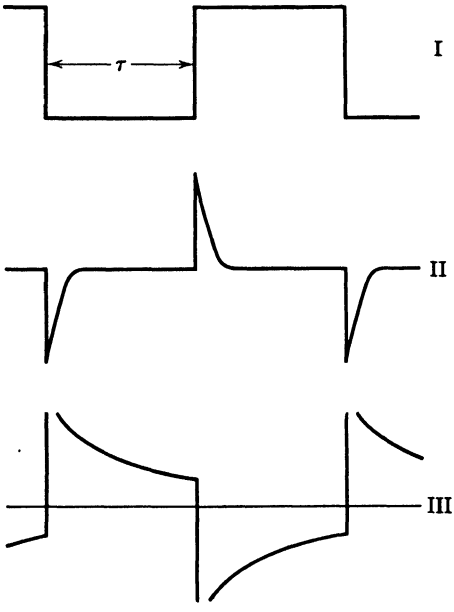

FIG. 5. Current–time and potential–time relationships in square-wave polarography.[1]

I = applied potential.
II = capacitance current.
III = faradaic current.

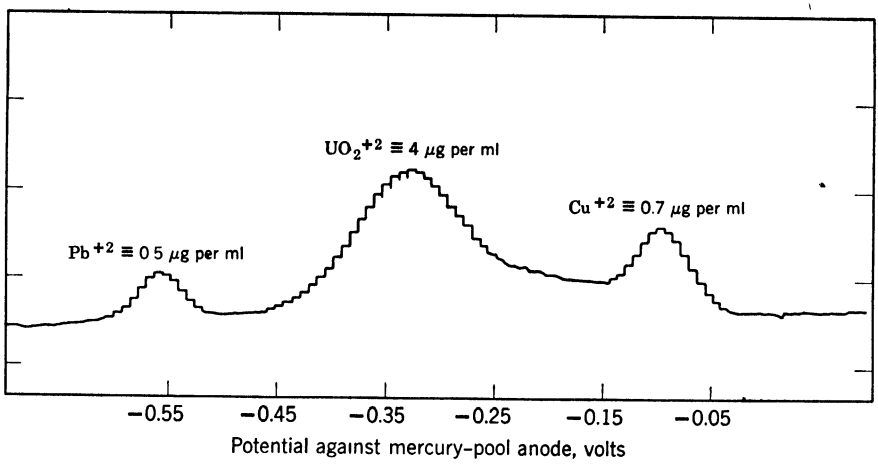

FIG. 6. Square-wave polarogram for UO_2^{+2}, Cu^{+2}, and Pb^{+2} in molar sulfuric acid.[11]

high sensitivity, even in the presence of other electroactive species, and the small influence of mild convection currents, the method promises to be useful for amperometric titrimetry.

Stirred Solutions

Stirring a solution increases the mass transfer of the species to the electrode and consequently increases the faradaic current. On the other hand, stirring does not increase the capacitance current, and consequently a better "signal-to-noise" ratio or useful sensitivity is obtained by stirring. A disadvantage of stirring arises in the difficulty of controlling the effectiveness of the stirring in a reproducible manner. Thus erratic or "noisy" currents may result with a lessening of the over-all signal-to-noise ratio. Furthermore, stirring causes more extensive electrolysis, and this factor may alter the nature of the surface of solid electrodes so that steady, reproducible currents are difficult to obtain. With transient methods employed in quiet solutions where the total amount of electrolysis is small, reproducible results are easily obtainable with solid electrodes.[49,51]

Rotating Platinum Electrode (RPE). Nernst and Merriam [45] were the first to describe the properties of a rotating platinum electrode. However, Laitinen and Kolthoff [37] were the first to apply this technique extensively for end-point detection. Platinum electrodes have the advantage of operating over a different voltage range (e.g., ~+1.1 to −0.5 volts vs. SCE in 0.5 *M* HCl) compared to mercury (e.g., ~+0.1

to −1.1 volts vs. SCE in 0.5 *M* HCl). The exact range depends strongly on the supporting electrolyte, but the general usefulness of platinum still remains in the positive potential regions.

Some typical amperometric titrations employing the rotating platinum electrode are given in Table III. A synchronous rotator and

TABLE III

TYPICAL APPLICATIONS (RPE)

Substance Titrated	Titrant	Amount Present	Accuracy, %	Reference
Arsenic	Potassium bromate	10^{-3}–10^{-4} *M*	0.1–0.3	37
Copper	Dichromate	0.02 mg	1	49
Vanadium	Ferrous ammonium sulfate	5	Very good	47
Fe(II)	Dichromate	20	1	48
Bromide, chloride	Silver nitrate	0.02–0.1 *M*	1	35
Iodine	Arsenic trioxide	0.0001–0.00001 *N*	1	28*b*
Cysteine	Silver	0.0005 *M*	0.3	30
Styrene	Potassium bromate	0.2–1.0 mg	3–5	22
Sulfhydryl groups	Silver nitrate	1–2	1–4	55
Mercaptans	Silver nitrate	0.2 mg S/100 ml over over 2 mg S/100 ml	1–2 0.3	2 23

replacement electrodes are available from E. H. Sargent and Company, Chicago, Ill.

The rate of stirring has a marked effect on the current sensitivity although a state of confusion exists as to its exact magnitude. Lord and Rogers [40] point out another factor: the rate of electrode reaction (potential independent mechanism, however). This effect is illustrated in Fig. 7. In these cases the rate of rotation increases the *limiting* current only up to a certain point. More rapid stirring then has little effect. Lord and Rogers logically concluded that at low stirring rates the limiting current is controlled by the convection and diffusion processes. At high speeds of stirring, the convection and diffusion processes are able to bring the species up to the electrode surface at a rate greater than the rate of electrode reaction. Compare curves 2 and 4. Although both the platinized and smooth platinum electrodes have the same projected area, and thus would have identical dependency upon convection and diffusion processes, the platinized electrode yields a much larger *limiting* (potential independent) current. Clearly the current is limited by conversion of the species to "active" form at the electrode surface and not by the rate of mass transfer or the electron-transfer step. The difference in curves 1 and 3 are similarly explained

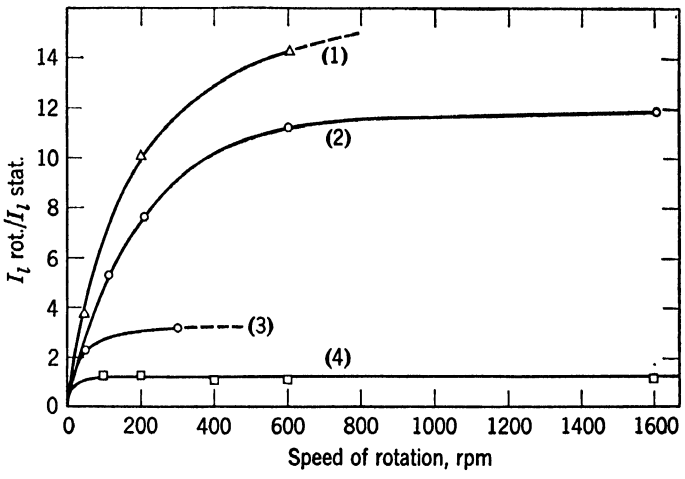

FIG. 7. Effect of speed of electrode rotation and rate of electrode reaction on height of limiting current.[40] (1) Fe(III) in 0.1 *N* HCl (smooth platinum), (2) hydroxylamine, 0.1 *N* H_2SO_4 (platinized platinum), (3) Fe(III) in 0.1 *N* H_2SO_4 (smooth platinum), (4) hydroxylamine, 0.1 *N* H_2SO_4 (smooth platinum).

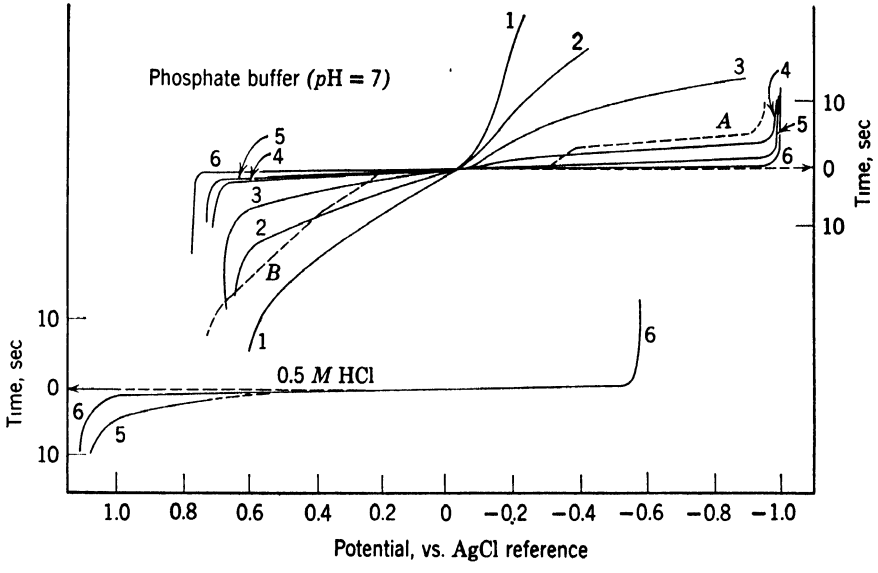

FIG. 8. Electrolysis of platinous oxide and sorbed hydrogen on platinum electrodes.[49] *A*, reduction of platinous oxide at 1.34×10^{-4} ampere; *B*, oxidation of sorbed hydrogen at 1.34×10^{-4} ampere; (6) electrolysis curve after reduction *A* or oxidation *B*.

by assuming that the rate of conversion to "active" form is greater for Fe(III) in hydrochloric acid than for Fe(III) in sulfuric acid. In conclusion, greater current sensitivity may be obtained in some instances by proper selection of background electrolyte, and by use of platinized electrodes with high rates of stirring.

A second effect to be considered when using platinum electrodes in dilute solutions is the possibility of distortion of observed currents due to electrolytic formation or destruction of platinum oxide layers on the electrode. Figure 8 [49] illustrates this effect. Curve *A* illustrates the reduction of the platinous oxide when a feeble cathodic current is passed. Once the reduction occurs, no further wave of this type will appear in subsequent polarizations (e.g., like curve 6). Curve *B* is attributed to reoxidation of platinum. After one or two polarizations this effect is also eliminated. Kolthoff and Tanaka [31] studied the platinum oxide effect in detail. They found that 10^{-4} *N* Fe(II) solutions yielded quite distorted anodic polarograms whenever a recording method was employed. However, by subtracting the "residual" current obtained with the supporting electrolyte alone, a well-defined anodic wave would be obtained.

Rotating Mercury Electrode. A rotating mercury electrode was devised and thoroughly studied by Lee.[38] The advantage of this method is that currents are obtained that are approximately 200 times larger than the corresponding diffusion currents obtained with the dropping-mercury electrode. This increased sensitivity is attributed to a larger (approximately 50-fold) electrode surface area and to an increase in mass transfer through stirring. Using this electrode, Lee found limiting currents for the reduction of lead, cadmium, and copper ions proportional to concentration in the range 10^{-4} to 10^{-7} *M*.

A limiting future of this method is in the rather large residual currents and a narrowed range of usable potentials. Also the electrode assembly must be fabricated with great care. In spite of these factors, the electrode promises to be quite useful for rapid titrations of dilute solution.

Vibrating Electrodes. Vibrating electrodes have been found to yield limiting currents proportional to concentration and yet practically independent of moderate stirring of the solution. This feature allows for more rapid amperometric titrations and for automatic recording of titration curves.

Lindsey and Harris [16,17,39] devised a vibrating platinum electrode suitable for amperometric titrations. Limiting currents were observed which increased with vibration frequency up to 40 cps and were con-

stant at higher frequencies. The limiting currents also increase with amplitude up to about 0.4 mm, becoming independent of amplitude beyond this value. This effect is reminiscent of the effect of speed of rotation on rotating platinum electrodes and may be attributed to increasing the convective and diffusion rates to a value greater than the rate of conversion to "active" form.

Berman, Saunders, and Winzler [1b] designed a vibrating dropping-mercury electrode specifically for procedures where agitation of the solution is required. With the rapidly vibrating (81 cps, displacement 0.012 inch) electrode, the drop size and time were greatly reduced with some reduction (about 2/3) in sensitivity compared to the DME. However, linear concentration–current relationships are obtained which are independent of moderate stirring. These authors performed a rapid amperometric titration of lead with chromate with inert gas passing continuously through the solution to illustrate the potentialities of the vibrating electrode.

Convection-Controlled Electrodes. Kolthoff, Jordan, and Prager [24,25] devised an electrode (platinum or mercury) system in which the current is controlled by the convective process of mass transfer. They state that the convection mercury electrode has at least the following advantages over the dropping-mercury electrode in amperometric titrations: (1) easier to construct and to manipulate and (2) greater sensitivity. The limiting currents were proportional to concentration (and independent of the diffusion coefficient of the substance) in the range between 5×10^{-6} and 5×10^{-4} *M*. For their mercury electrode, they found a convection current of $(20.6 \pm 0.4) \times 10^3$ μa per equiv per liter for all three species.

Stirred Solutions. In several applications it was found more convenient or desirable to stir the solution and allow the electrode(s) to remain stationary. This is especially true where electrodes of large area are employed to obtain sufficient sensitivity for titrations of very dilute solutions. In each case the rate of stirring is carefully controlled in order to obtain steady current values.

Sensitive End-Point Procedure with Platinum. Cooke, Reilley, and Furman [4,5,14] describe a sensitive end-point procedure which they applied to the coulometric titration of microgram and submicrogram quantities of materials. The indicator circuit is shown in Fig. 9. The sensitivity of the method is illustrated by the fact that addition of 1 μg of manganese gave a galvanometer deflection equivalent to about one-half mile! Using this procedure, Cooke, Reilley, and Furman were able to titrate solutions of 0.001 to 0.0005 μg of manganese

per milliliter with an accuracy of about 5%. The method was also applied to the titration of 0.01 μg per ml of iron and to microgram quantities of vanadium. The chief limitation of the useful sensitivity of the method occurs in the form of a current "drift" attributed to aging of the platinum electrode, attack by chloride at high potentials, thermal effects, etc.

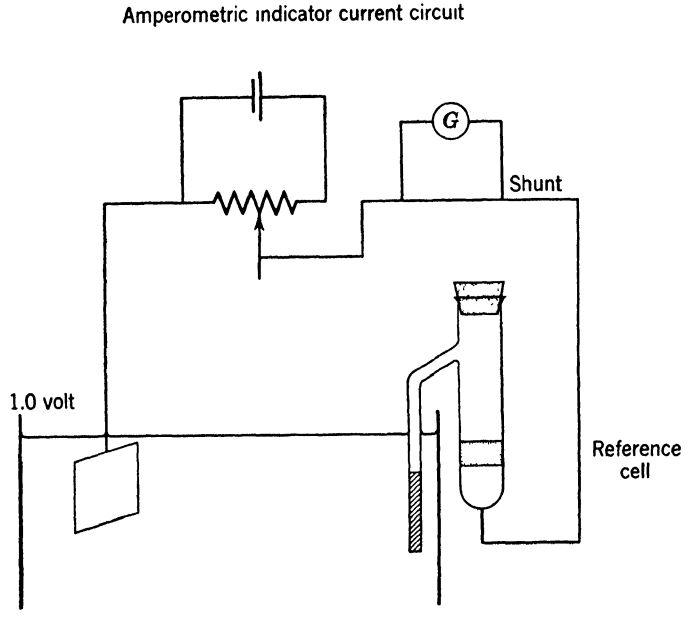

FIG. 9. Indicator circuit for sensitive end-point procedure (platinum).[4]

Sensitive End-Point Procedure with Mercury Pool. Nikelly and Cooke [46] employed a mercury-pool cathode with controlled stirring of solution to effect the amperometric titration with EDTA of metal ions approaching dilutions of 10^{-7} *M* in favorable cases. For example, a solution containing 0.02 μg of cadmium per milliliter can be titrated with an average error of 0.0001 μg. Titrations of copper, lead, nickel, and zinc were also performed. The titration cell and typical titration curves are illustrated in Fig. 10.

High-purity Linde nitrogen issues steadily from the frit and keeps the solution oxygen-free and in a *constant* state of agitation. Greater sensitivity could be obtained by using a larger pool and less volume of solution, but Nickelly and Cooke found that the electrolysis in the indicator circuit would then consume an appreciable fraction of the metal ion.

Dual-Indicator Electrodes. (*1*) *Direct-current polarization.* In this method, two indicator electrodes, polarized by an external emf of 0.02 to 0.3 volt, are immersed in a stirred solution, and the resulting current is measured. The method probably has its origin with Solomon,[54] who employed two polarized silver wires as electrodes for

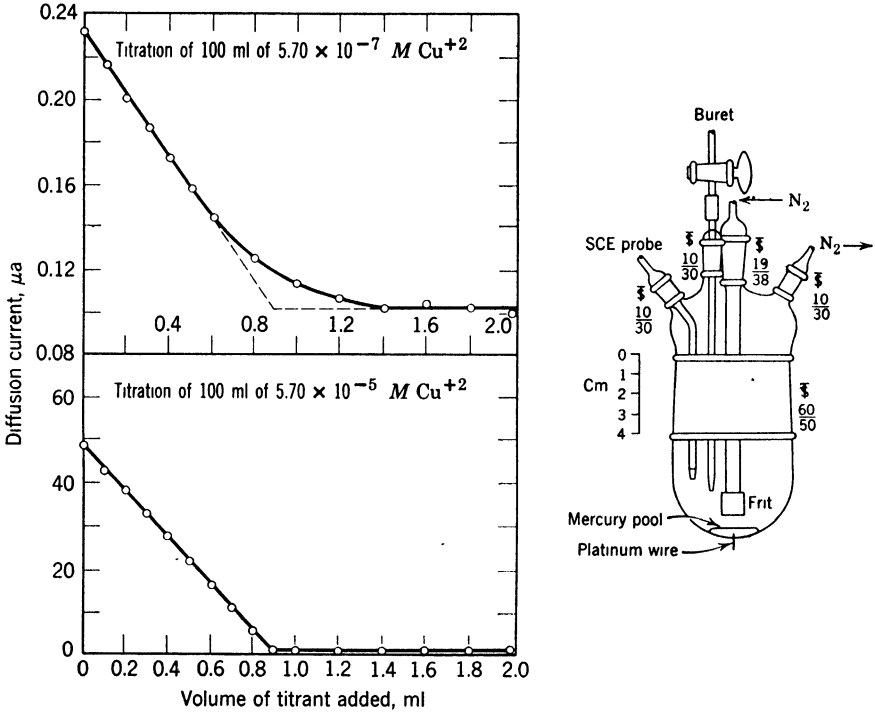

FIG. 10. Amperometric titrations and titration cell using the sensitive end-point procedure (mercury pool).[46]

argentometric titrations. Widespread use was not made of this technique, however, until the work of Foulk and Bawden [12] appeared.

The chief advantages of this technique are that the reference electrode is dispensed with, affording easier operation at high temperatures, in nonaqueous media, and eliminating the reference electrode as a source of contamination. Another advantage is that electrolysis at the indicator electrodes usually does not deplete the concentration of the substance being titrated. For example, in the titration of Fe(II), some Fe(II) is oxidized at the anode but an *equal* amount of Fe(II) is generated simultaneously at the cathode. The principle of the method was first presented qualitatively by Delahay [7] and quantita-

tively by Reilley, Cooke, and Furman.[50] Several authors have since developed the theory further.[2,9,15,20,21] The method has been extensively applied in iodimetric determinations and in the Karl Fischer titrations.[44] Stone and Sholten [59] have demonstrated the wide scope of the method.

Swift and co-workers [3,10,43,56] have used this method exclusively for very sensitive end-point detection in coulometric titrations. Bromide, for example, could be detected with a sensitivity of about 15 μa per

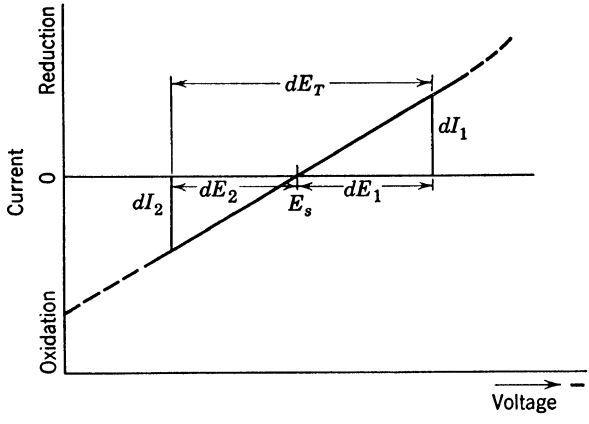

FIG. 11. Polarization curve for explaining dual-electrode system.[50]

μg per ml, using platinum indicator electrodes of 6 to 9 cm^2 area each. Microgram quantities of thiodiglycol, arsenite, chromate, vanadate, antimonite, iodide, aniline, and thallium were estimated accurately using this end-point technique in conjunction with coulometric generation of titrant.

The principle of operation becomes clear from a consideration of Fig. 2 and Fig. 11. Figure 11 illustrates an enlarged polarization curve —e.g., equimolar mixture of Fe(III/II)—in the vicinity of zero current. The dual-indicator electrodes immersed into the solution with no applied emf would both exhibit the solution potential E_s. When a definite emf, dE_T, is applied between the two electrodes, a resulting current, $dI_1(=dI_2)$, will flow. It is clear that the current would, as a first approximation, be proportional to the slope of the polarization curve in the vicinity of zero current. From Fig. 2 this slope, and thus the current, is seen to be small at the beginning of the titration, becoming steepest halfway in the titration, becoming small at the end point, steep again past the end point. In agreement with these con-

clusions, Fig. 12 shows the complete, dual-electrode amperometric titration curve reported by Stone and Sholten [59] for the titration of potassium ferrocyanide (reversible system) with ceric ion (reversible system).

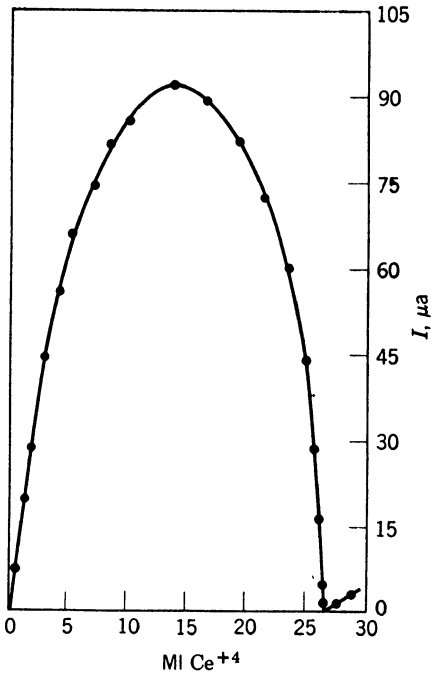

FIG. 12. Amperometric titration of $K_4Fe(CN)_6$ with ceric ion,[59] dual-indicator system.

(2) *Alternating-current polarization.* Polarization of dual-indicator electrodes (*smooth* platinum) may be effected using alternating rather than direct current. This method has not been used to any extent although it forms the basis of the end-point detection system for the Beckman model KF-2 aquameter (an automatic titrimeter for Karl Fischer titrations). The bridge circuit shown in simplified form in Fig. 13 is employed. *A* is a source of alternating potential operating at 60 cps. The bridge is originally adjusted by varying resistor *B* so that a sufficient output at the end point will occur at *D*, which in turn operates the solenoid controlling the flow of titrant from the buret into the cell. *C* represents the dual-indicator electrodes in the titration cell. Alternating current is advantageous in this situation since

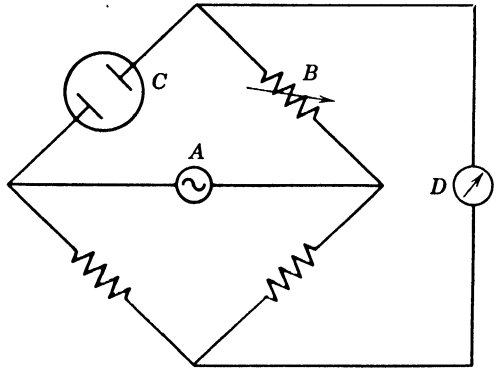

FIG. 13. Simplified circuit for a-c end-point detection.

modern electronic circuits can amplify alternating currents more simply and since the *phase* of the amplified signal can be used to designate the solution condition as "before" or "after" the end point.

Frank [13] has applied a similar polarization technique to a wide scope of titrations. He found excellent end-point detection for the titration

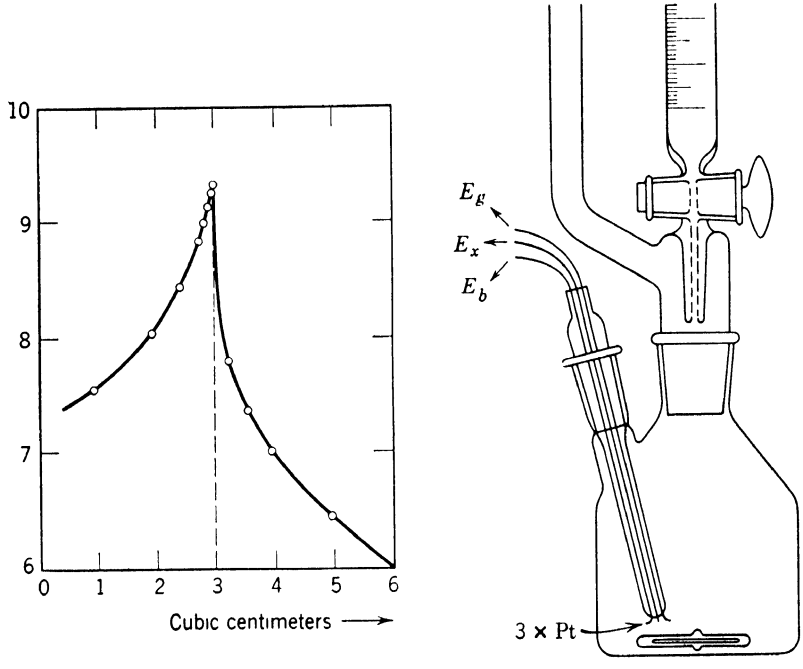

FIG. 14. A-c polarization curve and cell for titration of 10^{-5} *M* iodide.[13] Alternating current is passed between E_g and E_x. A-c potential is measured between E_b and E_x.

of 10^{-5} *M* iodide with silver ion, although he reported that no visible precipitate appeared to be formed for such dilute solutions. This titration curve and the 5-ml vessel employed are illustrated in Fig. 14. In this method he passes a "constant" alternating current of 1 to 50 μa through two platinum electrodes and measures, after suitable amplification, the alternating potential between one of these electrodes and a third platinum electrode acting as a reference. Best results are obtained using frequencies of 0.1 to 20 cps, although good results were obtained with the more convenient 50 cps. The method merits further study.

DISCUSSION

Discussion Leader
I. M. Kolthoff
University of Minnesota

In recent years a new electrode has been developed in our laboratory which has many interesting characteristics and which should be particularly suitable for trace analysis. A detailed description in the form of a manuscript by Dr. W. Stricks and myself has been submitted for publication to the *Journal American Chemical Society*. A brief summary of the main characteristics of the electrode is given below.

The rotated dropping-mercury electrode (RDME) has a sensitivity of the order of ten times as large as that of the conventional dropping-mercury electrode at a speed of rotation between 200 and 300 rpm and is suitable for trace analysis and amperometric titrations at high dilutions.

In the absence of a suitable capillary-active substance, no constant diffusion region is obtained except at an ionic strength greater than about 1.0. At a lower ionic strength, the limiting current has a maximum at the isoelectric point of mercury. At any potential, the limiting current is proportional to the concentration of electroactive substance and to the square root of its diffusion coefficient. In the presence of a suitable capillary-active substance, like gelatin, a diffusion current is observed which is smaller than the limiting current in its absence. This diffusion current is constant until the supporting electrolyte is discharged; it is independent of ionic strength, proportional to concentration and to $D^{1/2}$. With other capillary-active substances, like detergents, camphor, the diffusion current remains constant until the capillary-active substance is desorbed, and then a second wave is observed.

The peculiar shape of the current–voltage curves in the absence of capillary-active substances is attributed to stirring effects at the surface of the mercury which are caused by the flow of mercury, the stirring having a maximum at the isoelectric point of the mercury and decreasing at both sides of this point. This stirring causes a "maximum of the second kind" at the conventional DME and is suppressed by capillary-active substances. No ordinary maxima (of "the first kind") due to a heterogeneous electric field around the drop, caused by the

screening effect of the glass wall of the capillary, have been observed at the RDME.

QUESTIONS AND ANSWERS

JAMES J. LINGANE: Have you had any experience with the electrode that Leveque developed?

I. M. KOLTHOFF: No. I thought you did.

JAMES J. LINGANE: No. This electrode consisted of mercury flowing down a platinum wire to increase the surface and eliminate the oscillation.

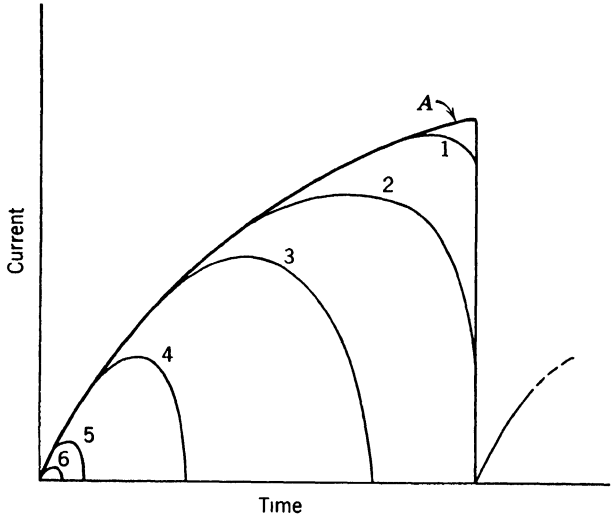

FIG. D1. Current–time curve.

I. M. KOLTHOFF: We had bad luck with the so-called amalgamated platinum electrode. The wetting properties of platinum regarding mercury are pretty poor.

PAUL DELAHAY: Would you discuss the cause of the gelatin effect? Do you have an explanation for the suppression of the second wave?

CHARLES N. REILLEY: Suppose we consider that the solution contains only the metal–EDTA chelate in a suitable buffer solution. The current–time curve (see curve *A* in Fig. D1) has the shape of a normal diffusion-controlled reduction. Very small concentrations of gelatin have no influence on this current–time response. If more gelatin is added, current–time curves are obtained where the current will tend to decrease just before the drop falls, as illustrated by curve 1. With larger and larger gelatin concentrations, the shape changes progressively from 2 to 3 to 4 to 5 to 6, and eventually practically no average current is flowing. The exact mechanism has not yet been evolved, but let us assume that, once the metal–chelate species gets to the electrode surface, it must then undergo some type of conversion to "active" form at the electrode surface prior to reduction. The rate of conversion

will then be dependent on the surface area available. Let us also assume that gelatin diffuses to the drop forming an accumulated film that decreases this available surface area. In curve *A*, the free surface area of the drop is sufficiently large so that the rate of conversion to active form on the electrode surface is greater than the rate of diffusion of the metal chelate to the electrode surface. In curve 3, during the early life of the drop, sufficient gelatin has not yet accumulated on the drop, and the limiting current is controlled by diffusion of metal chelate to the drop. Later in the drop life, the gelatin has accumulated on the surface to such an extent that the rate of conversion of metal chelate to active form (on the decreasing area of "free" surface) has now become the limiting step in the over-all electrode process. At higher gelatin concentrations, this current diminution would be expected to commence at an earlier time in the drop life, and this effect is observed experimentally. Similar results are obtained with other surface-active agents such as Triton X-100. The effect of a given quantity of gelatin depends considerably on the particular metal chelate involved, and with no apparent correlation with size of the metal chelate or other steric factors. This led us to believe that the current is kinetically controlled by a slow rate of conversion at the small free electrode surface available after gelatin has covered the remains of the total surface of the drop.

I. M. KOLTHOFF: That would be specific for a particular component?

CHARLES N. REILLEY: That is right.

I. M. KOLTHOFF: Not specific for the gelatin but for the electroactive species?

CHARLES N. REILLEY: That is correct. For the same concentration of gelatin, e.g., 0.03%, bismuth ions are reduced in a diffusion-controlled manner, but no reduction wave for the bismuth–EDTA complex is obtained. In a solution containing 0.002% Triton X-100, the cadmium–EDTA will not reduce while copper–EDTA yields a normal wave. In 0.005% Triton X-100, the reduction of copper–EDTA is just eliminated. Cadmium–EDTA has a reduction wave in the absence of surface-active agents. Lead–EDTA waves are eliminated less easily than those of cadmium–EDTA.

I. M. KOLTHOFF: It would seem probable that the gelatin forms a film on the surface of the mercury and that this film interferes with the electrode reaction. Several examples of this type of effect are known.

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Emission Spectrochemical Analysis *

Basic principles and applications

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ABSTRACT The more prominent physical processes occurring in the d-c arc are outlined. This includes the separate processes: melting, boiling, chemical combinations at higher temperatures, electron–atom collisions, atom–atom collisions, excitation of atoms, light emission, and the mutual interaction of these phenomena as well as their dependence on electric field in the arc.

The manner in which these various factors manifest themselves in the intensity-concentration curves set up as practical analytical working curves is illustrated by selected examples encountered in the spectrochemical analysis of mineralogical, geological, agricultural, chemical, metallurgical, biological, and medical fields, such as ores, rocks, soils, waters, inorganic chemicals, metals, plant and animal tissues. These examples are all based on the determination of intensity ratios derived by microphotometry of photographed spectra. The pitfalls and complex ramifications of these are reviewed, especially as affected by varying intensity level and the photographic process itself. The features of the photographic and arcing processes outlined are used to explain the many unique shapes and variable nature of the

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analytical calibration curves for some lines. The application of other means of excitation, such as sparks and flame, are outlined and illustrated.

INTRODUCTION

The determination of chemical composition by means of optical emission spectroscopy is based on the measurement of light emitted by atoms of the elements to be determined when energized to raise their valence electrons above their normal stable levels. The energies required to excite valence electrons to levels sufficiently high to cause light emission are relatively small (range 1 to 10 ev), compared to those required to excite electrons in deeper electronic orbits (10 to 100 kv). Emission spectrochemical analysis methods thus depend on the behavior of the peripheral electrons of atoms as distinct from the properties of the entire atom (mass and charge) on which mass spectroscopic methods depend, or electron transitions between deeper electronic orbits of the atoms which provide the basis for X-ray fluorescent analysis. Emission spectrochemical analysis methods are particularly well suited for the analysis of materials in the solid state (less so for liquids and gases), and have been widely applied during the past 25 years wherever impurity composition is of technical importance or of scientific interest.

INHERENT SPECTROGRAPHIC SENSITIVITY FOR D-C CARBON ARC

The amount of light emitted by a single atom as a result of a single electron transition is too small to detect. To detect the presence of an individual atom, light radiation emitted by a large number of electronic transition cycles would have to be integrated—in fact, something like 10^{14} transitions from the lowest excited state to the ground state in case of a 10-mg sample for a metal of atomic weight 60. Aside from the time required for this number of transitions to occur, it is impossible to retain a vaporized sample within the excitation environment of an unenclosed arc because of its high rate of diffusion in the gaseous state. Any practical analytical method must therefore depend on the total radiation emitted by a large number of impurity atoms within a time period of 1 to 3 minutes.

The intensity of optical emission lines is primarily determined by the number of atoms emitting light in unit time for a given excitation source. The intensity is thus obviously determined primarily by the

number of atoms of the species whose concentration is desired, present in a sample of such a size as can be introduced into the excitation source. The entire sample normally melts in a d-c source and volatilizes into the actual excitation environment. Because of the usual widely differing boiling points of a sample, leading to a fractional distillation of its constituents, light emission is not constant over the excitation time and must be integrated over its entire time of volatilization and excitation. Samples of 5 to 15 mg size may be volatilized conveniently in a conventional d-c source. Samples of either smaller (1 mg) or larger (100 mg or over) size may be employed, but then special techniques must be utilized. Larger samples produce high-continuum intensities which mask weak lines, and lead to serious self-absorption of many lines. The inherent sensitivity of an emission spectrochemical method is usually expressed as the lowest concentration of an impurity element that can be detected in some other material when that material is introduced into the excitation source in such amounts as can be conveniently and uniformly excited to give a spectrum of usable reproducible intensity. The impurity concentration is most frequently expressed relative to the total sample in whatever units are traditional for the material; for example, as weight per cent of element or some other molecular (oxide) group, parts per million (ppm); milligrams per kilogram (mg/kg), micrograms per gram (μg/g); gammas per milliliter or gram (γ/ml) or (γ/g), grams per ton (g/T), etc. On the other hand, solid-state physicists express impurity concentration as number of atoms per cubic centimeter (n/cm^3). Expression of sensitivities in absolute weights of an element has no general meaning for spectrochemists, unless some element can be isolated from a sample and transferred to an electrode and its mass determined by spectrographic means.

The majority of impurity analyses are expressed as weight per cent of sample or as parts per million; otherwise sensitivity data would be still more difficult to compare. It so happens that a given concentration of impurity expressed in a variety of the above-mentioned units, has the same numerical value; for example:

$$\begin{aligned} 0.0001 \text{ wt } \% &\equiv 1 \text{ ppm} \equiv 1 \text{ part in } 10^6 \\ &\equiv 1 \ \mu\text{g/g} \equiv 1 \ \gamma\text{/g} \\ &\equiv 1 \text{ g/T} \equiv 1 \text{ mg/kg} \end{aligned}$$

and these in turn $\equiv 1 \times 10^{14}$ atoms in a 10-mg sample of at. wt. 60.64 (based on atomic population of 10^{20} atoms).

The above-listed concentration of 1 ppm (or its equivalent in other units) represents the approximate lowest limit to which almost any element can be determined by excitation in a d-c arc. Special techniques may extend this limit to lower values. Unfortunately, the lowest limits for other elements lie an order of magnitude higher while a few lie two orders higher.

It so happens that As and Sb, two elements of interest as impurities in germanium semiconductors, belong to these less sensitive elements in d-c source. The elements Ag and Cu of special interest in phosphor research are two of the most sensitive elements. In biological work some important elements such as Zn and P fall in the lower-sensitivity group, while Co is in an intermediate-sensitivity group. Others such as Cu, B, Ag, Na, Ca, etc. belong to high-sensitivity group elements. Thus, when samples of 5 to 15 mg size are packed into holes drilled in carbon rods, and the composite is made an electrode of a d-c arc, spectral lines in the emitted light reveal the presence of impurities down to levels ranging from approximately 1 to 100 ppm, depending on the element. It is a very simple matter to produce such spectra and use them for qualitative analysis purposes.

Three major problems immediately arise when more serious applications are attempted with spectral lines radiated by impurities:

1. Extending sensitivity to lower levels.
2. Stabilizing line intensities.
3. Calibrating for quantitative analysis.

This chapter discusses some selected limited aspects of the various problems with special reference to the spectrochemical determination of trace elements, and, insofar as possible, as they are (or may be) encountered in the medical field.

EXTENDING SENSITIVITY LEVELS

The necessity of increasing sensitivity of any technique constantly arises in the problem of trace analysis, and no analyst must be reminded of this problem. Therefore, only a few specific examples, where trace-metal analyses must be pushed to unusually low limits, will be mentioned later in this chapter as various means of accomplishing sensitivity extensions are discussed.

By Physical Means within Light Source

Cathode Enrichment in D-C Carbon Arc. One of the first usable extensions of sensitivity in emission spectrochemical analysis depends

on a cyclic excitation of atoms in the region of the d-c arc cathode. This is due to the circumstance that some atoms of the sample material are converted to positive ions in the arc gas column, in amounts depending on the arc temperature, which in turn is determined by the ionization potential of the major sample constituents. The positive ions migrate in the electric field and become enriched near the cathode where, after neutralization by recombination with electrons, they build up a concentration gradient in the cathode area to an extent that enhances light emission there with a corresponding extension of sensitivity. To this increase in sensitivity, as a result of increased concentration in the region of the cathode, is added another increase due to the possibility of repeated excitation of the same atom by virtue of its remaining longer in the arc gas column. The net enhancement of line intensity results in a frequent expansion of sensitivity by a factor 10, and sometimes up to 100 in the region near the cathode, which has nothing to do with any special excitation (primarily of spark lines) caused by the cathode fall at the immediate cathode surface.

The enhancement, as expected, parallels the decrease in ionization potentials of the atoms in question. The effect is pronounced for small-sized samples (1 to 5 mg), so that it has found its principal use in determining impurity content of samples available only in small amounts, such as encountered in geochemical and mineralogical research carried out by V. M. Goldschmidt and his students and followers. The effect was first described by R. Mannkopff and Cl. Peters,[1] and studied by Mannkopff and his students [2-5] in the early 1930's at Göttingen. Strock [6] has described the effect in English as the "cathode-layer effect."

Repeated Excitation in Hollow-Cathode Closed System. There seems to be more likelihood of expanding emission sensitivity by a cyclic excitation process in a more controllable manner, and by a more substantial factor by employing a hollow-cathode source. The atoms to be excited are not able to escape the excitation environment of the hollow-cathode source as readily as from an open-atmosphere arc. If each atom can be made to participate in 10^3 or 10^5 excitation cycles, the sensitivity of detection of those atoms will be increased to permit their detection at corresponding lower concentration levels: namely, 10^9 to 10^{11} atoms. The hollow-cathode source affords more selective control of excitation energy applied to the sample. It has not been widely applied as yet, except as a source of narrow spectral lines in physical spectroscopy, but deserves more serious attention as

a means of excitation in methods directed to expanding emission sensitivity.

A good review and description of hollow-cathode sources is given by S. Tolansky,[7] who refers to the discovery and use of the source

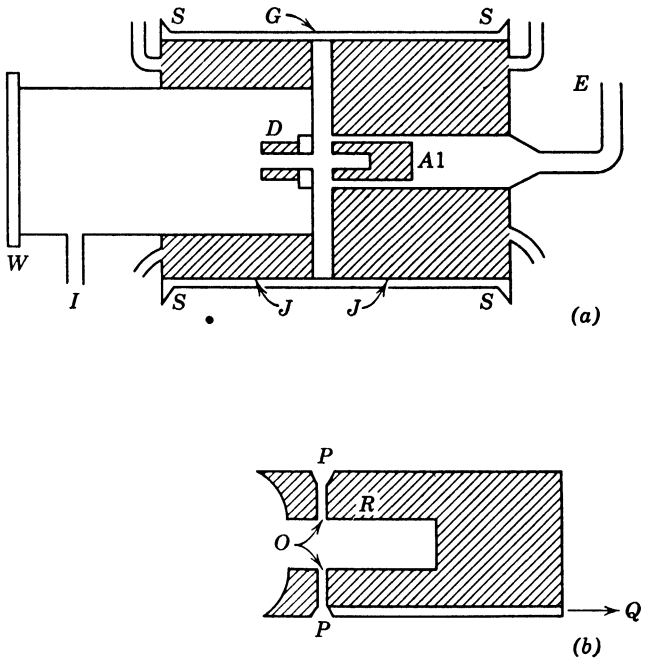

FIG. 1 Hollow-cathode discharge tube (*a*) and replaceable sample holder *Al* detailed in (*b*) after Schuler and Gollnow (see Tolansky,[7] p. 62). Anode (*left*) and cathode (*right*) sections of water-cooled assembly *J* are spaced by wax seals *S* to glass envelope *G*. Circulating gas entrance is *I*, exit is *E*, and observation window is *W*. Replaceable hollow-cathode sample holder *Al* with 15-mm-by-4-mm cavity and radial canals *R* and circular groove *P* for distributing gas flow from *O* to *Q* is detailed in (*b*). Current capacity of tube is 100 mA, and tube is useful for 1- to 10-mg samples. Collar *D* on anode traps volatilized sample.

in 1916 by F. Paschen [8] and the substantial improvements, adaptations, and applications of H. Schüler.[9,10] Schüler has used a hollow cathode specially designed for small-sized samples, which may be subsequently recovered, illustrated in Fig. 1. J. R. McNally, G. R. Harrison, and E. Rowe [11] used this source for detecting 0.2- and 1.0-μg quantities of Cl and S, respectively, in 20-mg samples. The hollow-cathode source differs from an open d-c arc principally in the mechanism by which a solid sample enters the arc column. In the open d-c arc the mechanism is thermal, and the sample is vaporized by a

microfractional distillation process. In the hollow-cathode source, the mechanism is one of cathode sputtering by positive ions impinging the cathode (sample) surface. Hollow-cathode excitation thus has as its primary task the production of positive ions in effective numbers and of the proper kinetic-energy range and excitation energy. These ions have two functions:

1. To first generate a cloud of sample atoms in a portion of the arc column, nearly surrounded by the cathode, into which space the positive ions of the column migrate.
2. To transfer their excitation energy to sample atoms reaching this space from the solid-cathode surface by the sputtering action of other positive ions impinging on the cathode surface.

An integral component of the source is thus a carrier gas (usually He or A when molecular spectra are undesirable) introduced in the correct amount to create the optimum number of positive ions required to balance the two opposing but necessary functions of the positive ions. On the one hand, there must not be so many ions that their mean free path is reduced by mutual interference of their motion to a point that prevents their reaching the cathode surface in adequate numbers to effect sputtering. On the other hand, the number of ions may not be so low that adequate numbers are not available to "atomize" sufficient sample into the arc column. Decreasing the number of sample atoms simultaneously with the number of positive ions rapidly decreases the chance of collisions between them, and thus the opportunity of exciting sample atoms and obtaining light emission. The gas is thus an essential component of the excitation mechanism of a hollow-cathode source, and its pressure must be confined to a unique and relatively narrow critical range—commonly a few millimeters.

By Means Outside the Light Source

Enrichment of Trace Metals in a Precipitate. The possibility of greatly increasing sensitivity by analyzing, by spectrographic methods, precipitates in which the trace-metal content of a larger sample has been isolated by chemical means is of crucial importance in the medical and biological field. In this way the trace-metal content may be determined in 10 or 100 grams (or more) size of samples of vegetable material, blood, tissue, water, soil, rocks, etc. Indeed the trace-metal contents at significant concentration levels, in most medical and biological materials, can frequently be determined only by such com-

bined chemical enrichment and spectrographic methods. Wide application has been made of such methods over a period of years by me and my previous associates in Saratoga Springs on a variety of materials, some of which will be referred to later.

This approach to extending emission spectrochemical sensitivity is usable only where the sample material can be gotten into solution and a suitable reagent can be found for precipitating the elements to be determined without precipitating the major constituents of the sample. The principle of this enrichment operation is such that such precipitating agents need not be selective other than for separating impurities from major constituents.

Cupferron was used for this purpose by L. W. Strock,[12] and L. W. Strock and S. Drexler [13] for isolating Ti, V, Sn, Cu, Zr, and Fe from waters. A real advantage, aside from extending sensitivity, is that the impurities are accumulated and converted to a uniform and homogeneous state for the spectrographic operation, irrespective of the nature of the original sample. This greatly simplifies calibration, and in many instances leads to a spectrographic operation which is essentially one of determining impurities in an iron oxide base. Cupferron, in strong acid solution, precipitates Ti, Zr, V, Hf, Cb, Ta, Mo, W, Fe, Pd, Ga, Sn, Sb, Bi, and Po. Obviously, little or no enrichment is achieved when any of the elements, precipitated by cupferron, is present in large amounts in the sample. For this reason, it is not well adapted for many materials (e.g., rocks) because it precipitates the abundant Fe. It has been used for determining trace elements in waters, soil leachates, and solutions of biological materials in the Saratoga Springs Commission Laboratories by G. E. Heggen and myself over the past 15 years.

There are many metals not precipitated by cupferron which can be precipitated by other organic reagents. 8-Hydroxyquinoline in acetic acid solutions precipitates Co, Zn, Ti, Zr, Hf, Th, Cb, Ta, Po, Mo, W, U, Cr, Fe, Ni, Pd, Cu, Ag, Cd, Hg, Al, Ga, In, Bi, and Ge quantitatively. Of these, the elements Th, W, Co, Ni, Cu, Ag, Zn, Cd, Hg, Al, and In are not quantitatively precipitated by cupferron. On the other hand Sn, Sb, and V are precipitated by cupferron but not by 8-hydroxyquinoline. We investigated the possibility of using a combination of these two organic precipitating agents early in 1946. Since the conditions for optimum operation of these two reagents are different, it is necessary to use the reagents in two steps and combine the precipitates in a single spectrographic operation. It was found that the formation of cupferron–metallic complexes are re-

tarded by 8-hydroxyquinoline, or partially soluble in its solutions; further, that 8-hydroxyquinoline, precipitating more elements, so depleted the analysis solutions, that the subsequent cupferron treatment failed to precipitate quantitatively trace elements remaining after the cupferron precipitation.

About this time, R. L. Mitchell and R. O. Scott [14] of the Macaulay Institute for Soil Research, Scotland, published a concentration method of spectrographic analysis using mixed organic reagents: 8-hydroxyquinoline, tannic acid, and thionalid (β-aminonaphthalide of thioglycollic acid). These mixed reagents quantitatively precipitate Ag, Al, Bi, Cd, Co, Cr, Cu, Fe, Ga, Ge, In, Mo, Ni, Pb, Sn, Ti, V, and Zn, in addition to others not investigated further. As listed above, the 8-hydroxyquinoline alone precipitates most of these elements, while tannic acid assures complete Cr and V precipitation, and thionalide does the same for Pb and Sn. This single-step precipitation has many advantages over our earlier two-step method, and was adapted to our analytical problems. We replaced the Mitchell and Scott variable internal Fe standard spectrographic operation by a fixed In standard one. For this purpose sufficient indium in solution was added to the analysis solutions before precipitation to provide 12.1% In_2O_3 in the ignited organic precipitate plus added carbon mixture (designated arcing mixture). In this manner all spectra are prepared from a spectrographic sample carrying a constant amount of In_2O_3 as a buffer and intensity standard.

This single-precipitation method has been extensively applied for the analysis of trace elements in soluble extracts of soils and biological samples whereby the total trace-element content of 50 grams plant or animal tissue, or available trace elements in 200 grams of soil are concentrated into a 50-mg sample subjected to spectrographic analysis. Obviously, a sensitivity extension factor of 1000 is achieved in this manner. Thousands of analyses have been made in Saratoga and Aberdeen by these methods. These methods, without the intricate and time-consuming analytical details, have been outlined here as another example of techniques currently available for extending the sensitivity of emission spectroscopy. I am not going into details on these methods since Dr. Mitchell is participating in this symposium. The method, as used at Saratoga, has been described and illustrated in a paper by G. E. Heggen and L. W. Strock.[15]

Enrichment by Volatilizing Major Constituent. An enrichment of trace metals can sometimes be achieved by direct volatilization of major components of a sample. The customary process of "ashing," too

commonly used and known to warrant enumerating its applications, is such an enrichment process. A nonmedical use of enrichment by volatilizing the major constituent has proved useful in the field of semiconductor germanium research. In the production of germanium most impurities must be removed to a couple of orders of magnitude lower level than previously was done for any other metal, where spectrochemical methods were used as a means of purity control. The reason is that Hall's measurements of *n*-type germanium were found to be affected by impurities down to 3.7×10^{13} per cm^3, which is equivalent to 6.9×10^{10} impurity atoms per 10-mg sample germanium. Based on 4.47×10^{22} Ge atoms per cubic centimeter = $8.34 \times 10^{19}/10$ mg (derived from Avogadro's number 6.064×10^{23}, density 5.35, and atomic weight 72.60) this number of impurity atoms per cubic centimeter represents an impurity concentration of 1.27 atoms per 1×10^9 Ge atoms; or 1.27×10^{-7} at. % (e.g. 1.11×10^{-7} wt. % Cu).

The resistivity of *n*-type germanium ranges from 0.2 to 20 ohm-cm for the impurity range 1×10^{16} to 5×10^{13} atoms per cubic centimeter. Spectrochemical methods for determining impurities in this range of resistivity must therefore operate in the 10^{-4} to 10^{-7} at. % range: i.e., to concentration levels three orders of magnitude below that achievable by direct analysis for the most sensitive elements, and still lower for some elements, including As and Sb in 20-ohm-cm material. The above numbers show that Cu (at 10^{-4}% = 1 ppm) in the most impure semiconductor germanium will be just detectable. A germanium metal sample (spectrographic analysis size) of 10 mg Ge (8.34×10^{19} atoms) with 1×10^{-4} wt. % Cu contains 1×10^{-5} mg Cu (1 mg Cu = 9.53×10^{18} atoms) which is 9.53×10^{13} atoms Cu or 1.14×10^{-4} at. % Cu. Germanium metal of this grade containing only Cu as impurity has a resistivity of approximately 0.2 ohm-cm. Higher-resistivity material contains much less impurity and could not be detected in a 10-mg sample.

In principle, it should be possible to determine any amount of impurity in a sample merely by exciting a sufficient quantity of the sample, so that the number of impurity atoms required to produce a detectable number of light quanta are added to and participate in the light-emission process. This solution has only very limited practical application. For example: To achieve this, it would be necessary to vaporize off, and excite a large number of samples of the usual 10-mg size and integrate the entire light output. For 20-ohm-cm germanium 100 to 1000 samples would be required, or the line intensity should be otherwise enhanced by this factor. Unfortunately,

aside from the mechanical limitations of time, electrode consumption, and accumulation of impurities, this does not provide a usable summation of impurity radiation from the samples. Band spectra (from arc gases and oxides) and continuous radiation would increase and mask any enhancement of impurity element line radiation in the integrated spectra. On the other hand, if the germanium (major constituent) could be fractionally distilled (as some elements can be) from the arc without its radiation being recorded, leaving a clear background on which to record radiation from the accumulated impurities, then the sensitivity of spectrographic emission methods could be extended almost without limit. This cannot be done with germanium metal or oxide samples directly in the arc. It is possible, however, to separate the germanium from most impurities, by a chemical step prior to the spectrographic analysis operation by taking advantage of the high volatility of $GeCl_4$ relative to that of the chlorides of practically all other metals. A large sample of Ge or GeO_2 is treated with HCl and heated to above the boiling point of $GeCl_4$ (86.5° C) but not over 120° C. The boiling points of the chlorides of all other metals (except $SiCl_4$ and $SbCl_5$) are above 120° C, and so they remain as a distillation residue, which may then be analyzed spectrographically.

A spectrochemical method for determining impurities in Ge, based on the above scheme, has been calibrated for 30 different impurity elements in 10-gram GeO_2 samples in the physics laboratory of Sylvania Electric Products Inc. at Bayside. A description of the method and preliminary results were described by L. W. Strock, V. A. Brophy, and T. E. Peters.[16] Careful precautions were taken to avoid impurity pickup, and means of evaluating those originating in reagents and apparatus were devised. The major problem was a mechanical one, of avoiding contaminations and making proper transfers from chemical to spectrographic operation. The method involves removal of the distillation residue from a quartz still and transferring it to a quartz dish for further evaporation after addition of carbon powder carrying an internal standard (Cb_2O_5). The actual spectrographic analysis is then made on this distillation-residue–carbon mixture.

Synthetic standards containing individual metals down to 10^{-6} wt. % (= 1 part in 10^8 or 4.5×10^{14} impurities per cm^3 Ge) were used in the initial preliminary calibration. It was found that some elements, e.g., Ag, Be, In, Mg, can be determined to an estimated order of magnitude lower without interference from residual impurities in the calibrating materials. Most impurities in germanium can be deter-

mined by this (impurity-enrichment) method in the 10^{-5} to 10^{-7} wt. % range: i.e., to 1 part in 10^7 to 10^9 atoms, which, based on 8.35×10^{22} atoms in 10 grams Ge, leads to an approximate range of 10^{14} to 10^{16} for the impurity-atoms sensitivity range of the method. This is the impurity range, which as pointed out above is involved for 0.2 to 20 ohm-cm Ge, with the expressed precaution that these analyses determine individual impurities, whereas the resistivity figures are related to total impurities.

The spectrochemical problem encountered in the analysis of semiconductor-grade germanium has been mentioned as an example of the extremely small amounts of a trace metal determinable by combined enrichment and spectrographic methods. Although 1×10^{11} atoms of no metal can be determined in a 10-mg sample by emission spectrography, when enriched by factor 1000 (by concentrating) to 10^{14} atoms in a 10-mg sample, impurities at these levels can be determined. Similar problems already confront biochemists and will multiply in number as metals are found to be essential components in rarer, biochemically significant compounds.

Other Means of Increasing Sensitivity. Various workers have found other means of increasing line intensities in various types of analytical problems which are difficult to classify in the above adopted scheme. However, the magnitude of sensitivity extension is usually very small compared with those just described, being well within an order of magnitude rather than several orders. Since most of the techniques developed (whether by using another means of excitation or special sample preparation) do lead to some extension of sensitivity, even though the prime emphasis was on "buffering" variations in sample composition, further discussion of them is included under Stabilizing Line Intensities below.

Aside from the d-c carbon arc and hollow-cathode sources, use has been made of other electrodes for the d-c arc, especially copper and aluminum. The replacement of carbon by Cu or Al as electrodes eliminates disturbing CN band spectra and also provides an arc column of essentially different temperatures. The temperatures of a Cu and an Al arc are 5100° C and 4000° C, respectively, compared with 7400° C for the C arc and 2900° C for a K arc. Mechanically interrupted arcs, and various types of higher-voltage discharges are of course commonplace as means of excitation. Spark discharges are widely used in laboratories that analyze metallic samples. Much use has been made of spark discharges in the analysis of powdered and

refractory materials by compacting them into a self-supporting electrode after mixing with some conducting materials such as powdered copper, graphite, etc. However, because of the greater sensitivity and versatility with respect to sample type which it can handle, the d-c arc using both carbon and copper or aluminum electrodes remains in the foreground for determining trace metals in medical and biochemical materials. The literature on spark sources and their analytical application is so numerous and varied that no survey of the subject has been attempted in this chapter. It seems equally inappropriate at this time to attempt any historically comprehensive review of numerous and detailed applications even of d-c arc methods. Such a review would reach the proportions of a monograph.

STABILIZING LINE INTENSITIES

For Trace-Element Analysis

Numerous methods and applications have been developed in spectrochemical laboratories in which substantial portions of other materials have been combined with samples before spectrographic analysis, the object being to "buffer" sample variations in the arc, or reduce them to a common "matrix" for the purpose of stabilizing and standardizing sample excitation.

It will be shown later in this chapter that additional and more specific justification is available for several refinements empirically introduced into spectrochemical methods over the years, particularly of powdered and refractory samples. By these are meant the attempts made to alter the sample as arced to remove its original strict dependence of line intensity on its own unique composition, rather than refinements on electrical components and circuitry in the excitation apparatus. The former may be generalized to *chemical control* versus the latter or *electrical control,* both determining the actual physics of the arcing process. Chemical control was found to be essential by early workers in the field. E. Preuss [17] showed in 1935 that addition of carbon powder to powdered silicate minerals greatly increases the burning qualities of a carbon arc, and suppresses fractional distillation from the arc. I had developed methods over 20 years ago [18] which made use of the enhancing effect of alkali metals on certain spectral lines as a buffering agent to assure comparable dependence of intensity of lines of other elements on their concentration in samples whose alkali content was small and variable. Exten-

sive use of this buffering principle has been made since then by myself and others.

The basic function of the buffer here is to produce an arc of a lower temperature, which is determined by the added alkali, and which is not influenced by variations in the sample itself. This would be temperature buffering. The general term buffering was originally employed to include any function that tended to make spectral line excitation less dependent on the sample composition. In other words, before analysis, the sample was admixed with other substances, which would themselves largely determine the light emission from the arc of the sample lines used in the analysis.

The problem of fractional distillation of the sample components, or even nonvolatilization of the more refracting components, was recognized by early users of d-c arcs. Some early methods (e.g., Preuss) actually split the light emission of a burning sample into separate photographs, recording first the high volatile elements such as Hg and Zn, and then on a separate photograph the more refractory elements. This, under such circumstances, would eliminate difficulties encountered when otherwise usable lines of the two groups coincided. Preuss stated that, from unbuffered clays in a carbon arc, small amounts of Pb, Sn, Ge, Ga, and Cu first appear in the spectra, followed by alkalies; then by Cr, Mn, Fe, Ni; then Mg, Si, Al; then Ca, Ba, Sr; and finally Ti and Zr. Preuss also recognized the dependence of volatilization from the arc on particle size and the crater depth.

By the addition of certain substances to the sample, convenient and more effective splitting of a sample into more volatile and less volatile elements was achieved. A convenient use of this principle has been made by B. F. Scribner and H. R. Mullin [19] in determining impurities in uranium by driving them out ahead of uranium whose spectrum is very rich in lines and renders their detection, identification, and determination difficult. Scribner used Ga_2O_3 as the "splitting" substance. In the United States wide use was made of this technique, popularized as "the carrier distillation method."

Many analytical techniques have made additions to samples for the purpose of driving out more refractory elements. In addition to carbon, Al_2O_3, SiO_2, and others were used in Göttingen in the early and middle thirties by Preuss, myself and others. More recently N. W. H. Addink [20–22] has described these as very useful for this purpose, along with NiO. Since 1940 I have also made extensive use of NiO in the determination of Be in mineralogical and metallurgical

products, finding it useful (along with Fe_3O_4) in stabilizing the intensity of the Be lines largely because of its parallel volatilization and excitation. Thousands of analyses have been made by me and later by my associates in BeO ranges from 0.0001 to 20%. We see thus that buffering may be used to control arc temperature, volatilization, and excitation.

The control of volatilization is not a direct consequence of temperature control, as it is also tied up with fluxing and decomposition of sample and reduction to metal, the atoms of which must actually be liberated from the sample and reach the arc gas for emission of atom spectral lines. In this connection, carbon is seen to be an important fluxing agent, in that it is active in reducing things like oxides to metals. Other substances like carbonates (Na_2CO_3, Li_2CO_3, $SrCO_3$, etc.) or known mineralizers like sodium tungstate are also practical fluxing agents which have been used. Some of these have been employed as buffers for different reasons: e.g., $LiCO_3$ because it lowers arc temperature, thus enhancing lines of low excitation potential. A. Kvalheim [23] used $SrCO_3$ for a buffer in analyzing silicates. Sodium tungstate is useful because its refractory oxide WO_3 tends to drive out more refractory metals. The same substance therefore added as a buffer may alter the arc in several ways, usually without the author's knowledge or intention. All such substances are referred to as buffers; which, as will be shown below, having correct ionization potential with respect to other metals present and in sufficient concentration, may also act as exciter particles and enhance certain lines by excitation of energy-exchange collisions.

For Major Constituent Analysis

The advantages of adding buffer substance, or of combining sample material with one or more other substances to produce samples, as arced, of a predominantly common matrix, are not limited to trace-element analyses. There are two other very important advantages:

1. To create favorable conditions for major constituent determination methods.
2. To provide the basis of a general semiquantitative method for analyzing samples of diverse composition.

Germanium was first suggested and widely used by me and my associates for these purposes.[24] Its selection was based on the several different points:

1. Observed high regularity of concentration calibration curves for minor constituents in germanium metal-powder synthetic standards.
2. Marked suppression of fractional distillation from a d-c arc rich in germanium metal.
3. The temperature range of the liquid state of many metals lies wholly or partially within the temperature range of liquid germanium metal. A germanium metal matrix thus affords the possibility of volatilizing many elements from a homogeneous metallic solution.
4. The ionization potential of germanium is above that of most other metals, thus lying between them and that of carbon of the electrodes. This reduces the necessity of the sample elements providing the conducting particles and competing with each other in the process, while still supplying more charge carriers in the arc than are available from carbon alone. The Ge arc temperature is 2000° C lower than that of the C arc.

Considerable work in both directions (major constituent analysis and/or general semiquantitative methods) was carried out between the years 1946 and 1951 in Saratoga Springs, initially by me, and later under my direction by Ladslav Dejnozka and Carl F. Edwards at Saratoga Laboratories and Strock Laboratories. By a tenfold dilution of sample with germanium metal, the analysis of a major constituent (1 to 50%) in a silicate rock, for example, reduces to determining concentration in the range 0.1 to 5% in a sample as it reaches the spectrograph, while the arcing process is dominated by the behavior of Ge in the arc. Thus effects of self-absorption are reduced, compared to those arising in the case of lines of major constituents of the same amount of undiluted rock fed into the arc. Choice of crater volume also is a further means of controlling the number of sample atoms used for analysis of Ge-diluted rock sample. In this work we determined two optimum crater sizes for covering the necessary concentration range: namely, $\frac{7}{64}$ and $\frac{1}{16}$ inch diameter by 4 mm deep in $\frac{3}{16}$-inch-diameter electrodes—the one accommodating approximately 60 mg, the other 10 mg of arcing mixture. Since the arcing mixtures contained rock + Ge + C in the ratio 1 + 9 + 20 (2 parts carbon per 1 part all other components of mixture) only $\frac{1}{30}$ of the arcing mixtures are rock sample, or 2 mg in one instance and 0.33 mg in another. As in all such methods, the sample is completely volatilized and its emitted light integrated. The only published [24,25] part of this work is that which has been interpreted as evidence for a contribution of collisions of the second kind to concentration calibration curves in spectrochemical

analysis. Further details are contained in Reports to The Office of Naval Research under the grants previously referred to.

Parallel studies were made on the same standards, using only carbon as the buffering material; i.e., at 60 × carbon dilutions. E. Preuss [26] had already shown the value and feasibility of calibrating an analytical method in terms of amount of sample: i.e., by incorporating weighed amounts of sample in a carbon powder matrix.

For a General Semiquantitative Method

The other advantage of combining an unknown sample with a common matrix (e.g., Ge–metal powder) is that materials of a wide diversity of composition present a common problem of excitation when they are minor constituents in that matrix. These can be employed as standards, calibrated in one operation, and applied to a large number of impurities or minor constituents. Naturally, some elements or compounds when present in amounts over 1% (= 10% in original sample) will affect the line intensities of other elements. In the great majority of cases, their calibration curves will not be shifted to an extent that prevents a very useful and approximate determination of all elements detectable in spectra of the spectra obtained. In brief, a quick and useful semiquantitative analysis of any powderable material can be made by mixing with Ge metal powder, by reference to a single set of standards. The dilution factor, of course, prevents the techniques from applying to trace elements in samples, whose lines are already on the verge of, or below, the limit of detection in spectra of their undiluted samples. Extensive use was made of this method for quick survey purposes in my Saratoga laboratories, and I understand that considerable use has been made of it by the spectrographic laboratory of the General Motors Corporation since my first report of the method at their Spectrographic Symposium in 1949.

The convenience of reducing samples to a common or nearly common base is obvious to all who have been confronted with the analysis of material of widely varying composition by spectrographic means. This was achieved by E. Minami [27] by enriching the rare-earth content of the rocks under study in a sesquioxide precipitate. Others employed X-ray emission analysis or optical spectroscopy. I found cupferron precipitation useful, and R. L. Mitchell [28] has similarly found precipitation with other reagents a help in the pretreatment of the sample prior to analysis. These and many other techniques can be employed to enhance the sensitivity, accuracy, and universality

of materials that do not lend themselves to simple electrode-arc analysis.

The requirements for intensity stabilization in quantitative methods are varied and frequently not obvious since an interplay of many factors is usually involved. An example encountered and described by me [13,29] may be mentioned, in which the elements Zr, Ti, Ce formed very stable carbides and nitrides, which could not be volatilized, and dissociated at temperatures reached on the carbon cathode,

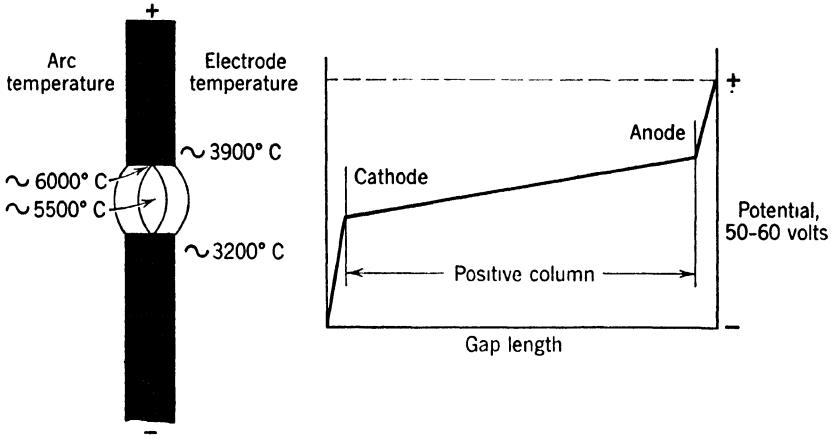

FIG. 2. D-c carbon arc in air, 2 to 12 amperes.

but which did volatilize from the anode. This also happens with some refractory oxides like tungsten. Thus a certain amount of temperature control is available by choosing between cathode and anode as sample carrier, which also affords a means of varying the rate of feeding sample into the arc. The rate of anode consumption is much greater and more dependent on current as reported by me [30] at the 1946 Spring Meeting of the Optical Society of America and in ASTM Symposium on Spectroscopic Light Sources. The maximum temperature of the anode (tip in contact with arc column) is the sublimation temperature of graphite or approximately 3900° C; this and other temperatures of the carbon arc are shown in Fig. 2.

By Close Matching of Standard and Sample Matrix

As soon as attempts are made to analyze samples in their raw state, special precautions must be taken to assure close similarity of sample and standard. A long list of techniques developed in my own lab-

oratories over the past 20 years could be described in this connection, where great effort was taken to prepare standards which would have as their major component not only the same elemental analysis as the unknown samples but also a closely similar mineralogical and physical composition. All these factors have been shown to have a measurable and sometimes serious effect on the reliability of a method and its application. These statements are accepted today as general truths, but they were presented and propounded originally to audiences conditioned to statements of manufacturers of equipment who finally had simplified chemical analyses by making available apparatus that had a unique response to any and all elements, regardless of their environment and combination.

Earlier serious contributions to the spectrographic literature seemed to have solved all problems for the quantitative analyst by stabilizing and controlling all unsuspected variables by use of homologous line pairs and internal intensity standards—and some people still write as if this were strictly true. The fact is that these earlier efforts were laudable and contributed greatly to the development, understanding, and use of emission spectroscopy in the field of compositional analysis. A good review of this earlier work is given in the books by the Gerlachs [31] through 1933. The inevitable accumulation of experience and exacting demands and the greater diversity of application have since shown that the virtues of such means of control are not sufficiently universal, delicate, or practical for present-day requirements. The analyst is still confronted with variations in sample composition and the highly sensitive manner in which its constituents partake in the arcing (or sparking process)—leading to variations in light-emission intensities, which depend on relative number of atoms, their rate of feed, gas temperature, surrounding atmosphere, as well as absolute numbers present.

Most of my personal experience in attempting to match samples and standards has been in the field of inorganic and geological materials. Rather than outline any of it here, I have selected an appropriate example from the literature on biological materials. This is a method described by K. Pfeilsticker [32] for the simultaneous determination of Na, K, Ca, Mg, and P in blood serum.

This work is referred to principally to illustrate the great effort that must usually be expended to match synthetic standards and raw samples. The excitation is in reality a low-voltage a-c spark, which Pfeilsticker developed from his well-known mechanically interrupted

arc by adding high-frequency ignition. This was necessary because films of serum caused poor ignition of the interrupted arc.

A special internal standard element is incorporated into the serum for each element to be determined as follows: Li for Na; Rb for K; Sr for Ca; Mn for Mg; and Sb for P. The standards were prepared with a base designed to approximate the serum with respect to both cations and organic content: namely, 5 to 20 ml impurity stock solution, 2.3 grams glucose–urea condensation product, 1.5 grams glycocoll, and 0.05 ml chloroform as preservative. This combination of organic materials provided a close match in C, H, O, and N content between serum and standards. Special Na standards carrying Na as both Cl^{-1} and HCO_3^{-1}, in citric acid were also employed.

Equal parts of serum and reference liquid (containing 200 mg Li, 40 mg Sr, 10 mg Mn, 2 mg In, 40 mg Sb, and 60 mg Rb, and 3 grams NH_4Cl in 50-ml solution), normally 0.5 ml each were used for an analysis. The mixture was transferred to the top of machined and cleaned electrolytic copper electrodes, placed in a thermostatically heated aluminum block for drying; then duplicate samples were arced for 25 seconds. Determinations were made with an average deviation of about 4%. Pfeilsticker examined in some detail the influence of the organic components on intensities, but gave no recognizable limits of sensitivity for the method (except that it covers well the normal range of the determined metals in blood serum). His work is quoted as an example of a problem arising from attempting to calibrate raw biochemical samples. I am not offering any comparison with flame photometer methods on serum.

The Gerlachs[31] made extensive applications of spectrographic analyses on medical and biological materials, especially of tissues and body organs. Their techniques involved the partial dehydration of a sectioned sample to a point where it readily absorbed a measured amount of a solution containing an element to serve as internal intensity standard, which, when further dried and fixed by H_2S treatment, was placed on a filter paper over a supporting electrode for excitation by means of a high-frequency spark. Part II of their book contains a large number of details and applications. Their strong partiality for a spark source was based on the nonavailability of pure carbon electrodes at the time (1930–33). Their method did not involve any chemical enrichment, and so determined only those metals detectable in essentially raw samples. They strongly emphasized the effect of sample composition on both line intensities and intensity ratios.

The above discussion of means employed for increasing sensitivity

and stabilizing line intensities is by no means a complete coverage of the subject, and perhaps not even a fair sampling of the literature, since it is weighted with work with which I have been personally associated. I believe it will give some idea of the complexity of the field of emission spectrochemical analysis and arouse the suspicion that the subject is liable to be overloaded with meaningless variations of operational details. This is of course true, and most published papers on "methods" are really details of a technique applied to a particular practical problem. Their value lies in passing working details on to others confronted with a similar analytical problem. Some unifying ideas concerning the basic processes on which a spectrochemical analysis depends are gradually emerging, partially as a result of the accumulation of empirical results, as well as of actual research on the methods themselves.

My own work on the role of excitation-energy exchange collisions in spectrochemical methods has prompted a study comparing the basic processes occurring in a d-c carbon arc and the conventional hollow-cathode source, and from this study has come a clearer understanding of the role of "buffer" as it has been applied on a rather empirical basis. For this reason, and since hollow-cathode sources have been stated earlier to offer a means of increasing sensitivity, the functioning of d-c carbon arcs and hollow-cathode sources are considered here in some detail, as indicating one direction from which more quantitative understanding may be applied to spectrochemical analysis methods.

Suggested Hybrid: D-C Carbon Arc and Hollow-Cathode Source

In addition to the work of H. Schüler [9,10] and J. R. McNally, G. R. Harrison, and E. Rowe [11] on hollow-cathode sources, N. E. Gordon Jr. and H. D. Cook [33] have designed a complete unit consisting of a water-cooled cathode tube, power supply, and carrier gas system for purposes of spectrochemical analysis.

The present most-established application of hollow-cathode sources is in the field of line spectroscopy where maximum line sharpness is essential. This is not required by spectrochemical analysts, in most cases, who have available instruments of adequate dispersion. In fact, extremely narrow photographed lines present difficulties for reliable microphotometric measurement. Line width in a photographed spectrum can be increased by use of wider slits in case their physical width is undesirably small. The principal promise of hollow-cathode sources is for increasing sensitivity by repetitive excitation of atoms in the

more confined hollow-cathode source, and for increasing accuracy by better control of the mechanism of transforming a solid sample to the arc column and its subsequent excitation. Sample sputtering from a cathode surface is apparently a less selective process than fractional distillation of a heterogeneous solid sample from a hot electrode of the d-c arc. Further, excitation in a hollow-cathode source is due to excitation-energy transfer from positive ions to sample atoms: i.e., "collisions of the second kind," whereas excitation in the d-c arc column is largely thermal: i.e., by direct collisions of unexcited atoms with conversion of kinetic energy to excitation energy. The hollow-cathode (cold) is a pure excitation source, in the sense of a single-excitation mechanism being involved, whereas in the d-c arc several different mechanisms are active, which may partially interact and interfere with each other. Thus radiation of a single individual line from a d-c arc may result from a composite excitation mechanism, only one of which may be subject to operational control while the others may fluctuate widely and be very sensitive to sample composition. Obviously, intensity stability for a given d-c arc method, for these reasons, can be of low order. Although much of the d-c arc radiation results from thermal excitation, there is good evidence that "collisions of the second kind" contribute substantially to the intensities of many lines in d-c carbon-arc spectra. These contributions either increase or decrease their normal thermally excited radiation, depending on whether the atoms involved have received or given up excitation energy in the collision event.

That "collisions of the second kind" should occur in the open d-c carbon arc is to be expected; but only recently [24,25] has the magnitude of their contributions been demonstrated from spectrochemical analysis data. It should not be surprising that excitation energy-transfer collisions contribute to d-c carbon-arc line intensities once the facts are known; that positive ions and excited atoms are derived not only from the atmosphere, but also from the sample itself, and exist in the arc column along with neutral or excited atoms derived from the sample. Just as in the hollow-cathode source, these positive ions are attracted to the cathode, where, if the sample is placed in the cathode, they mingle with a dense cloud of sample material vaporized from the cathode. It is immaterial to these positive ions by what mechanism sample atoms have reached the arc column. The only difference is that, in the open d-c carbon arc, the sample atom gas is hotter, enabling the atoms to excite each other before giving up or accepting excitation energy from the colliding positive ions or a more highly excited metal

atom. Under these conditions, the atoms being in more violent motion, the lines radiated will show more Doppler broadening than in the much cooler hollow-cathode source. This constitutes, in fact, the main value of the latter as a source in the study of line spectra.

Excitation by "collisions of the second kind" in the open d-c carbon arc are not confined to examples where the sample is vaporized from cathodes. In other words, the sample atom cloud does not have to be densest in the portion of the arc column where positive ions tend to migrate. Published experimental evidence for contribution of "collisions of the second kind" have been obtained from d-c carbon-arc spectra of arcs carrying the sample in the anode carbon. It would seem that lines radiated from arcs with samples as cathodes should contain still higher proportions of "collisions of the second kind" excitation, thus approaching hollow-cathode excitation to a greater degree.

With the knowledge that atmospheric open d-c carbon arcs do contain a certain amount of hollow-cathode excitation, some considerations have been given to how the former differs from, or may be altered to acquire more of the advantages of, the latter source, or vice versa. In other words can a "hybrid" source be imagined, whose several mechanisms merge, in a constructive manner, the best features of both sources? In the first place, as already mentioned, the gas pressure of the carrier gas is confined to a narrow critical range in the hollow-cathode source. It must be low so that the positive carrier-gas particles reach the cathode and "atomize" the sample into the arc column where excitation is then effected by other ionized gas particles. This places an upper limit of 2 to 3 mm on gas pressure. Lower limits of an order of magnitude below these values are determined by the necessity of atomizing sufficient sample and having available a sufficient number of charged particles to collide with sample atoms. Now, if another mechanism is provided to furnish samples into the arc column, then higher gas pressures (at which their mean free paths are too short to permit sufficient numbers reaching the cathode for sputtering off sample) may be used. A higher number of positive ions are thus available to effect more collisions with sample atoms and thus more intense radiation from them, thereby increasing sensitivity. In the open d-c carbon arc, the sample atoms actually enter the arc gas by a separate mechanism (or agency) from the exciting one, as in the case of the collision-excited component of the radiation: namely, thermal volatilization. In hot hollow-cathode sources this also occurs to a certain extent—so there is an increasing similarity between hot hollow-

cathode and open d-c carbon-arc sources. When the sample is fed to the arc column by a thermal process, two advantages of the hollow cathode are immediately sacrificed: (1) narrow line width and (2) absence of selective sample volatilization. As pointed out, the first is no disadvantage in spectrochemical analysis, but the second definitely is.

Fractional distillation is the source of much trouble in attempting to compensate for intensity fluctuations in the burning arc. This disadvantage, in the case of sacrifice 2, must be weighed against other advantages which are inherent in the case of "thermal feeding" of a sample into the arc column of an open d-c carbon arc. One obvious advantage is that samples of much larger size can be fed into the arc in a short time by thermal, compared with a sputtering, mechanism. Hollow sources require long times (½ to 1 hour) to develop maximum intensity of radiation, an impractically long time for anything but occasional research-type analyses. Therefore, an important time-of-analysis factor favors the more rapid thermally fed open d-c arc. The vapor-richer column of the d-c carbon arc however, causes self-adsorption in many lines radiated from it. This is a serious disadvantage to counterbalance the favorable time-of-analysis factor.

It thus appears, that a compromise between these advantages and disadvantages lies in altering the conventional open d-c carbon-arc source in a manner that maintains thermal feeding of sample but limits the amount of sample in the arc column by use of smaller-sized samples. The resulting lower intensity of lines radiated, because of small sample size, can be compensated by confining the arc column in such a way that the same atoms are repeatedly excited; that is, the total vaporized sample remains in close proximity to the arc column for a longer time, thus increasing the chance for a greater portion of the atoms to become excited either thermally or by collisions. Such confinement of the arc column will prevent sharp temperature gradients across a narrow arc cross section, thus promoting thermal excitation in a large arc-column volume. Simultaneously, excitation by "collisions of the second kind" can be enhanced by feeding into the confined d-c arc a carrier gas (just as in conventional hollow-cathode sources but at higher pressures), since creation of long mean free paths of carrier-gas ions is not required for sputtering the sample, thus providing more positive ions to effect more collisions with sample atoms, and thus more excited atoms and light emission from them. To accomplish this physically, the cathode should almost completely surround the arc column, providing a cup enclosing the sample and

arc for the purpose of attracting positive ions—which at the same time creates a furnace and provides more opportunity for thermal excitation throughout a larger arc-column volume. With the arc so enclosed in a cathode cup, the addition of a carrier gas to provide a controlled gas atmosphere is easily accomplished.

The use of helium or argon has the added advantage that both oxygen and nitrogen may be excluded from the arc-column environment, both of which gases are undesirable. Oxygen readily combines with sample metallic atoms or ions, and this process not only robs them from the arc gas, thus depleting the number of radiating atoms in a short time, but these oxides produce undesirable molecular band spectra which mask or interfere with atomic line spectra. Nitrogen combines with carbon to yield intense CN band spectra which interfere with the use of many atomic lines, completely blanking out large otherwise useful wavelength ranges. The addition of carrier gas, for the purpose of promoting excitation energy-transfer collisions, thus simultaneously provides atmosphere control of the arc. It has been found advantageous to use mixtures of He and A as carrier gas in hollow-cathode sources. Argon is useful because its larger mass leads to more effective sputtering of cathode material. Its ionization potential of 15.69-ev limits its exciting ability largely to atomic spectra.

Spectral lines of higher energy (spark lines) can be excited by using helium as carrier gas because of the higher excitation potential of He (24.48 ev). Thus, in practice, carrier-gas mixtures of He and A are used—A to achieve maximum cathode material feed by sputtering, and He to achieve higher energy excitation. Since high energy excitation is seldom required nor desired in spectrochemical analysis methods, there is no general need to use helium as a carrier gas. The 15.69-ev excitation potential of argon atoms suffices to excite the atomic lines of all metals—it being close to carbon (11.20 ev) itself.

Further consideration of this same point suggests an additional improvement of a hybrid d-c-arc–hollow-cathode source. Since hollow-cathode excitation results from sample atoms colliding with positive ions of carrier gas, a more generalized source is one in which excitation occurs by collisions between neutral or excited atoms or ions and other atoms or ions while excited to higher excitation energy. In such a generalized source, the carrier gas of the hollow-cathode source may be replaced by any element whose principal function is to provide an abundant and constant number of excited particles which will transfer their energy to the sample atoms on collision. The probability of such collisions occurring increases with the number of such

atoms introduced into the arc. Such atoms added for this purpose may now be designated as excitation energy donors, or exciter particles, and are a generalized substitute for the usual carrier gas in the normal hollow-cathode source. The material added as exciter particles, to be useful in analytical methods, must have an excitation potential above that of most metals to be determined, yet not so far above that very few of their atoms are excited or ionized relative to the number of lower ionization-potential atoms present. The addition of energy-donor atoms of ionization potential higher than the atoms to be excited assures that all possible excited states of the donor atoms are available for satisfying the requirement of close energy-level match in order that energy transfer occurs on collision.

It so happens that germanium metal meets these requirements with the ionization potential of 7.85 ev. The use of a solid has the great advantage, over a gaseous element as an exciter particle, in that it can be, intimately and in exactly controlled proportions, incorporated mechanically with the sample and fed into the arc gas simultaneously with it. Further, far greater numbers of exciter particles can thus be added to the arc column, thus increasing the number of sample–exciter-particle collision events, and thus creating more collision radiation. Other favorable properties of a germanium-rich carbon arc include (1) the ability of molten germanium to dissolve large amounts of most other metals, thus forming a homogeneous melt in the crater before thermal feeding of the sample into the arc column, (2) the large number of metals whose liquid range is covered by the temperature range of liquid germanium, (3) high purity of metal available from technical developments in its production as semiconductor material (being the purest of all available metals), (4) its brittleness which enables it to be powdered and ground together with the sample, (5) the relative simplicity of its arc spectrum, (6) large selection of lines available for intensity standardization, and (7) its favorable ionization potential relative to most other metals. The use of Ge added as exciter particles eliminates the necessity of using a gas as exciter, but argon should still be used to provide an oxygen- and nitrogen-free atmosphere in the source. It is to be expected that germanium metal fed into the arc independently, through volatilization from a second source, would provide a maximum stable light source. The reason is that the rate of thermal feed of exciter Ge particles will be independent of the sample, always vaporizing from a charge of pure Ge metal. A practical manner of achieving this is to vaporize the Ge from a cratered, center-post

cathode, i.e., the sample being in a surrounding annular crater. A schematic diagram of such a device is shown in Fig. 3.

The above discussion emphasizes the fact that the light source is a very crucial component of spectrochemical equipment and the analytical process. It is the stage in the entire analytical operation that determines the sensitivity and reproducibility of a method. A proper

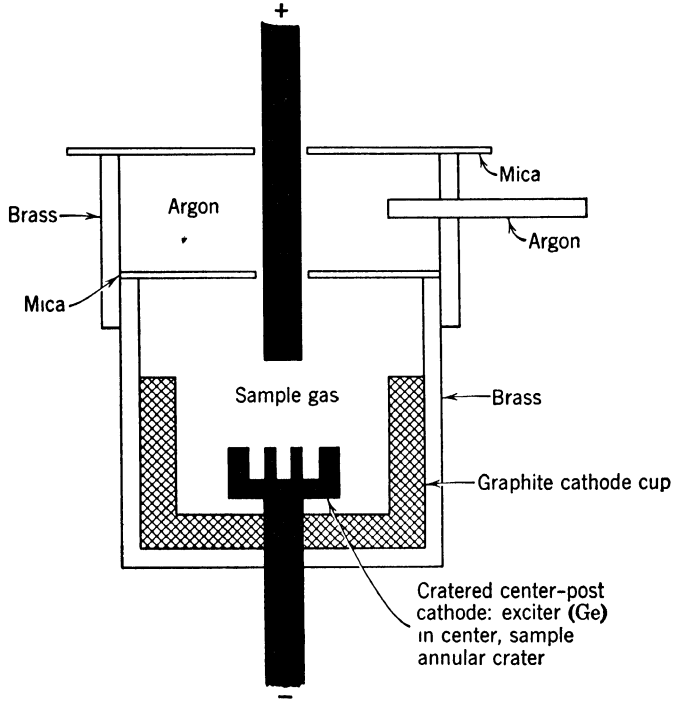

FIG. 3. Cathode-enclosed carbon-arc column with argon seal.

understanding of the fundamental process active in it is thus required to determine the optimum design and operation of a light source for analytical purposes. Attempts have been made to develop a light source combining the essential and advantageous features of the conventional hollow-cathode source and the open-atmosphere d-c carbon arc. Operation-wise, it is a cathode-confined arc column with thermally fed exciter particle and controlled-gas atmosphere. It is really a hollow-cathode carbon arc at atmospheric pressure in argon.

That germanium could play a direct role in the excitation of sample atoms was not obvious when it was first used as a light-emission regulator; but the above analysis of the separate processes, active in the hollow-cathode carbon arc, shows that it was an ideal choice as exciter

particle as well. It is now evident that a portion of any beneficial effect observed by the addition of "buffers" to spectrochemical samples may be attributed to their functioning as exciter particles in the source in addition to any other purpose they might serve, the relative importance of their contribution varying, of course, with each case. The role of "buffers" in the light source can now be understood and applied more rationally in a quantitative manner.

BASIC PRINCIPLES OF CALIBRATION FOR QUANTITATIVE ANALYSIS

General Remarks

The intensities of some spectral lines radiated by any given element present in a sample placed in an excitation source are dependent on the number of atoms of the element in the sample. This is the simple physical criterion for a quantitative spectrochemical method of analysis. The practical problem of utilizing this relationship is not so simple, since the manner of response of the detectors employed to measure the irradiated intensity enters the problem. It is therefore necessary to convert numbers resulting from measuring the response to others representing the number of atoms in a sample that produced the particular spectral line selected for an analysis. This requires a *calibration* procedure, in order to relate intensity to concentration or number of atoms present in a sample. This is consistently referred to in this chapter as *concentration calibration.* A prior step of *plate calibration* is required when a photographic emulsion is used as an intensity detector, in order to relate the optical density of the photographic effect produced by a spectral line to its intensity. Since intensity ratios only are needed, the plate-calibrating intensities may be relative ones only. They are therefore more correctly referred to as photographic line intensities to distinguish them from actual light-intensity values. The entire process of calibration is thus one of *objective photographic photometry* for determining the intensity ratio of two lines in the same spectrum—one line used as intensity standard, the other of unknown intensity and varying with the concentration to be determined.

The same basic problems and operations underline all quantitative methods of spectrochemical analysis, but practical procedures vary in considerable detail according to (1) method of recording radiation from the excited sample (photoelectric as in direct readers or photo-

graphic), (2) means of excitation (spark or arc), (3) type of sample and combination of elements to be determined (which largely determines kind of excitation, supporting electrodes, "buffers," exposures, etc.), (4) optical equipment available (prism or grating and its mounting which determine means of plate calibration), (5) technique of intensity calibration, (6) analysis load, and (7) speed required. It is impossible to present an adequate discussion of all these operational factors within the scope of this chapter, so that, here again only those aspects are stressed that apply most generally to the subject of trace-element analysis in the medical and biochemical field. This means that special procedures and equipment utilized in many metallurgical laboratories for handling heavy analysis loads at high speed, such as direct readers, short exposures, rapid photographic processing, and minimal photometering, need not be considered as of prime importance for which all else must be sacrificed or minimized. Accordingly, in the medical field, procedures may be employed in which basic features of the method are less masked and remain subject to more obvious control. The nature of the samples encountered is such that the d-c arc is the most widely used light source (along with more recently improved flame sources described by others at this symposium).

This chapter describes intensity measurements and standardizations as they may be readily made in d-c arc methods. Whether prism or grating spectrographs are used is (except in rare instances) immaterial, except that the type of grating mounting determines the plate-calibrating technique.

In industrial laboratories, having heavy analysis loads, and of necessity, relying on rather routine-type operators, specific devices have been developed for processing data. All such devices are specifically tailored to special problems, laboratories, and equipment. In his excellent and complete account of spectrochemical analysis with particular reference to the aluminum industry prior to the advent of direct readers, J. R. Churchill [84] has described and illustrated the operation of various calculators for rapidly converting spectrograph information to analytical results. Instead of a review of these special "short-cut" devices here, the basic photographic photometry underlying their operation is stressed.

Use of Intensity Ratios in Quantitative Methods

As stated above, a quantitative method of analysis involves the use of *photographic photometry of line intensities* to determine the rela-

tion of the number of parent atoms and the intensity of their spectral lines emitted from a light source.

Since the line intensities are calibrated against known concentrational standards, it is only necessary to determine them against some fixed-reference intensity standard. This permits the determination of intensity ratios of lines in both unknowns and concentrational standards relative to the fixed intensity standard. Therefore, both in setting up a method and in analyzing unknowns, the basic problem is in determining the intensity ratio of a line selected for study to some fixed light intensity.

Several intensity standards have been resorted to: (1) total light radiated by the source, (2) background or continuum in the neighborhood of a line used, (3) an external light source, (4) an internal intensity standard: i.e., spectral line of a sample constituent or of some added substance. In this chapter only the fourth standard need be seriously considered.

Internal Intensity Standards. The *internal-standard* principle was introduced by Walther Gerlach [35] 30 years ago for the purpose of referring the intensity of the analysis line to another line (used as a reference standard) in the same spectrum. Both lines, properly selected, would be subject to the same uncontrollable fluctuations in the excitation and photographic process; so that, if the reference line originated from an element present in the sample in a fixed or known concentration, an intensity ratio based on it should be a reliable index of concentration of the other element. Part I of the Gerlach and Schweitzer book lists "homologous pairs" of many lines for pairs of element: i.e., lines that have comparable excitation conditions, or otherwise whose intensity ratios are not too sensitive to variations in the light source. By 1933 Gerlach had recognized that intensity ratios may be strongly affected by sample composition, but that "homologous pairs" were subject to less change than others.

The application of the internal-standard principle immediately puts spectrochemical analysis on a more precise basis. Over the years it has been demonstrated that it is not always easy or possible to select an internal intensity standard material (or line) that meets the ideal requirement of exactly following intensity fluctuations of an analysis line during the excitation. The reason is both physical and chemical and is inherent in the photographic process. This has been emphasized on several occasions by L. W. Strock.[36,37] Difficulties in finding a good internal-standard element (or line) are not confined to d-c arc excitation, or even photographic recording methods.

Emulsion or Plate Calibration: Density versus Intensity. In photographic recording methods, the light radiated from the light source is detected as a blackened area on the emulsion. It is thus necessary to calibrate the emulsion for its response to light of various intensity levels for the wavelength range covered by the lines involved in the method. This step of emulsion calibration is frequently termed "plate calibration" in the literature. It is a necessary step in quantitative work to convert photographic density, as experimentally determined

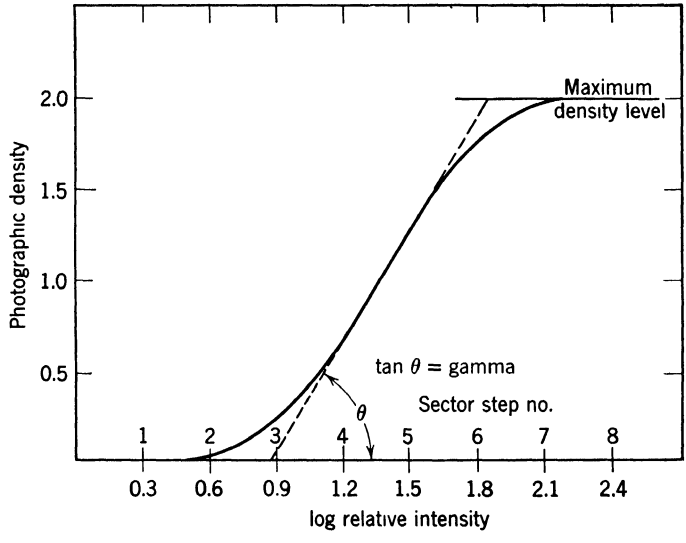

FIG. 4. Emulsion characteristic curve.

from the photographed spectra, to an intensity value for each line, so that its ratio to the selected intensity standard line may be determined. The fact that intensity ratios are actually needed simplifies the experimental problem.

To determine intensity ratios by photographic photometry, it is only necessary to impress on the photographic emulsion to be employed in the analysis a series of photographic exposures—ideally, exposures of varying intensity at some constant time. The resulting densities (as measured on a microphotometer) plotted against these intensities is the "characteristic" curve of the emulsion. A typical curve is shown in Fig. 4. The intensity units are relative values, which is all that is needed in quantitative methods using intensity ratios and known standards for concentration calibration.

The necessity of emulsion calibration follows from the fact that

the characteristic curve is not linear (if at all, only over a limited middle portion); that its slope rapidly decreases to much smaller values, and eventually to zero, both at higher and lower intensity levels; that the shape and slope of the curve depends on the development process (materials, temperature, time, age), wavelength, and size and density gradient of an exposed emulsion area. All these factors might be rigidly controlled and held constant for purposes of a specific routine application if it were not for the additional facts that a given emulsion varies from batch to batch, with its age and storage conditions, as well as from plate to plate, or even from area to area on any given plate.

Since the slope, position, and shape of the emulsion's characteristic curve is rather sensitive to a number of factors which may vary in an unexpected and uncontrollable manner, the value of a photographic line intensity determined from a measured density via such a curve is strongly dependent on many factors other than its intensity in the light source. So that any method involving the determination of the relative intensity of an analysis line via a characteristic curve determined when a method is set up, or when a new batch of emulsion is obtained, and presumed to hold over a subsequent time interval, cannot be used as an index of concentration except for semiquantitative purposes. However, its ratio to another line in the same spectrum, using an internal-standard method, will be independent of fluctuating and deteriorating photographic conditions and thus useful for quantitative method of analysis.

There is a relatively wide difference in the degree of completeness with which the emulsion is calibrated by various workers, and thus in the degree to which intensity ratios are uniquely determined from measured density ratios.

Emulsion-Batch Plate Calibration. At one extreme is the case where intensity ratios are derived by converting density to intensity on the basis of an emulsion calibration curve prepared once and for all for a given emulsion batch for various specific wavelengths over a wavelength range. Many early workers used continuous spectra, while others used line spectra from gas-discharge tubes for preparing "intensity marks": i.e., plate-calibration exposures on the emulsion. Such methods specified that all subsequent spectra used for analyses were to be photographed with the same material and processed in the same manner. This provided a set of "plate calibrations": i.e., emulsion characteristic curves, used in the method to determine the intensity of any line, relative to another in the same spectrum, at approximately

the same wavelength. With such a plate-calibration curve, intensity ratios can be determined from single photometer measurements of a very short line (e.g., 1 mm) on a plate carrying spectra of 50 or more samples to a 4 × 10-inch plate. This technique of "plate calibration" provided a convenient basis for the earlier application of photographic spectrography to high-speed, high-volume analysis of metals in the steel, magnesium, and aluminum industries.

The basic and practical weaknesses inherent in these applications need not be discussed here, which necessitated the frequent checking with concentration standards interspaced with the work loads. The technique of short-line single-step exposures was largely necessitated by and suited for spark excitation. It did not adequately take care of variations and changes in a given emulsion batch, different plate areas, dependence on wavelength, or strong dependence on the geometry of the photographed line (sharp, diffuse, varying background which modifies or exaggerates the Eberhard or development rate effect).[36] Nor did it correct for nonparallelism of characteristic curves at different intensity levels, nor give an immediate check on photometer reading errors since basically only one point was experimentally determined on the characteristic curve of an analysis line.

Individual-Plate Plate Calibration. A partial correction of these deficiencies was achieved by placing on each plate a stepped line spectrum of the same quality and origin (e.g., a duplicate spectrum of the material to be analyzed) as used for the analysis. This provided the following: (1) a characteristic curve of the emulsion for each line (thus at closely spaced wavelength intervals) in the spectrum, (2) a plate calibration for each individual plate of an emulsion batch, each photographic processing operation, and (3) identical spectral lines with those used in an analysis. This was done by E. Preuss [17] in 1935. This still permitted the use of single-step analysis spectra as must result from spark excitation (unless intensity filters are used instead of rotating stepped sectors), while giving more complete plate-calibration control and more reliable intensity ratios. Before this, individual plate calibration was sometimes done using a continuous light source.

Individual-Line Plate Calibration. The other extreme in emulsion control of the density–intensity relationship is possible by one further step in the succession of refinements listed above: namely, by stepping all lines in all spectra, making it possible to determine the characteristic curve for any line in the standard and analysis spectra. With this degree of plate control in deriving numerical values of intensity ratios from experimentally measured density values, such ratios can

then be critically evaluated in terms of the excitation process and the materials excited.

For the purpose of this chapter, the derivation of intensity ratios by the complete plate-calibration technique is outlined, from which any of the less complete calibration procedures (and practical short-cuts) will be readily understood. Since the use of stepped filters can lead to some lack of generality and difficulty in calibrating, the stepped exposures are more often achieved by varying time rather than intensity of exposure. This is achieved by rotating stepped sectors placed in the light path in such a way as to sharply divide a spectrum line vertically into a number of graded exposures by varying exposure time. The photographic response is not identical in the two cases, and the consequences of this fact on spectrochemical methods was discussed by L. W. Strock.[38]

When rotating stepped sectors are used for plate calibration, the resulting characteristic emulsion curves are density:relative exposure-time curves. These permit experimental densities to be converted to their corresponding relative exposure times and relative exposure-time ratios or their logarithms; designated as log RET ratios (as $\Delta \log T$ in my earlier publications), which are actually reciprocals of the corresponding intensity ratios. For most practical purposes the two expressions may be used as if they were identical. I have always insisted on maintaining their correct identity as time ratios, whereas in the literature data referred to as "intensity ratios" are almost always *actually time ratios* or their reciprocals, having been determined by a rotating sector technique.

Photographic Photometric Procedures

Preparation of Spectra. The above basic principles of complete emulsion calibration are inherent in the following spectrographic operation procedure which follows whatever preliminary sample preparation was required. The sample, properly prepared, is packed into an electrode crater and fastened in electrode holders of the excitation unit. Details as to excitation vary with materials, but in a d-c arc the sample is normally burned to completion (with a minimum loss by spattering) by careful selection of current, electrode spacing and regulation, etc. The light from the sample source must illuminate the spectrograph slit uniformly over its entire length (10 to 12 mm). This may be done, as in some prism spectrographs, by a lens placed just in front of the slit, which focuses the arc on the spectrograph

prism. The portion of the light source desired for analytical purposes may be selected by masks placed in front of the spectrograph objective inside the spectrograph. It is best to mask out the incandescent anode in order to reduce the continuum which it contributes to the spectra. With this arrangement a rotating sector placed just in front of the slit lens provides a convenient means of stepping line exposures.

A more flexible method is one used extensively in my own laboratories whereby a two-lens illuminating system, earlier employed by

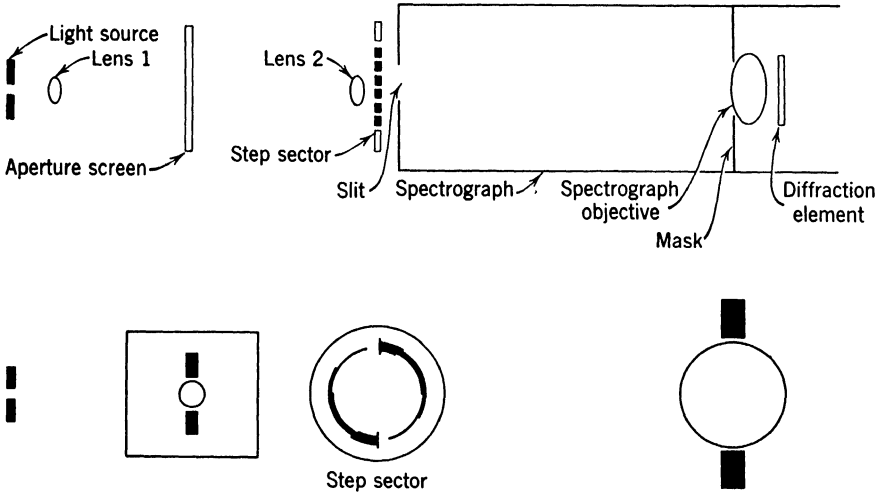

FIG. 5. Spectrograph illumination.

E. Preuss [17] and originally described by F. Twyman and F. Simeon,[39] produces an intermediate arc image on an aperture screen placed on the optic bench by a short-focus objective. By this means radiation from the incandescent electrodes can be excluded from the spectrograph and the entire positioning of the arc image viewed and constantly controlled during the exposure. This permits using light from the entire arc column, or selection of limited portions (e.g., cathode-layer area alone) at will. A second and longer focus lens placed close to the slit then focuses an image of this aperture on the spectrograph prism as before, and permits placing a sector between the slit and the second lens. The arrangement is shown schematically in Fig. 5. A sector providing steps with a factor 2 in exposure time is a very useful one, and especially one with inside cut to permit steps differing by an exposure factor 4 being recorded as adjacent steps in the spectrogram. This greatly facilitates identification of steps in

subsequent photometering and in determining step boundaries, especially for intense lines and emulsions of lower contrast. It is possible to photometer two or three steps, covering a density range up to $D = 1$, in routine work, using plates of a contrast like Eastman "Spectrum Analysis no. 1." In actual practice it is not necessary to calculate photographic density D, where $D = \log I_o/I_l$, i.e., intensity of photometer light through unexposed emulsion I_o/light through exposed area (line) I_l. Instead, galvanometer readings can be plotted on a logarithmic scale directly, arranging for full deflection of unexposed area to read 100. The immediate plotting of galvanometer readings reduces bookkeeping labor and reveals reading errors at once.

Photometer Curves. The time-stepped spectral lines can be photometered in any step of convenient density, which permits the measurement of lines over a wide intensity range in the integrated spectrum

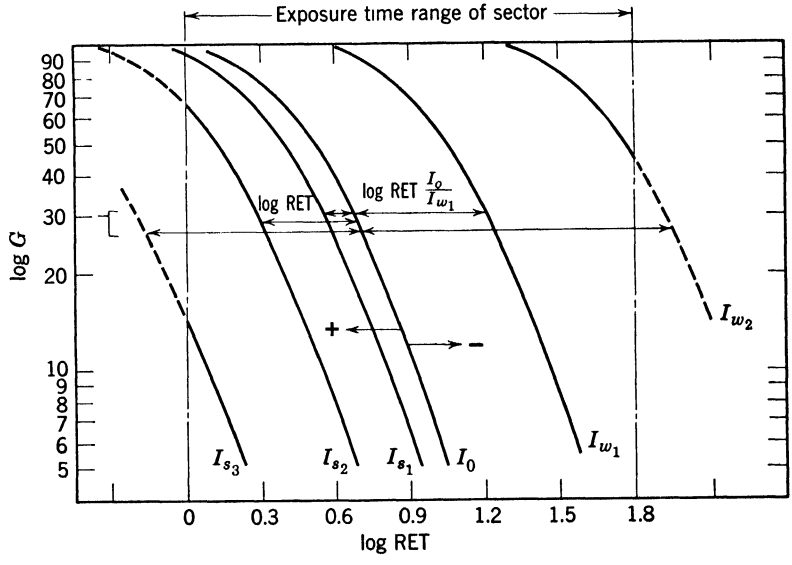

FIG. 6. Photometer curves for lines of different intensity. Log galvanometer reading versus log relative exposure time.

of a sample burned to completion. Maximum use is made of the photographed spectra by photometering three or four steps (except for the weakest lines where fewer steps are measurable). Log plots of galvanometer deflections for several steps in lines of different intensities produce a family of "photometer curves" displaced from each other on the time axis by an amount equal to the log of their exposure-time ratio at any given density (i.e., galvanometer deflection

level) as illustrated in Fig. 6. This time ratio is inversely proportioned to the intensity ratio. For most practical purposes these *time ratios* can be considered as numerically equivalent to *intensity ratios* and as such used as an experimentally determined index of concentration. They are designated as log RET ratios (in earlier publications as $\Delta \log T$).

Derivation of log RET Ratios from Photometer Curves. The log RET value of any line relative to another line, taken as intensity standard, is the logarithm of the ratio of the exposure times required for each to produce equal densities in a photographed spectrum. In practice, the time ratios are expressed so that the log RET ratio is *positive for lines more intense* than the intensity standard, and *negative for lines weaker* than the intensity standard. Thus for lines I_w and I_s, weaker and stronger, respectively, than the intensity-standard line I_o, the following relation exists:

$$\text{For } I_s, \quad \log T_o/T_s = \log T_o - \log T_s = + \log \text{RET ratio}$$

$$\text{For } I_w, \quad \log T_o/T_w = \log T_o - \log T_w = - \log \text{RET ratio}$$

The log RET ratios, along with their algebraic signs, are read graphically (see Fig. 6) from logarithmic plots of galvanometer deflection (maximum = 100) versus log relative exposure-time values of the stepped sector employed. The horizontal separations of the resulting photometer curves from the curve for the intensity standard line are quickly and routinely measured by means of a scale calibrated in the same units used on the horizontal axis of these plots. These values, so obtained, are the log RET ratios. Their sign is determined readily, being (+) for curves to the left (lower RET values, i.e., stronger lines) and (−) for curves to the right (higher RET values, i.e., weaker lines) of the intensity standard curve. Since these log RET ratios provide the basic spectrographic numerical data, their physical and practical derivation has been included in this chapter. This method is particularly valuable when use is made of a number of lines of widely varying intensity, or where the concentration of some elements may vary by several orders of magnitude. When sufficient sample is available, log RET ratios averaged from two or three spectra are best used for an analysis. If considerable chemical work preceded the spectrographic operation, the value of the usual practice of duplicate analyses is obvious.

Concentration Calibration Curves. The initial use of the averaged log RET ratios is to construct a concentration calibration curve, whose

general features are shown in Fig. 7. This curve shows the relationship between log RET ratios (i.e., spectral line intensities) and log concentration of the element producing the line. This provides the practical "working curve" used in routine analysis. Before it can be so used, however, it must be critically studied and checked for reproducibility and stability with respect to normal variations in operating conditions as well as chemical and physical differences in materials to which it will be applied. This latter point is of crucial

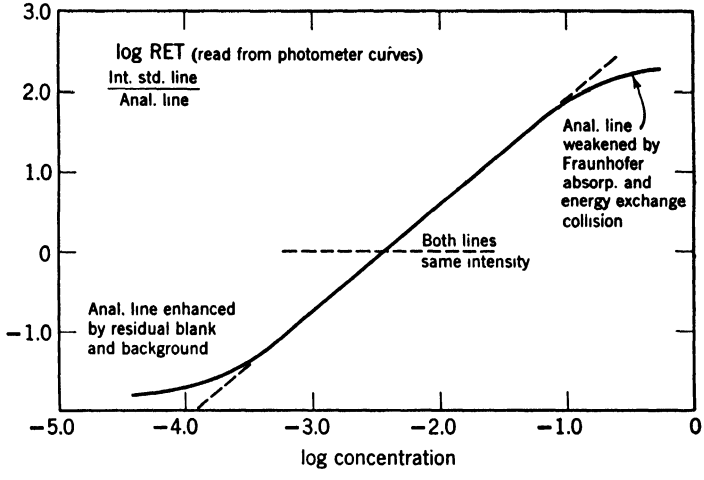

FIG. 7. Concentration calibration curve. Internal intensity standard method (schematic).

importance in the medical field, where considerable labor may be expended in developing a method involving considerable sample preparation, where it is desirable to apply one method to a number of different problems. The reliability of concentration calibration curves can only be determined by replicate analyses, cross-checking with results obtained by other techniques, recovery experiments, and skillful use of synthesized standards.

Some Examples of Concentration Calibration Curves from Actual Methods

Actual concentration calibration curves, along with illustrations of stepped spectra, photometer curves, manner of deriving the log RET ratios from them have been published from time to time and may be referred to for more specific information. One rather detailed exam-

ple, "Quantitative Spectrographic Determination of Minor Elements in Zinc Sulfide Ores" by L. W. Strock,[40] illustrates a method that in-

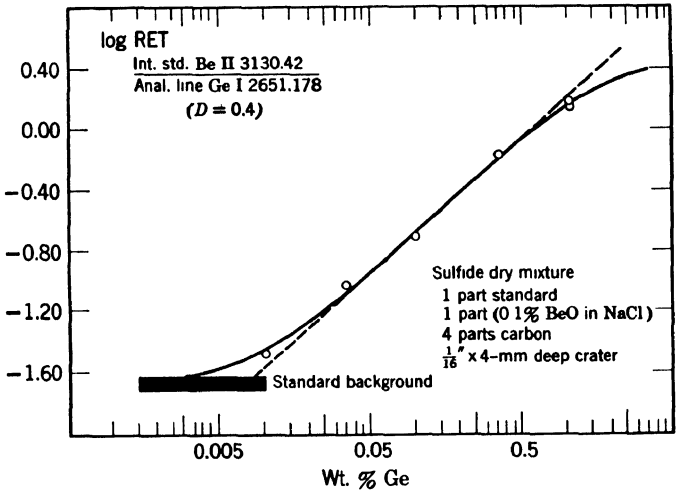

FIG. 8. Concentration calibration curve for germanium. Synthetic zinc sulfide standards.

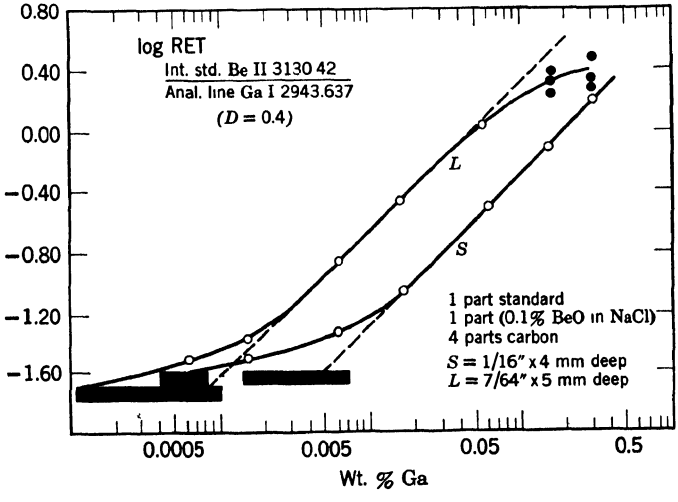

FIG. 9. Concentration calibration curve for gallium. Synthetic zinc sulfide standards.

corporates NaCl mechanically with the dry powdered sample in 1:1 ratio and an added substance (0.1% BeO in the NaCl) as intensity standard. The method was calibrated for Pb, Mn, Cu, Cd, Co, Sb,

Ag, Sn, In, Ga, Tl, Hg, Ni, and Ge in a matrix designed to approximate the samples both chemically and mineralogically. The paper illustrates stepped spectra and photometer curves, and gives calibration curves for Sb, Bi, Sn, Ge, Ga, In, Ni, and Co. The effect of sample size on the arcing process is shown by a displacement of curves of the two different-sized samples. The analytical difficulties encountered where the curves flatten off into the spectrum background or into a residual blank value are also illustrated in this paper. The curves for Ge and Ga are reproduced here as Figs. 8 and 9.

An earlier paper by L. W. Strock and S. Drexler [13] on "Geochemical Study of Saratoga Mineral Waters by a Spectrochemical Analysis of Their Trace Elements" described an early attempt to extend sensitivity to lower levels, by a chemical enrichment using cupferron as a concentrating agent for trace metals as discussed earlier in this chapter. The paper also illustrated the difficulty of constructing a calibration curve for metals which are not volatilized at the lower temperature of the cathode after forming very stable compounds with constituents of the air as zirconium and titanium nitrides, and carbides with carbon of the electrode.

In another paper L. W. Strock and G. E. Heggen [41] have shown, as illustrated by their respective concentration calibration curves, the effect of attempting to standardize a method by using as an internal intensity standard an element that does not volatilize parallel with the elements to be determined. This paper compared the calibration curves using two different intensity-standard materials (BeO and Cr_2O_3) and studied the results when completely volatilized samples versus selected fractions of the emitted light were used for determining alkaline-earth metals in saline brine residues.

Other examples of concentration calibration curves have been published and peculiarities of their shapes discussed by L. W. Strock in "Quantitative Evaluation of a Metal Base Method for Determining Major Constituents in Nonmetallic Samples," [24] and in "Some Experimental Evidence of Collision Processes in Spectrochemical Analysis." [25] A summary of the combined chemical enrichment and spectrographic method mentioned earlier in this chapter, which we applied very widely in Saratoga Springs (and which is still used there by G. E. Heggen and C. F. Edwards) has been published by G. E. Heggen and L. W. Strock. Information on standards is given along with typical results on the trace-metal content of such various materials as leaves, coal, coke, flue dust, petroleum, brine, rat livers and blood, and hog

liver. Figure 10 is the calibration curve obtained for two lines of copper in this work.

More complete information is available in the several Progress Reports to the U. S. Public Health Service prepared by the Spectrochemical Group [42] at Saratoga Springs. These reports contain the results of many analyses, history of samples, and discussion of results for a variety of human and animal pathological materials. Included are

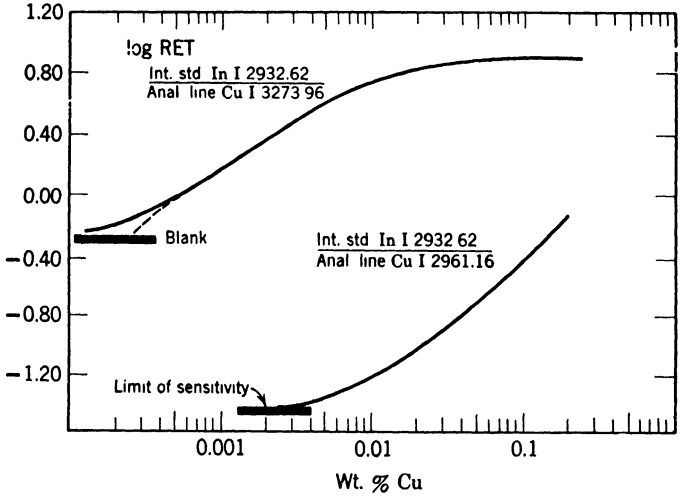

FIG. 10. Concentration calibration curves for copper. Trace elements by organic precipitation.

cancerous human tissues, human and rat blood, rat tumors, rat livers with "butter yellow" tumors, induced lymphosarcoma from rats, results on "butter yellow" feeding experiments in rats as measured by the Zn, Fe, Mo, Cu, Cr, and Ni content of livers, kidneys, and spleens. In most "butter yellow" experiments, Zn showed a decrease in liver in the initial stage of tumor development and a subsequent increase at a later stage of development, which may be related to the enzyme anhydrose content of the liver.

Two series of experiments were made (see Reports 4 and 5) to determine the trace-metal content of various organs in a series of rats after irradiation with X rays (total dose 600 roentgens total body radiation) up until 12 days' postradiation time. The elements Fe, Zn, Cu, Mo, and Mn were determined in spleens, kidneys, lungs, livers, and testes. The Zn and Cu content of the spleens showed a strong decrease in the immediate postradiation period, while Fe and Mn showed a marked

increase relative to the controls. Other data of this sort are included in Report 5.

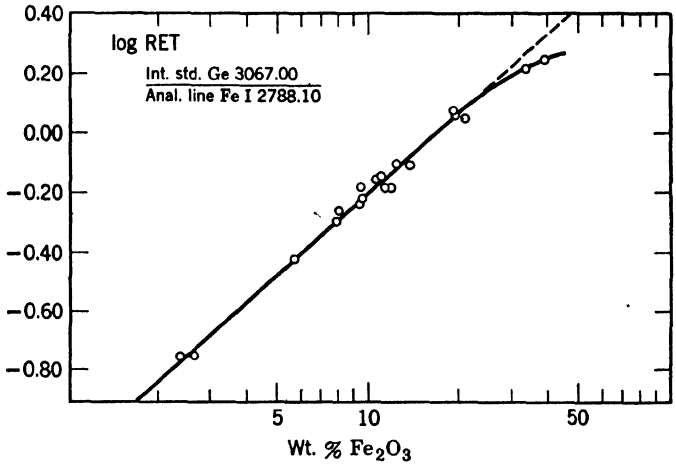

FIG. 11. Concentration calibration curve for calcium oxide. Mineral standards—germanium metal powder base.

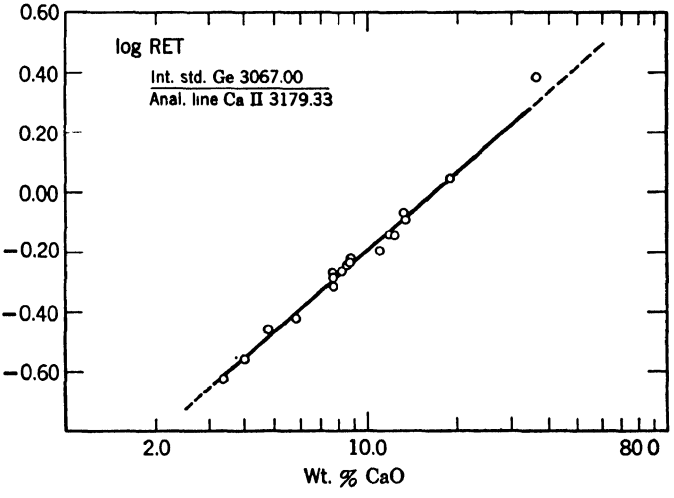

FIG. 12. Concentration calibration curve for iron oxide. Mineral standards—germanium metal powder base.

Finally, two reports to the Office of Naval Research by L. W. Strock [48] contain many examples of actual calibration curves for analyzing geological materials by several methods investigated, with data on reproducibility attainable and on difficulties encountered in absence

of standards of a common matrix composition. Certain aspects of this work have been published, and calibration curves for the Ca II–3179 and Fe 2788 lines are reproduced here as Figs. 11 and 12 from one paper,[24] which illustrates the curves using a heterogeneous set of rocks and minerals as standards. Figure 13 is a calibration curve for the same Ca:Ge pair plotted on a greatly expanded scale for a very homogeneous series of synthetic silicate standards taken from another paper.[25]

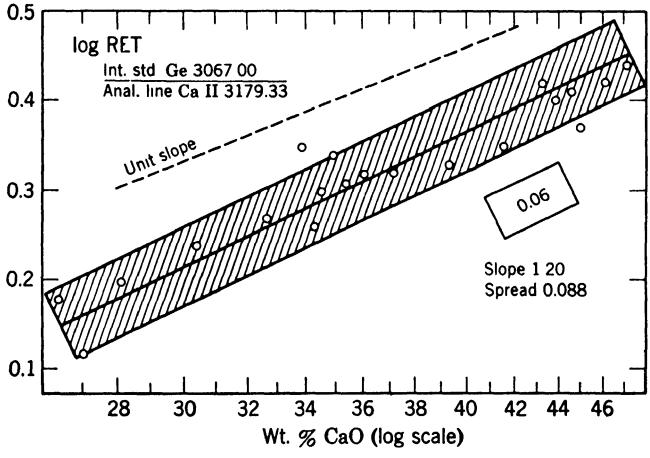

FIG. 13. Expanded concentration calibration curve for calcium oxide. Synthetic silicate standards.

The above applications of spectrochemical methods, with which I have been closely associated, have been listed for the benefit of those desiring more specific information than could be included in the main body of this general survey type of chapter.

No effort has been made to present a comprehensive type of review of the published literature on emission analysis. Neither has this chapter made any pretense at becoming a compendium of laboratory operations. Rather, the emphasis has been on general principles, typical applications, and the basic aspects of photometry underlying all photographic recording methods.

The chapter has been developed around the conviction that the d-c arc is a most useful and versatile method for medical and biological problems. The necessity of extending methods to increasingly lower concentration levels has been recognized and discussed, and means of accomplishing it have been illustrated by preconcentration techniques. A detailed analysis of the d-c carbon arc and hollow-

cathode sources have been made in a search for a special source combining the special advantages of each. Present results justify making further attempts to improve the d-c arc as an analytical light source, with respect to both sensitivity and stability. An attempt was made to give a fair impression of efforts which many persons have made to compensate for sample variation and dependence on light intensity on minor differences in composition from one sample to another, and for differences between samples and standards—all with emphasis on the principles involved without analytical details.

BASIC REFERENCE WORKS AND TABLES

The spectrochemical analyst has frequent need of reference tables containing data on wavelengths, energy levels, and ionization potentials. The compilations by Charlotte E. Moore [44] are indispensable; as well as the many original papers on the analysis of line spectra by W. F. Meggers and others at the National Bureau of Standards. The table of wavelengths with 100,000 entries by G. R. Harrison [45] is also a standard spectrochemical laboratory reference. The detailed and beautiful reproductions of the arc and spark spectra of iron by A. Gatterer and J. Junkes [46] at the Astrophysical Laboratory of the Vatican are extremely useful, as are the spectra of 30 chemical elements,[47] including the rare earths published by them.[48]

The American Society for Testing Materials publishes the *Index to the Literature of Spectrochemical Analysis* compiled by W. F. Meggers and B. F. Scribner [49] of the National Bureau of Standards. A similar bibliography, *Spectrochemical Abstracts,* compiled by F. Twyman and E. H. S. van Someren,[50] is published by Adam Hilger Ltd. of London. The "ASTM Symposium on Spectrographic Light Sources," [51] contains a useful series of papers on this subject and belongs in the category of basic references. Those interested in details of the analysis of metals and alloys will find in two long papers by J. Sherman and J. W. Jenkins [52] a useful treatment of the subject. In the textbook field, the book by Nachtrieb [53] is outstanding in its attention to the actual problems of spectrochemical analysis. The monograph by R. L. Mitchell [28] on the "Spectrographic Analysis of Plants, Soils, and Related Materials" is a fine summary of work from his laboratory and a valuable source of detailed information on methods and applications in the field of agriculture.

DISCUSSION

Discussion Leader
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Philips Research Laboratories
N. V. Philips' Gloeilampenfabricken
Eindhoven, Netherlands

The method which we apply for the determination of major constituents as well as of trace elements is characterized by the following:

1. A complete evaporation of a weighed amount of material (5 mg or 10 mg) through:

2. A d-c carbon arc of relatively constant temperature (column temperature 5000 ± ~150° K,* temperature of the outer zone for example 4000 ± ~70° K,† graphite electrodes of large diameter, consuming much carbon [54]).

3. A speed of evaporation (stream velocity) of less than ‡ 1 meter per second.

4. For the determination of the concentration only faint lines [55] are measured, and corrections for background are always carried out (microphotometrically or with the spd scale.[56] In most cases the measurements of faint lines prevent disturbing effects of self-absorption.

5. The light coming from the center of the arc (gap width 9 mm) is sent to the spectrograph and is analyzed (see Fig. D1*A*). K values give a relationship between the concentration in weight per cents and the intensity (standardized with a standard light source) §: $c = K \times i$.

6. For the determination of trace elements an arc with a gap width of 2 mm is used (see Fig. D1*B*). All the light is transmitted to the spectrograph. Q values give a relationship in this case: [57] $c = Q \times i$.

The relationship between K and Q values is found on the basis of experiments to be (cf. also Fig. D2):

* This fluctuation in temperature has been derived from data published by me (see Table 1 of ref. 21) through the application of Boltzmann's equation to the arsenic K values, because of its high ionization potential, arsenic is excited in the column. As determined by us, the absolute temperature has been found to be 6100 ± ~ 100° K.

† The same calculations were made in respect to aluminum K values; because of its low ionization potential, aluminum is excited in the mantle of the arc.

‡ Assuming a theoretical cross section of the column of 1 mm².

§ Cf. ref. 22. A version in English has been published in *Applied Spectroscop., 10,* 128 (1956).

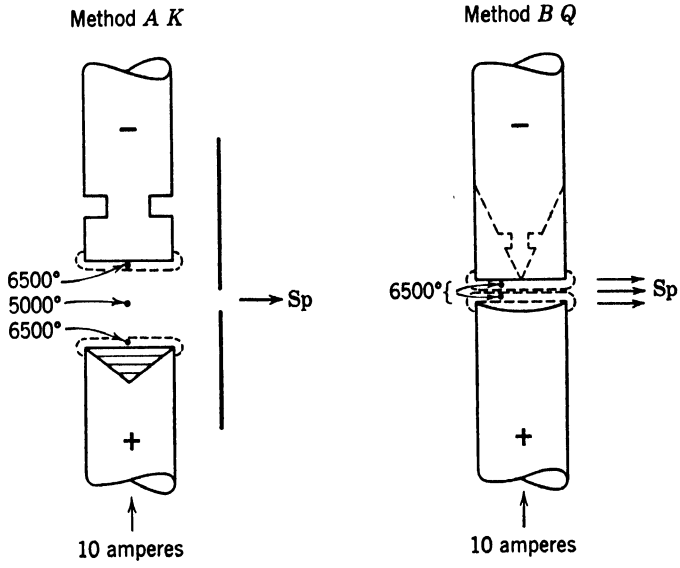

FIG. D1. Diameter of the graphite rods 10 mm, depth of the conical hole (method *A*) 3 or 4 mm, depth of the concave hole (method *B*) 1 mm.

A. *K* values. 5 mg of the sample with or without subsidiaries to retard or to enhance complete evaporation. The whole is always covered with a layer of pure graphite powder (about 7 mg) to prevent loss caused by spluttering during the ignition period. Temperatures are given in a relative scale.

B. *Q* values. 10 mg of the sample are deposited on top of the anode. The top of the cathode is shaped either flat or, in cases of less volatile materials, pencil-like (broken line).

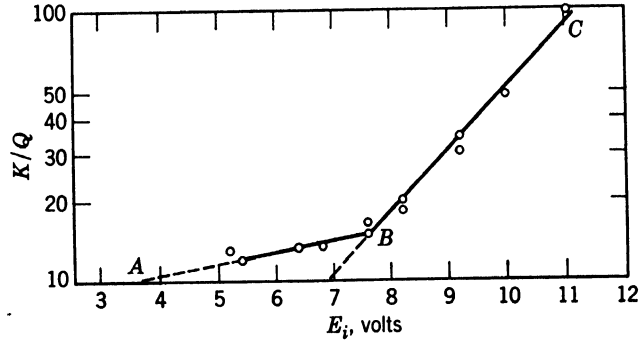

FIG. D2. The relationship log K/Q and the ionization potential E_i of the element is shown. Note the point *B*, below which elements are excited in the mantle of the arc; elements with an $E_i \geq 7\frac{1}{2}$ volts are excited in the column.

1. For those elements that are excited in the column (ionization potential $E_i \geqq 7\frac{1}{2}$ volts):

$$\log \frac{K}{Q} = 0.24E_i - 0.64$$

and from this equation the temperature difference between the center of the column and the electrode layers is found to be about 1000°, through application of Boltzmann's equation:

$$N_E/N_0 \sim e^{-E/kT}$$

where N_E = the number of atoms in the excited state
N_0 = the number of atoms in the ground state
E = the excitation energy
k = Boltzmann's constant
T = the absolute temperature

2. For elements that are excited in the outer parts of the arc ($E_i \leqq 7$ volts):

$$\log \frac{K}{Q} = 0.04E_i + 0.86$$

Temperature differences between center and layer amount to about 100°, likewise according to Boltzmann's equation. For these elements (the alkalies, the earth-alkalies, Al) ionization is so large (Saha) that arc lines are emitted almost exclusively from the mantle and not from the center.

For the sake of completeness some Q values are shown in Table DI, calculated from the K values as well as determined experimentally.

Influence of Major Components. As to the determination by means of K values (Fig. D1*A*), no influence has been found, except for very large quantities of sodium, potassium, etc.; for example, K values of elements evaporating at the same time as sodium have to be multiplied by 1.5 if the sodium content amounts to 50%.

In respect of the Q-value method (Fig. D1*B*), corrections are much larger: for instance, Q values have to be multiplied according to an example shown in Fig. D3. A correction factor <1 means that elements with an ionization potential $E_i < 7$ volts are excited in a larger volume of the mantle (a larger excitation zone) in this particular case than in a relatively pure carbon arc, whereas elements with an $E_i > 7$ volts are excited to a lesser extent because of the smaller excitation zone, or because of lower temperature.

TABLE DI

Element	Wavelength, A	$Q_{calc\ from\ K}$, %	Q_{exp}, %
Ag	3280	0.00007	0.0001
Al	3082	0.0008	0.0009
As	2860	0.009	0.01
Ba	3071	0.12	0.1
Be	3130	0.0002	0.0002
Bi	3067	0.0004	0.0006
Cd	3261	0.003	0.003
Co	3465	0.0009	0.0008
Cr	2677	0.005	0.004
Cu	2961	0.026	0.03
Fe	3020	0.0005	0.0005
Ge	2754	0.0025	0.0025
Mg	2781	0.0074	0.008
Mn	2933	0.003	0.0025
Na	3302	0.03	0.02
Ni	3414	0.00017	0.0002
P	2535	0.0023	0.002
Pb	2873	0.015	0.01
Sb	2878	0.0043	0.006
Si	2528	0.001	0.001
Sn	2850	0.016	0.015
Ti	3242	0.0017	0.002
V	3185	0.001	0.002
Zn	3345	0.003	0.003

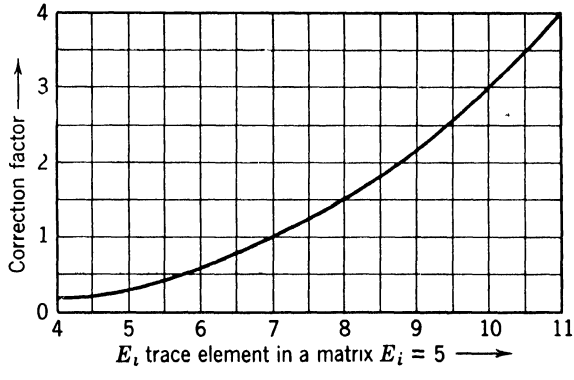

FIG. D3. The concentration of an element found in the application of Q values (the element has for instance an excitation potential $E_i = 9$ volts) has to be corrected with a factor $\cong 2$, if it is present in a matrix of $E_i = 5$. This factor corrects for the decrease in temperature caused by the matrix.

The K and Q values and their temperature gradients having been determined once and for all, all kinds of materials can be analyzed according to both methods, covering concentration ranges from parts per million to 100%.

The cathode layer may be used, according to our experiments, if very small amounts over a certain period are carried into the arc (i.e., up to 30 μg). An amount of 0.2 mg carried into the arc is sufficient to disturb the layer.[57]

Our Q values are comparable, to a certain extent, to those mentioned by Mitchell and by Ahrens.[58] In many cases where the amount of material is very small, they can produce fairly good results in respect of the over-all composition.[57]

In recent publications[59] examples are given of collisions of the second kind: excited atoms of the element A do not lose their energy by radiation but by transferring this energy to other atoms of the element B in the ground state, for experimental data show that the working curve of A has a shallow slope, whereas B has a steep one.

These facts can also be explained in a much simpler way: in the particular cases mentioned,[59] spectral lines of A were chosen, which show a strong self-absorption, and this corresponds to a working curve

TABLE DII

ANALYSIS OF A PASTURE HERBAGE
97780 (OUR NO. 9026)

Parts per million

	Spectro, Mitchell	Spectro, Eindhoven	Remarks
Fe	53	57	With K values
Mn	123	132	With K values
Mo	0.71	$\sim$2 *	With K values
Cu	4.4	3	With K values
Co	0.12	$<$0.2	With Q values; steps of 15 seconds
Ni	0.86	0.9	With Q values
Sn	$<$0.6	0.2	With Q values
Pb	1.6	2	With Q values
Zn	29	30	With Q values
V	0.13	$\leq$2	With Q values
Ti	4.1	$\leq$3	With Q values
Ba	21	18	With Q values
Sr	14	$\sim$20	With Q values

* Determined chemically, since the air in the spectrograph room contained traces of Mo.

with a gradual slope, while the element *B*, being excited in the mantle of the arc, extends this excitation zone with increasing concentration, and this results in a steep working curve.

In many cases chemical preconcentration by separation of trace elements from the major components (see Mitchell [60]) can be avoided through the application of our *K* and *Q* values without any pretreatment. The material had only to be dry-ashed (15 minutes, 490° C), and about ½ gram of the original material was needed for a spectrochemical analysis in triplicate. Table DII shows that both methods produce practically the same results.

According to our experiences with our method of determining zinc in blood ash,[61] it may be stated that the standard deviation amounts to 5 to 10%.

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Emission Spectrochemical Analysis

Determination of trace elements in plants and other biological materials

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ABSTRACT For the spectrochemical determination of metallic trace elements in biological materials at levels in dry matter of the 0.01 to 10 ppm order it is generally necessary to combine a chemical concentration with a sensitive spectrographic technique. The purpose of the pretreatment is to eliminate major constituents such as alkali and alkaline-earth metals, phosphates, sulfates, and halides, and to produce a concentrate of uniform major chemical composition in a base suitable for subsequent spectrographic treatment. It has been found that a satisfactory precipitation is effected by a mixture of organic reagents (8-hydroxyquinoline, tannic acid, and thionalide) at *p*H 5.2 (ammonium acetate-buffered) in the presence of about 15 mg of Al to provide a suitable matrix. Complete recovery of microgram quantities of Co, Ni, Mo, Zn, Sn, Pb, V, Cr, Ag, Au, Ga, Fe, Cu, and certain other elements is obtained.

The spectrographic technique found most useful is d-c arc excitation, with the sample (about 3 mg mixed with twice the amount of carbon powder) packed into a deep narrow boring in a carbon (*not* graphite) cathode electrode. When amounts are adequate, as for instance with Cu, Mn, Ba, and Sr in plant materials, direct analysis of the ash mixed with a suitable buffer can be employed. Provided conditions are rigorously controlled, good reproducibility is obtained by the arc technique and single determinations on standard solutions, including both chemical pretreatment and spectrochemical analysis, should not vary by more than ±10%. Methods for the preparation of different types of sample are described, and comparative chemical and spectrochemical determinations are quoted.

At the Macaulay Institute for Soil Research we are interested in the determination of trace elements in agricultural and biological materials. The basic method that has been developed involves a chemical concentration technique followed by spectrographic analysis by d-c arc excitation. The chemical pretreatment is an essential part of the process, as it makes possible at one and the same time the removal of unwanted major constituents, the introduction of a matrix material suitable for the subsequent spectrochemical analysis, and the addition of any internal standards which may be required. The advantages that ensue include the ability to analyze almost any type of biological material against one set of spectrographic standards and the fact that the precipitation process which all samples undergo insures uniformity in physical conditions.

The method is applicable within appropriate concentration limits at the microgram level, to Co, Ni, Mo, Sn, Pb, Zn, Ti, V, Cr, Ga, Ge, Be, Ag, and Au. Other elements probably also recovered, W and Bi for instance, have not been investigated fully. Al, Fe, and Cd are precipitated but not determined, being used as carrier and spectrographic standards. The essential feature of the process is the separation of alkali and alkaline-earth metals, phosphates and other anions, and also Mn which could cause difficulties with certain plant materials. In the space available it is impossible to describe the method in detail; in what follows emphasis is given to certain points with which other workers appear to have had some difficulty, or where recent minor modifications have been introduced. Fuller details will be found in the various publications cited.

PRETREATMENT AND CHEMICAL CONCENTRATION

The first step is to bring the trace elements into solution in dilute acid. The amounts of alkali and alkaline-earth metals that are present are, within very wide limits, quite immaterial, the presence for instance of 4 grams of Na_2CO_3 or $KHSO_4$ from a preliminary fusion having no influence on the precipitation.[7]

Plant Materials. Twenty to forty grams of dry matter are ashed overnight in a silica-lined muffle furnace at 450° C in platinum basins covered loosely by silica clock glasses. At least during the initial heating there should be provision for a slow movement of air through the furnace. The ash is extracted by hydrochloric acid, the residue filtered off, re-ashed, fused with 1 gram of Na_2CO_3 in platinum, and dissolved in hydrochloric acid, any silica remaining being filtered off.

The extraction and re-ignition prior to fusion have been found desirable because, if carbon is left after the first ignition, reducing conditions during fusion may lead to the loss of Ni, Pb, and other metals which alloy with platinum. We prefer a dry-ashing technique because of the difficulties involved in the purification of perchloric acid and other reagents in the quantities required for wet ashing 20-to-40-gram quantities of dry matter. We have had no difficulty in obtaining complete trace-element solution after dry-ashing.

Animal Products. Animal organs are ashed as described above after freeze-drying. For most determinations about 1 gram of ash is required. With blood, an ether extraction to remove Fe, present in excessive amount, is included before precipitation, and in this process a few trace elements, e.g., Mo, may be partially lost, but the method is applicable to the determination of Co. Materials such as bones and dried milk may cause difficulty because of their high phosphorus content, resulting in precipitation of calcium phosphate, and the operations should be carried out at a greater dilution, adhering however to the reagent concentrations and acidity conditions specified.

Soil Extracts. The soil extractants employed include 2.5% acetic acid, 1 *N* ammonium acetate, and dilute hydrochloric acid. The extract from 20 to 50 grams of soil is taken just to dryness, organic matter is removed by HNO_3, and the residue dissolved in HCl, nitrate being removed by repeated evaporation to avoid interference with the subsequent precipitation.

Waters. A volume of 1 to 5 liters, depending on the type of water, is evaporated to dryness and treated as are soil extracts.

The solution should contain Al (equivalent to approximately 30 mg Al_2O_3), Fe (1 to 4 mg Fe_2O_3), and Cd (0.4 mg). The Al provides the carrier for the trace-element precipitates and the Al_2O_3 produced is a very satisfactory matrix for the spectrographic determination. Fe is employed as internal standard for all elements except Zn, for which Cd is used. Experience soon teaches if Al or Fe need to be added: Cd, never present in significant amount, is always added at this stage.

Concentration is effected by precipitation by means of a mixture of 8-hydroxyquinoline (oxine), tannic acid, and thionalide (β aminonaphthalide of thioglycolic acid)[6,7] at *p*H 5.1 to 5.2. Thionalide is required to precipitate Pb; tannic acid brings down Cr, V, Be, Ge, Sn, and Cd; most other elements are precipitated by 8-hydroxyquinoline alone. Numerous other reagents have been tested but none have proved satisfactory. Thioacetamide does not recover Pb under the conditions employed.

Precipitation is carried out at room temperature, the extract being approximately normal with respect to HCl, and the volume about 150 ml. After the addition of 10 or 15 ml of 5% 8-hydroxyquinoline in 2 *N* acetic acid, 10 *N* ammonia solution is added dropwise until the color of the solution just changes from yellow to emerald green at a *p*H of 1.8 to 1.9. This color change which occurs when iron is present in the solution and is presumably due to formation of an 8-hydroxyquinoline–iron complex, allows the *p*H of the solution to be established sufficiently accurately to enable final buffering to *p*H 5.1 to 5.2 to be effected. This requires further 50 ml of 2 *N* ammonium acetate, but at this stage only 30 ml are added, with stirring. A bulky precipitate of Al and Fe oxinates forms and soon coagulates. To the mixture are now added, with continuous stirring, 2 ml of 10% tannic acid in 2 *N* ammonium acetate and 2 ml of 1% thionalide in glacial acetic acid, followed immediately by sufficient ammonia to neutralize the glacial acetic acid and thereby bring the total amount of ammonium acetate added to 50 ml. Tannic acid and thionalide should be freshly prepared.

After standing overnight, the precipitate is filtered through filter paper (Whatman 540) or a porous porcelain crucible and washed with water. The purity of batches of filter paper must be rigorously checked, as must all reagents employed. After drying, the precipitate is ashed in porcelain at 450° C. The ashed precipitate is free and easy to manipulate, never adhering to the crucible. It is weighed on a torsion balance after it has reached air-dry moisture equilibrium in the balance room. It should weigh between 30 and 50 mg: if below 30 mg, it is made up to 40 mg with Al_2O_3. A concentrate weight of 40 mg corresponds to a 500-fold concentration if 20 grams of original material were taken. Weights of 100 mg or over may occur with some soil extracts or even with plant materials which are higher than normal in Al or are soil-contaminated. The precipitate is mixed thoroughly in an agate mortar before subsamples are taken for analysis. The Fe content is determined on a 5-to-10-mg aliquot as described by Scott,[12] using a sodium salicylate colorimetric procedure.

SPECTROGRAPHIC ANALYSIS OF TRACE-ELEMENT CONCENTRATES

Even in this concentrate the determination of trace elements demands a reasonably sensitive technique. At the 0.1-ppm level in the original material, a 20-gram sample provides no more than 2 μg,

which in a 40-mg concentrate corresponds to 50 ppm. Many elements, including Ni, Mo, Sn, Pb, V, and Cr, occur in plants at this level, while there may be only one fifth as much Co, in which case the amount introduced into an electrode is about 0.03 μg. The spectrographic technique must be capable of giving a satisfactory accuracy at this level, and we have found that adequate sensitivity and accuracy are obtained only by a method [2,6] based on the cathode-layer-excitation d-c arc technique of Mannkopff and Peters.[5] The use of smaller initial sample and concentrate weights leads to manipulative difficulties, but is possible for specific purposes.

The normal method involves excitation of 3 to 4 mg of concentrate mixed with twice its weight of carbon powder, in a 2.8-mm-diameter carbon electrode with a boring 1.0 mm wide and 8 mm deep. The electrodes, it cannot be emphasized too often, are made from carbon and not from graphite, which because of its greater heat conductivity, does not burn away at a satisfactory rate. With a properly designed tool,[15] the cutting of electrodes in carbon presents no difficulty and a production rate of 60 per hour is readily achieved.

The electrodes are filled by packing with a blunt needle, care being taken to insure that the tightness is uniform. Some practice is necessary, but, once the skill is acquired, filling by hand is superior to any form of mechanical packing. The time expended in packing electrodes of this design is justifiable in view of the reproducibility obtained, compared with that from other shapes of electrode used for d-c arc work.

The electrodes are burned as cathode in a 9-ampere d-c arc supplied from a 250-volt source, the anode being a 5-mm carbon rod. In our opinion greater stability is obtained with a well-ballasted 250-volt source than with a lower voltage, such as 110 volts, although the potential over the gap is no more than 80 volts. To make an exposure, the electrodes are brought together with sufficient resistance in circuit to reduce the current through them to 3 amperes. After some 10 seconds, they are separated slowly and deliberately to a 10-mm gap, and simultaneously the current is raised to 9 amperes by adjustment of a tapped resistance. The rate of separation and current control can be judged by the sound and appearance of the arc. If either operation is not properly performed, some of the sample may be ejected. The arc, which burns quietly and steadily, is run at a constant gap length and current until the sample and its supporting electrode walls are completely consumed after about 3 minutes. The arc is kept in adjustment by an image on a conveniently situated

screen, and the arc stand must have provision for independent movement of each electrode in all directions.

The method requires a spectrograph with at least the dispersion of the Hilger large quartz spectrograph (E1, E492, or E742) with a spherical condenser at the slit to image the arc on the collimator. A 6-step 1:2-ratio 500-rpm sector is introduced between lens and slit, giving stepped exposures down to $\frac{1}{64}$ of the uninterrupted intensity. A mask at the collimator passes light from the tip of the cathode and the adjoining third of the arc column. The spectrograms are recorded on 10×4-in. Ilford Chromatic G30 plates, covering the wavelength region 2800 to 4800 A.

Photometry follows standard principles. Three steps of analysis line, internal standard line and, if necessary, background around a density of 0.4 are measured and plotted, using a Seidel scale as described by Black.[1] Either the linear separation of the Seidel blackening curves at a density of 0.4 or a background corrected intensity ratio [6,9,13] is used to produce the working curves. These are prepared from standard mixtures of the trace elements in a matrix of Al_2O_3 and Fe_2O_3 and trace-element contents of 1, 3.16, 10, 31.6, 100 ppm etc., up to 3160 ppm, apart from Zn for which the range is 0.1 to 10% and for which the base also contains 1% Cd. For most trace elements, satisfactory standard mixtures are made by incorporating oxides into an Al_2O_3–Fe_2O_3 base, but, for certain of the more volatile elements, it has been found necessary to prepare standards by precipitation. This applies in particular to Ag and Ga which give very high results if oxide standards are used against precipitated samples, indicating that the effect cannot be due to incomplete precipitation, but rather to enhanced excitation in the precipitated samples.

To enable iron, present in the concentrate in inconstant amount generally between 2 and 15% Fe_2O_3, to be used as internal standard, the variable internal-standard technique has been devised. Series of working curves covering the usual trace-element ranges but with varying contents of iron were found to be displaced by constant amounts dependent on the iron content, and it is therefore possible to prepare curves or tables from which a correction value for any iron content can be obtained, enabling a single (generally 5% Fe_2O_3) working curve to be employed. The method is described fully by Davidson and Mitchell,[2] Scott,[11] and Mitchell.[6] It is possible to reduce the process to a single curve by employing trace-element-to-iron ratio instead of trace element for the working-curve abscissa,[16] but we consider the method originally described to be more convenient in

routine operation and to be more widely applicable. The same corrections apply to all elements using the same Fe line as internal standard.

SENSITIVITY AND APPLICATIONS

The elements that may be determined by the concentration technique followed by the above spectrographic procedure include Co, Ni, Mo, Sn, Pb, Zn, Ti, V, Cr, Ga, Ge, Be, Ag, and Au. Precautions are necessary during pretreatment to avoid loss of volatile Ge as chloride and of Be with any insoluble silica.[7] Cu is probably recovered, but reagent contamination problems cause difficulties. The recovery of the rare-earth group is incomplete.

The wavelengths and concentrations found most suitable in analytical practice are detailed in Table I. The sensitivity quoted refers to

TABLE I

ANALYTICAL RANGE AND SPECTROGRAPHIC SENSITIVITY OF TRACE ELEMENTS IN CONCENTRATES FROM BIOLOGICAL MATERIALS

Trace Element	λ	Sensitivity, μg in Electrode	Range, ppm in Concentrate	Internal Standard	λ
Cr	4254.4	0.03	10–3000	Fe	4250.8
Co	3453.5	0.03	10–3000	Fe	3451.9
Ni	3414.8	0.03	10–3000	Fe	3413.1
Zn	3345.0	3	1000–100,000	Cd	3261.1
Ag	3280.7	0.01	3–1000	Fe	3306.4
V	3185.4	0.03	10–3000	Fe	3196.9
Mo	3170.4	0.03	10–1000	Fe	3196.9
Be	3130.4	0.1	30–3000	Fe	3116.6
Ge	3039.1	0.3	100–3000	Fe	3116.6
Ga	2943.6	0.03	10–3000	Fe	2929.0
Sn	2840.0	0.15	50–3000	Fe	2838.1
Pb	2833.1	0.15	50–3000	Fe	2838.1
Au	2675.9	0.15	50–5000	Fe	2680.5

the lowest convenient quantitative determination: the qualitative sensitivity is at a considerably lower level.

The method as described is applicable to plant and animal materials, many fertilizers and chemical products, soil extracts, and natural waters. The ranges of contents in the original materials that can be covered assuming a 500-fold concentration (20 grams to 40 mg) are, in parts per million: Cr, Co, Ni, V, Ga: 0.02–6; Ag: 0.01–2; Mo: 0.02–2; Sn, Pb: 0.1–6; Au: 0.1–10; Ge: 0.2–6; Zn: 2–200. For waters,

where 2 liters are concentrated to 40 mg, the above figures represent the ranges in parts per hundred million. The upper limit is readily extended by dilution with Al_2O_3–Fe_2O_3 base.

Although several authors of compilations of methods have suggested the use of this method for the determination of the total contents of trace elements in soils or silicate rocks after Na_2CO_3 fusion, we have in fact never so used it, it being impracticable because of the high Al and Fe contents.

REPRODUCIBILITY AND ACCURACY

There are many statements to the effect that reproducible results cannot be obtained with d-c arc excitation. This is completely false and appears to be based on preliminary findings of inexperienced operators. In Table II are presented results obtained by a nongraduate girl assistant for 12 replicate arcings for five elements, a concentrate of a tomato-root sample being used for Co, V, Mo, and Sn, and of an oil palm-leaf sample for Zn. For Co, V, and Mo, elements for

TABLE II

REPRODUCIBILITY OBTAINABLE IN THE D-C ARC. REPLICATE SPECTROGRAMS OF PLANT-MATERIAL CONCENTRATES

Results in parts per million in the Concentrate

Element	Co	V	Mo	Sn	Zn
Internal-Standard	Fe	Fe	Fe	Fe	Cd
Background	Corrected	Uncorrected	Uncorrected	Uncorrected	Corrected
	436	354	159	258	7940
	441	378	168	267	6870
	437	363	166	275	7640
	437	372	180	270	8220
	441	363	161	278	7940
	430	363	166	258	7870
	448	372	173	338	8530
	448	358	163	295	8150
	443	363	170	279	8300
	447	363	163	258	7590
	448	358	170	279	7760
	447	354	163	279	8220
Mean	442	363	167	278	7920
Standard Deviation	6.0	7.4	5.8	21.9	434
Coefficient of Variation	1.35%	2.03%	3.49%	7.88%	5.47%

which Fe is a reliable internal standard, the coefficients of variation of 1.5 to 3.5%, particularly those for Co and V, are as good as any other excitation source using photographic recording will provide. The coefficient of variation for Zn against Cd is 5.5%, which we consider reasonable for the determination of Zn in a d-c arc. The corresponding coefficient of variation found for Zn against Fe was over 20%, owing chiefly to one high value (11,200 ppm) resulting from some irregularity in the burn. In our routine analysis, Zn is read against both Cd and Fe. The former value is employed for Zn determination, the comparison being used to indicate the reliability of the burn for the determination of other volatile elements against Fe. A poor burn, generally due to a splutter, results in high Zn:Fe figures. Pb, Sn, and other volatile elements are affected to a considerably less extent.[7] We prefer to apply this Zn check for those elements whose characteristics are intermediate between Zn and Fe rather than introduce a third internal standard.

The coefficient for Sn reported in Table II is just acceptable, but it should be noted that it is affected by one value (338 ppm) which would, in fact, have been rejected in practice because of the very considerable difference between the Zn results against Fe and Cd observed in this spectrogram. If this Sn value is ignored, the coefficient of variation for Sn falls to 4.5%. It is to be noted that in this burn the elements for which Fe is an appropriate standard are not affected.

The results quoted in Table II fall near the center of the range of determination of the elements in question. Figures reported by Scott [13] indicate a coefficient of variation of 6.8% for Co at the lowest determined level of 10 ppm.

The reproducibility reported here cannot be obtained by an operator who has not had adequate experience and training in filling, arcing, and photometric techniques, but we find that after a few months girls of senior school grade, aged 17 to 18, can be relied on to produce such precision provided the arc technique described above is followed.

The accuracy of the method has been assessed by examining the recovery of trace elements from standard solutions and by comparing analytical results from different laboratories employing different methods. The type of result consistently obtained in recovery experiments will be found in earlier publications,[6–8, 10] and it is unnecessary to repeat these here: the errors of individual determinations of most elements seldom exceed ±10%. The coefficients of variation calculated from the published tables are generally between 5 and 10%,

with the higher values given by the more volatile elements when measured against Fe, as the results for precision would suggest. It should be noted that these coefficients of variation cover the whole concentration range and include any error in both the standard mixtures and the solutions used for the tests, as they were calculated against the theoretical contents.

Space does not permit the presentation of many comparative sets of analytical figures obtained by spectrographic and chemical methods. Table III shows spectrographic results for Mo and Zn in some

TABLE III

COMPARISON OF SPECTROGRAPHIC AND CHEMICAL DETERMINATION OF MOLYBDENUM AND ZINC IN PLANT MATERIALS

As parts per million in Dry Matter

		Molybdenum					Zinc		
		Spectrographic			Chemical		Spectrographic		Chemical
	Sub-sample	Individual		Mean	Individual	Mean	Individual	Mean	Mean
Apple leaves	*a*	0.09	0.11	0.10	0.07	0.08	29 27	27	—
	b	0.11	0.10		0.09		27 26		
Pasture herbage	*a*	0.68	0.75	0.71	0.71	0.73	34 32	29	26
	b	0.71	0.69		0.74		24 25		
Wheat grain	*a*	0 05	0.07	0.05	0.05	0.05	44 45	42	43
	b	0.05	0.04		0 05		39 38		
Wheat straw	*a*	0.19	0.19		0.16		16 17		
	b	0.16	0.15	0.17	0.15	0.16	16 15	17	13
	c	0.16	0.16		0.16		19 18		
Broccoli leaves	*a*	0.10	0.12	0.10	0.11	0.08	61 51	53	49
	b	0.10	0.09		0.05		50 49		
Cauliflower leaves	*a*	0.56	0.58	0.54	0.47	0.50	68 65	65	52
	b	0.51	0.51		0 53		61 64		

plant samples compared with chemical values obtained by Dr. D. J. D. Nicholas of Long Ashton Research Station. Mo was determined as the dithiol complex [11] and Zn as dithizonate.[4] Reasonable agreement between the two methods is shown for both elements.

By the arc method described by Farmer,[3] it is possible to determine Cu, Mn, Ba, and Sr directly in the ash of plants and animal organs: together the two techniques provide a comprehensive trace-element survey covering some 20 elements; typical results are illustrated in Table IV.

The concentration method described thus makes possible the determination of many important trace elements in plants and bio-

TABLE IV

TYPICAL EXAMPLES OF TRACE-ELEMENT ANALYSES BY SPECTROCHEMICAL METHODS
On a Dry-Matter Basis unless Otherwise Stated

		Co	Ni	Mo	Fe	Pb	Sn	Zn	V	Ti	Cr	Ag	Cu	Mn	Ba	Sr
Pasture herbage	ppm	0.09	1.3	2.1	75	2.3	<1	30	0.08	3	<0.1	0.04	7.8	120	15	34
Oat grain	ppm	0.05	3.2	0.21	76	1.1	<1	30	0.06	3	0.2	<0.1	1.5	51	4	4
Oat straw	ppm	<0.02	0.16	0.10	26	<0.5	<1	14	0.12	8	0.1	<0.03	1.2	71	31	28
Sugar-beet leaf	ppm	0.29	1.1	0.54	390	3.2	1.5	57	1.3	40	0.9	0.2	10.6	64	11	40
Swede turnip tuber	ppm	0.06	0.51	0.09	45	<1	<1	19	0.04	2	0.1	<0.06	2.5	6	10	17
Peas (shelled)	ppm	0.06	2.3	0.34	65	0.5	<1	72	<0.03	2	<0.1	<0.1	13.7	11	2	2
Tea leaf	ppm	0.07	3.2	0.09	59	<2	<2	37	0.3	13	0.5	<0.2	13.3	640	77	101
Conifer needle	ppm	0.46	2.4	<0.1	162	6.0	<2	42	0.38	23	0.7	<0.2	5.5	520	19	5
Seaweed stipe	ppm	0.48	0.3	0.29	446	5.4	<5	59	2.5	26	1.3	0.9	5.0	47	43	2500
Sheep-liver ash	ppm	4.0	12.9	71	2680	56	<10	3300	<0.7	18	2.2	8.2	1600	260	—	—
Casein	ppm	<0.02	0.11	0.07	14	0.6	2.7	29	<0.02	<0.5	<0.1	<0.1	1.2	<0.3	0.3	<0.1
Water (drinking)	μg/liter	0.3	1.8	1.7	—	8	<4	20	1.7	10	1.1	0.5	1.1	—	—	—
Water (lake)	μg/liter	<0.1	0.3	<0.1	—	<2	<1	36	<0.1	<0.5	<0.3	<0.2	4.9	2.1	2	4

logical materials. Greater accuracy might be obtained for individual elements by other techniques, but the advantage of simultaneous determination is so considerable that in our experience no other method approaches it for comprehensive trace-element surveys. It is not a method that can be set up and employed in the course of a few weeks; it is applicable rather to long-term investigations in which change of operational personnel is reduced to a minimum, as several months' training is required before reliable results are obtained. This caution, however, applies in greater or less degree to any spectrographic method.

DISCUSSION

Discussion Leader
Cyrus Feldman
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I have been asked to include in my remarks some comments on the application of the porous-cup technique to trace analysis.

As a trace-analysis method, it shares certain advantages with the other thin-film devices for exciting liquids: it is free from preferential volatility effects, and permits the use of high total-solute concentrations and small-sample volumes (0.3 ml). It is easily adaptable to use in enclosed chambers such as are needed when special atmospheres or radioactive solutions are employed. It can be used by technicians with a minimum of scientific training.

There are essentially no restrictions on the nature of the solute. We at one time hesitated to expose perchloric acid solutions by this technique, fearing explosion. One day, however, we found that we had exposed a sample which, unknown to us, contained a substantial amount of perchloric acid. No explosion had occurred, so we investigated its use in routine work. We found it completely satisfactory as an acid and thickening agent, and superior to sulfuric acid in that it forms no insoluble salts in normal concentration ranges.

In terms of micrograms per milliliter of solution, the sensitivities obtained with the PCE are approximately the same as those obtained by other methods in many cases, but not so great in others. Provided that the maximum permissible power level is not exceeded, a great deal can be done to improve PCE sensitivities by proper choice of excitation conditions.[17]

A few recent modifications of PCE technique may be of interest in this connection.

Where it is desired to prolong the exposure for any reason, we have employed a solution reservoir of a type originally suggested by Dr. Mitchell. This consists simply of an appropriate length of plastic tubing fitted over the upper end of the PCE. This has permitted exposures as long as 15 minutes in connection with recent temperature investigations.

We guard against contamination of solutions by electrode clips by wrapping the latter in platinum foil, which is frequently removed and cleaned. We guard against cross-contamination between solutions by the same means, and by waterproofing the side walls of the PCE. Uniform porosity of electrode material is very important in obtaining good precision; we have found National Carbon Company no. L4048 entirely satisfactory in this regard.

Where ultimate absolute sensitivity in terms of number of micrograms per exposure is needed, we have found it advisable to concentrate chemically and isolate the species in question, dry the entire amount on the surface of a waterproof graphite electrode, and spark the sample in argon–oxygen mixture, using a large number of small discharges per half-cycle.

* * *

I should like now to discuss a few points in connection with retention of trace elements during the ashing of tissue. Reliable data on this subject are few and widely scattered; however, an excellent review of the present status of the matter has been published by Middleton and Stuckey.[18]

Most chemists undertaking the ashing of tissue for trace analysis have justifiable fears of loss of trace elements by volatilization. Vanadium pentoxide is known to accumulate in the smokestacks of vessels using oil for fuel; boron can be separated from plant material by ignition: the vapors produced contain the boron.

In testing an ashing procedure for recovery of trace metals, two approaches may be adopted.

1. Comparison of Wet and Dry Ashing. In this approach, it must be assumed that nothing is lost or added in wet ashing, and that the material in question can actually be completely decomposed in this manner. The validity of these assumptions may vary from element to element and from material to material.

2. Recovery of Known Amounts of a Trace Element Added to the Sample before Ignition. If the difference between the trace-element concentrations found in the treated and untreated aliquots is equal to the amount of trace element added, it is assumed that none of the trace element was lost in ashing. This assumption ignores the fact that the element is seldom added in the same chemical form in which it exists in the tissue. (Recent determinations of cobalt in blood by Thiers, Williams, and Yoe [19] are an exception in this rule.) Where such a difference exists, there is a possibility that equal amounts of the element originally present in its natural-state combination might be volatilized in metallo-organic or oxide form from each sample, whereas this inorganic form would remain. This would leave a difference in trace-element concentration between the treated and untreated aliquots corresponding to the amount added, giving the appearance of complete recovery, but the figure obtained from the untreated sample would actually be low.

It may be possible to circumvent this difficulty, when it exists, by (1) igniting the material, trapping the vapors and analyzing the trap liquid, with preliminary chemical concentration if necessary, or (2) igniting the sample in an oxygen bomb.[20] Such ignition may be incomplete, but materials that are not volatile at room temperatures can be recovered.

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Gamma-Ray Spectroscopy

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ABSTRACT Gamma-ray spectroscopy is the newest tracer-chemistry technique of examining the nuclear radiations emitted by trace elements. A description of the method is used to intercompare the methods of counting and spectroscopy with both beta and gamma rays. The increased sensitivity and selectivity obtained with gamma rays can be applied to advantage in experiments where the desired chemical elements can be made gamma-radioactive by neutron-pile or cyclotron bombardments. The basic tools needed to perform gamma-ray spectroscopy are discussed with particular emphasis on the characteristics and design of the essential component, the scintillation crystal. Calculations and measurements with sodium iodide crystal sizes up to a 5-inch diameter and a 9-inch length are presented.

INTRODUCTION

The rapid development of scintillation counters since 1949 has made the energy measurements, or spectroscopy of gamma rays, a practical tool for trace analyses. In this technique, the nuclear gamma rays from the trace element are detected and sorted according to their energy just as an optical spectrograph analyzes a spectrum of the light from an atomic source. The light spectrum from an arc source serves to identify and analyze the atoms in the arc. In a like manner, the

energy spectrum derived from a gamma-ray spectrometer identifies and analyzes the atoms by the gamma rays from the atomic nucleus.

In some respects, gamma-ray spectroscopy is the most universal tool of any of the radioactive techniques because of the characteristics of the gamma rays compared to those of alpha and beta particles. The important characteristics are the greater penetrability of the gamma rays in bulk materials and the uniqueness of the identifying energies of the gamma rays from the emitting isotope. Both characteristics lead to high-detection sensitivities for radioactive materials. When properly applied, the technique also provides features of speed, simplicity, and reliability, which can augment the presently available methods of trace analyses in those limited instances where the radioactive isotopes of the trace and host materials have favorable decay characteristics.

This report describes the detailed characteristics of gamma-ray spectroscopy using scintillation counters. A consideration of the characteristics of gamma-ray sources is given first in order to provide a discussion of the possibilities of this method. Then the design and operation of gamma-ray spectrometers [3] will be studied. The examination of a trace-element source with the spectrometer will illustrate the influences of the radioactive impurity and the bulk material on the sensitivity attainable.

GAMMA-RAY SOURCES

Gamma-ray sources are usually applied in any one of the three following ways:

1. Natural Occurrence of the Radioelements in the Materials under Study. An example is the study of the natural radioactivity in dogs and humans now underway at the Argonne National Laboratory and the Los Alamos Scientific Laboratory. The investigators at the latter laboratory [11] were able to measure with reasonable accuracy the potassium content of the body. They employed a gamma-ray spectrometer with good-detection efficiency, but a poor gamma-ray energy definition, in order to measure the 1.45-Mev * gamma ray [8] from K^{40}. A 70-kg person contains about 1.8×10^{-2} gram of K^{40}. The sensitivity to the K^{40} impurity was at least 1 part in 4 million. In the same experiments, the sensitivity to the radium content of the body was at least 1 part in 7 million million. Both sensitivities could have been made higher

* The abbreviations Mev and kev stand for energy units of million electron volts and kilo-electron volts.

by several orders of magnitude if a spectrometer with better energy definition had been used. However, the large physical dimensions employed in the experiment would make the cost of an improved spectrometer prohibitive.

2. Production of Radioactive Isotopes of the Impurity Element by Nuclear-Particle Bombardment of the Host Material. (*a*) *Neutron Activation in a Nuclear Pile.* The impurities in germanium can be determined quantitatively by this method. By a combination of gamma-ray counting and other radiochemical techniques, sensitivities as great as 1 part impurity in 100 million are obtained.[7] The real problem is the undesirable activation of the host material that always accompanies the activation of the impurity being studied. A selective gamma-ray spectrometer should permit sensitive differentiation between the radioactivity in the host material and the trace element and should help improve the sensitivities now obtainable.

(*b*) *Cosmic-Ray Production of the Trace Element in the Earth's Atmosphere.* An interesting and rare example of this trace technique [1] is in the studies of atmospheric Be^7. This isotope is produced some 9 miles above the earth's surface by cosmic-ray collisions and has a radioactivity with a 54.4-day half-life, a 0.48-Mev gamma-ray and no beta-ray activity. Since many meteorological processes have time scales of the order of weeks, Be^7 is a sensitive and practical trace element for weather processes.

3. Injection of the Radioactive Material into the Host Material in Order to Study the Passage and Fate of the Trace Element. In this technique, a small quantity of the radioactivity can be procured and brought to the laboratory separately from the host material. Since this method is the simplest to describe of the three listed above, the descriptions to follow will concentrate on the characteristics of the radioelements available for it.

CHARACTERISTICS OF GAMMA-RAY EMITTERS

Radioactive elements emitting gamma rays are available from the U. S. Atomic Energy Commission [10] or from commercial sources that will process the material according to desired specifications. In order to appreciate the characteristics of the radiations from such active source materials, one can follow the history of the production of 5.2-year Co^{60}, which in its decay gives rise to two gamma rays. These gamma rays, the 1.17- and 1.33-Mev rays, are used extensively in medicine and industry.

The isotope of cobalt with atomic weight of 60 can be produced by neutron bombardment of Co^{59} in a nuclear pile. The various radioactive by-products that result from the capture of neutrons by the Co^{59} nucleus are shown in Fig. 1. As shown, the Co^{59} plus a neutron becomes Co^{60} in an excited state. Excited Co^{60} (designated by Co^{60*})

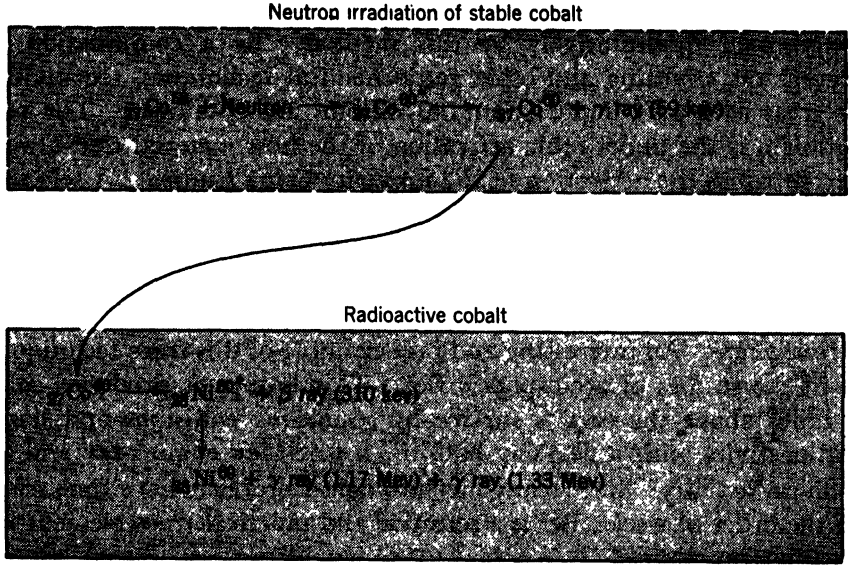


FIG. 1. Neutron irradiation of cobalt to produce the Co^{60} gamma rays.

almost immediately decays to Co^{60} in the ground state by the emission of 59 kev of energy. The resultant Co^{60} nucleus is, however, unstable and decays by the emission of a beta ray to Ni^{60} in an excited level (Ni^{60*}). The maximum energy of the beta ray is 310 kev, and the half-life of this decay process is 5.2 years. The excited Ni^{60} (Ni^{60*}) decays quickly by the successive emission of two gamma rays with energies of 1.17 and 1.33 Mev.

The energies of the beta rays emitted from Co^{60} range continuously from the maximum of 310 kev down to zero. This is in contrast to the two gamma rays that are single-energy gamma rays arising from the de-excitation of a nucleus from one energy level to another. Therefore, the measurement of the gamma-ray energies is a more positive and simple procedure of identification than the determination of the end point of a beta-ray spectrum.

The length of time of 5.2 years for the radioactivity of Co^{60} to decay to one-half of its original activity is characteristic of the beta-decay

process in this case. In general beta-decay half-lives predominate over gamma-ray half-lives and determine the lifetime of a given beta- and gamma-ray decay chain.

Another important characteristic of gamma rays is their penetrability through thick materials. Gamma rays are exponentially absorbed in

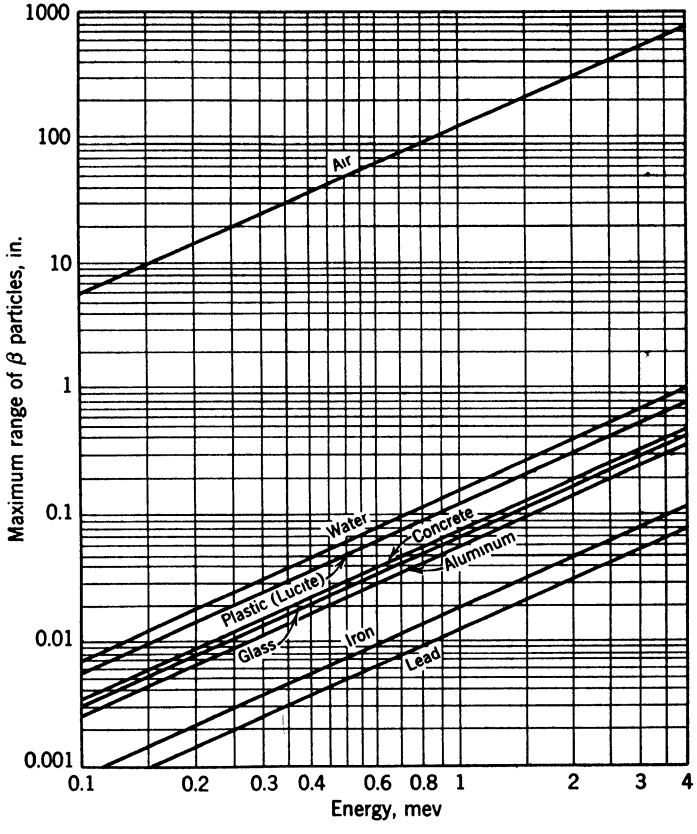


FIG. 2*a*. Penetration of beta radiation. These curves were taken from *Radioisotopes in Industry*.[4]

matter. This is in contrast to the finite range of beta rays of a given energy. The striking difference between beta-ray and gamma-ray penetration is shown in Figs. 2*a* and 2*b*.[4] In Fig. 2*a* is plotted the maximum range of beta rays versus energy. Since most of the beta rays from radioactive elements have energies less than 4 Mev, the beta ray ranges will be less than 1 inch in most substances. Therefore, any experiment in which the trace element is surrounded with 1 inch of host material will be unable to give a detectable indication

of radioactivity by the detection of beta rays. However, Fig. 2*b* shows that, even if several inches of material surround a gamma-ray emitter, a sufficiently large intensity of gamma rays will escape to provide an identification of the radioactive element.

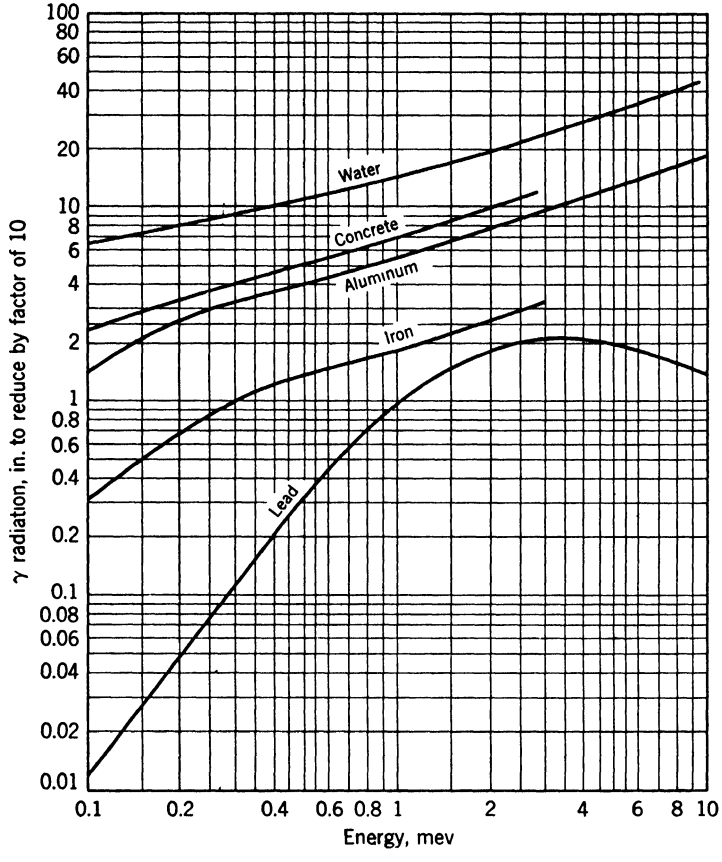


FIG. 2*b*. Penetration of gamma radiation. These curves were taken from *Radioisotopes in Industry*.[4]

The Co^{60} isotope is but one of approximately one hundred radioactive isotopes that are now available from the Atomic Energy Commission. The various substances have varying gamma-ray energies, half-lives, modes of production, and chemical properties. Table I is an arrangement of the various gamma-ray emitters available from the Oak Ridge National Laboratory.[10] They have been grouped into four sets of half-lives on the abscissa scale: (1) ½ to 5 days, (2) 5 to 50 days, (3) 50 to 500 days, (4) longer than 500 days. In the vertical direction

TABLE I

GAMMA RADIOISOTOPES AVAILABLE FROM OAK RIDGE NATIONAL LABORATORY

The radioisotopes are listed according to the maximum gamma-ray energy and the half-life.[10]

	½ to 5 days		5 to 50 days		50 to 500 days		Above 500 days	
0–500 kev	Au^{199}	24, 51, 70, 156, 207, 231	Os^{191}	39, 127	As^{73}	52		
	Hg^{197}	77, 135, 165, 273	Ru^{97}	220	Sn^{113}	90		
	Hg^{203}	286	Ce^{141}	141, 315	Co^{57}	14, 119, 131		
	Mo^{99}	141, 181, 360	Cr^{51}	320	Ce^{144}	130		
	Au^{198}	411	Hf^{181}	87, 133, 136, 344, 480	W^{185}	134		
			Ru^{103}	494	Se^{75}	67–405 10 γ rays		
					Be^{7}	480		
500–1000 kev	Sb^{122}	570	Cd^{115m}	500	Ir^{192}	137–651 12 γ rays	Sb^{125}	35, 110, 175, 425, 465, 601, 637
	Cd^{115}	340, 520	Ba^{140}	160, 306, 540	Zr^{95}	708	Ba^{137}	662
	Sm^{153}	69, 103, 610	Nd^{147}	35, 580	Co^{58}	810		
	W^{187}	71, 133, 478, 615, 680	As^{74}	590	Mn^{54}	835		
			Nb^{95}	758				
			I^{131}	80, 160, 284, 364, 720				
1000–2000 kev	Br^{82}	550, 610, 690, 770, 820, 1040, 1310	Rb^{86}	1100	Zn^{65}	1110	$Eu^{152-154}$	300, 1200
			Mn^{52}	730, 940, 1460	Sc^{46}	890, 1120	Na^{22}	1280
			Ba^{131}	260, 500, ~1200	Ta^{182}	46–1237 33 γ rays	Co^{60}	1170, 1330
	Cu^{64}	1, 340	Fe^{59}	1100, 1300	In^{114}	192, 548, 715, 1270	Cs^{134}	568, 602, 794, 1350
	Ir^{194}	1430, 380						
	K^{42}	1, 510			Ag^{110}	116–1516 9 γ rays		
	Pr^{142}	134, 329, 490, 624, 1590						
	Re^{186}	132, 275, 1700						
Above 2000 kev	As^{76}	567, 1250, 1800, 2100			Sb^{124}	121, 600, 650, 714, 1700, 2060, 2300		
	Ga^{72}	631–2500 9 γ rays			Pr^{144}	700, 1500, 2200		
	Na^{24}	1380, 2758						
	La^{140}	93, 335, 490, 820, 1600, 2550, 2900						

of this figure, the radioactive isotopes are arranged according to the value of the highest-energy gamma ray from the isotope sample in question. The energies of all gamma rays emitted are also given.

Although Table I contains an impressive arrangement of radioactive isotopes, not all chemical elements are represented. To illustrate this representation schematically, Fig. 3 gives the periodic arrangement of the elements with the light blocks to show those elements that are represented in Table I. One can assume that those elements in the dark blocks in Fig. 3 cannot be used or detected in trace studies and also will not cause confusing activities in host materials, since they are unavailable from Oak Ridge National Laboratory with sufficiently intense and practical radioactivities.

The gamma-ray emitters that have been described are available in various chemical forms for trace analyses and in approximately known radioactive source strengths.

Standard sources of calibrated activities are available for some of the radioactive isotopes from the National Bureau of Standards.[9] Table II gives a list of the available standards and their certified activities. An appropriate one of these gamma-ray sources can be

Group	IA	IIA	IIIB	IVB	VB	VIB	VIIB	VIIIB			IB	IIB	IIIA	IVA	VA	VIA	VIIA	VIIIA
Period 1	H																	He
2	Li	Be											B	C	N	O	F	Ne
3	Na	Mg											Al	Si	P	S	Cl	A
4	K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
5	Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
6	Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
7	Fr	Ra	Ac															

Rare earths	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Ac series	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf					

FIG. 3. Periodic classification of the elements. The elements in the light blocks are those that appear in the list of available gamma emitters given in Table I.

used for calibration and standardization of the detection equipment in a quantitative analysis.

Because of the gamma-ray characteristics of penetrability and uniqueness of energy, the use of gamma rays in trace analyses makes possible the attainment of great sensitivities. As an example of the orders of magnitude detectable, let us assume that the 1.17- and 1.33-Mev gamma rays from Co^{60} can be detected with a 100% detection efficiency and that 100 gamma rays per minute must be detected for an accurate measurement. Since the specific activities of Co^{60} available from Oak Ridge[10] are of the order of 1 curie per gram,† the weight of cobalt "solids" necessary to give this decay rate will be

† Some radioisotopes such as Ta^{182} are available with specific activities as high as 50 curies per gram.

TABLE II

GAMMA-RAY STANDARDS AVAILABLE FROM THE NATIONAL BUREAU OF STANDARDS

Radiation	Nuclide	Nominal Activity	Volume
$\gamma(\beta)$ *	Co^{60}	10^5 dps †	5.0 ml
$\gamma(\beta)$	Co^{60}	10^6 dps	5.0 ml
$\beta(\gamma)$	I^{131}	10^5 dps/ml	‡
$\beta(\gamma)$	Au^{198}	10^5 dps/ml	‡
$\beta(\gamma)$	Na^{22}	10^4 dps/ml	‡
$\gamma(\beta)$	Na^{22}	10^6 dps	5.0 ml
$\beta(\gamma)$	Na^{24}	10^5 dps/ml	‡

* The first symbol refers to the primary radiation for which the sources are calibrated, and the bracketed symbol to the other radiation from the sources. γ stands for gamma ray and β for beta ray.

† Dps is the abbreviation for disintegrations per second.

‡ Approximately 3 ml of low-solids carrier solution containing the active nuclide in a flame-sealed ampoule.

In addition, radium gamma-ray standards with a volume of 5 ml and a radium content of 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, and 100 μg are available.

2.3×10^{-11} gram. If this weight of cobalt were imbedded in 20 grams of other material, one would be detecting approximately one part of cobalt impurity in one million million parts of host material.

The example of imbedding the radioactive material in the host material is a hypothetical one and is not that followed in usual practice. The more conventional procedure in trace analysis is the irradiation of the trace and host elements by neutrons in a pile and the production of a radioactivity in both sets of elements. The quantity of trace material must be evaluated from a measurement of its radioactivity. Consideration must be given to the possible confusion of radioactivities in the host material.

The Co^{60} example was selected to show the numbers of cobalt gamma rays that must be detected to achieve a given sensitivity. The high sensitivity described can now be achieved with gamma-ray scintillation spectrometers.

CHARACTERISTICS OF A GAMMA-RAY SCINTILLATION SPECTROMETER

The problem until recent years has been the sensitive detection and energy measurement of the gamma rays. Until the advent of scintillation counters, the available gamma-ray spectrometers, such as pair

spectrometers and Compton-line spectrometers, were devices that detected and measured gamma-ray energies by measuring the energies of secondary electrons produced by the gamma rays. The detection efficiency for the gamma rays was very low, and approximately one gamma was measured for 10^5 gamma rays incident on the instrument. When one included in the consideration the gamma rays emitted in all directions from a source, the detection efficiencies were of the order of 1 in 10^7, or $10^{-5}\%$. Therefore, in the cobalt example cited above, the sensitivity to radioactive nuclei would be one impurity atom to 10^5 host atoms in place of a sensitivity of the one in 10^{12} for a 100% efficient detector. Gamma-ray trace analyses with a Compton-line spectrometer were clearly not as sensitive as almost any other quantitative analyses technique.

Scintillation counters for the measurement of gamma-ray energies have changed this picture because they afford the possibility of the detection and measurement of the energy of almost *every* gamma ray emitted from a sample. Gamma-ray spectroscopy, therefore, becomes a very desirable tool for determinations of small quantities of elements because it makes possible sensitivities to certain impurities that are several thousand and perhaps a million times larger than those of other analytical techniques.

The individual gamma rays from a radioactive substance are emitted at the rate of 3.7×10^{10} gamma rays in 1 second for 1 curie of gamma activity. Each of these gamma rays emitted in 1 second carries with it a unique energy for radioactive isotopes that emit but one gamma-ray energy. If the individual gamma rays are directed at a large block of scintillating material one at a time and if none of the gamma-ray energy escapes from the block, the scintillator will emit a light pulse whose magnitude is proportional to the gamma-ray energy. Statistical fluctuations on the number of light photons emitted by the hypothetical scintillator are the only theoretical limit on the one-to-one correspondence of the gamma-ray energy and the light pulse magnitude.

Scintillating materials that can be used in the manner described are the thallium-activated crystals of NaI, KI, CsI, and CaI_2 or the crystals [6] of $CaWO_4$ and $CdWO_4$. Of these, work with sodium iodide has progressed most rapidly. The data and calculations to be presented relate wholly to sodium iodide.

The use of a large sodium iodide crystal [5] in a spectrometer to measure gamma-ray energies is shown schematically in Fig. 4. The complete sodium iodide scintillation spectrometer consists of the sodium

iodide crystal, a photomultiplier light detector, an electronic voltage amplifier, and a pulse-voltage discriminator.

The individual gamma rays being emitted from the source on the left are shown as arrows that are admitted by a hole in a lead wall to the central axis of the sodium iodide crystal. These gamma rays have one or several discrete energies, depending upon the gamma-ray emit-

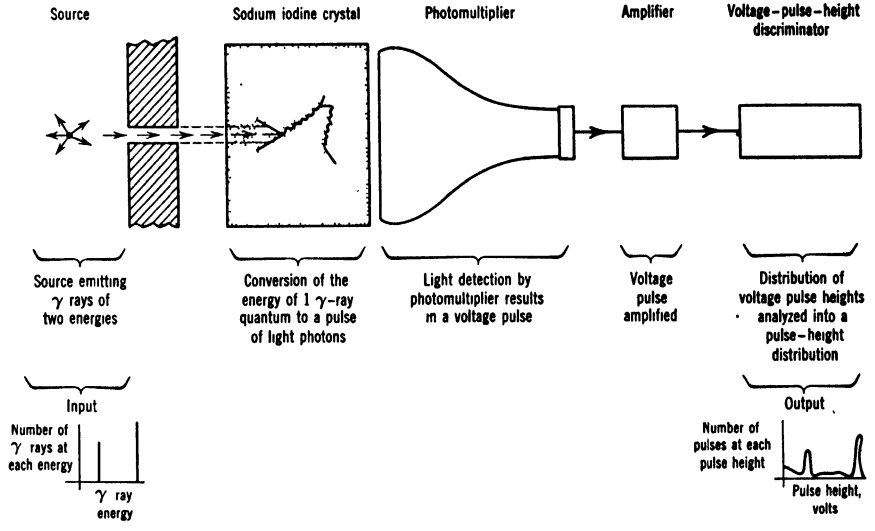


FIG. 4. Principle of a sodium iodide scintillation spectrometer. The source as schematically shown is emitting two monoenergetic gamma rays whose energies and intensities are to be measured by the spectrometer. The output of an ideal spectrometer, which is a voltage-pulse-height distribution, will be in one-to-one correspondence to the gamma-ray energy distribution input to the crystal.

ting nucleus. In the example of Fig. 4, two gamma rays are being emitted, and they are assumed not to be simultaneous in time. If one were to plot the number of gamma rays versus gamma-ray energy, the plot would contain two lines as shown in the figure below the source. The ideal spectrometer would reproduce as its output on an energy, or possibly a wavelength scale, the same two narrow lines.

The photomultiplier detects the light pulse from the crystal, linearly amplifies the light-pulse amplitude, and transmits a voltage pulse into an electronic amplifier. The output of the amplifier is sent into a voltage discriminator that is used to determine the number of output pulses of a given voltage-pulse height. The information from the discriminator is used to plot a so-called "pulse-height distribution" which, for the scintillation spectrometer, is exactly analogous to the

densitometer trace of a spectroscopic plate. On the plate, the peak densities correspond to intensities of lines at given wavelengths. In the pulse-height distribution shown on the right of Fig. 4, the experimentally determined peaks correspond to gamma-ray lines at specific energies. The more nearly ideal the scintillation spectrometer, the greater the similarity will be between the original gamma-ray spectrum (on the left of Fig. 4) and the final pulse-height distribution obtained from the spectrometer (on the right of Fig. 4). Therefore, the pulse-height distribution can be used as an identifying fingerprint for the trace element emitting the gamma rays.

CRITERIA FOR DETERMINING THE OPTIMUM DIMENSIONS OF THE SODIUM IODIDE CRYSTAL

The most important component of a scintillation spectrometer is the sodium iodide crystal. The purpose of this crystal is to absorb, as completely as possible, the energy of an individual gamma-ray quantum and to convert that energy into a light pulse. If the crystal is large in physical dimensions, every gamma-ray incident on the axis of the crystal will be absorbed, and most of the absorbed energy will remain in the crystal. Therefore, the output from the large crystal spectrometer will be a narrow pulse-height distribution. Such a distribution makes the identification and analysis of the gamma-ray source a simple and a fast procedure.

However, the size criterion on what constitutes a "large crystal" varies strongly with the gamma-ray energy. In order to design efficiently the smallest crystal size for a specific energy, Berger and Doggett [2] have performed Monte Carlo calculations for various crystal sizes and gamma-ray energies. The gamma-ray source and crystal geometries assumed for the calculations are those shown in Fig. 4 in which a well-defined gamma-ray beam was used. The calculations predicted the shapes of the pulse-height distributions as shown in summary form in Fig. 5. The height of the voltage pulses from the spectrometer is given on the abscissa scale in relative units to locate the main peak of the pulse-height distribution at the same place on the abscissa scale for all energies. The ordinate scale is the logarithm of the relative number of pulses of a given pulse height. For greater details regarding these results, the original paper by Berger and Doggett [2] may be consulted.

As will be observed from Fig. 5, the gamma rays and energies below 1 Mev, which are of primary interest in this discussion, will produce

distributions with sharp and prominent peaks with a spectrometer containing a 4-inch-long-by-2.5-inch-radius cylindrical crystal. This well-defined distribution is to be contrasted with the curve labeled 1.17 Mev and L = 0.5 in., R = 0.25 inch. In this case, the subsidiary peak at a pulse height of 50 units will be almost as large as the main

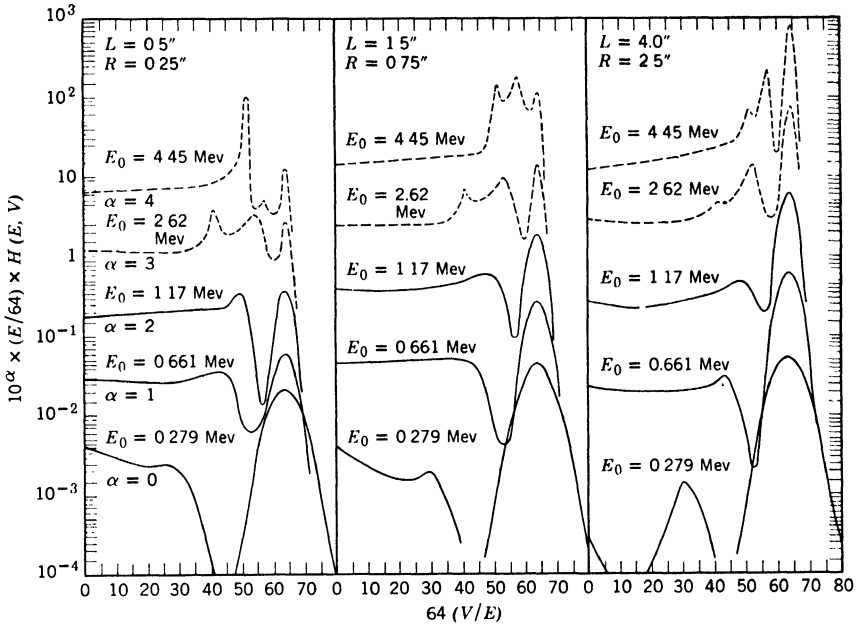


FIG. 5. Predicted pulse-height distributions for a sodium iodide spectrometer.[2] The parameters on these curves are the gamma-ray energies producing the distributions and the dimensions of the cylindrical crystals of the spectrometer. $H(E_1V)$ on the ordinate scale is the probability that a gamma-ray quantum with energy E will produce a light pulse that registers on the spectrometer output as a pulse height with an apparent energy of V.

peak at a pulse height of 64 units. The identification of the number and relative strengths of the gamma rays from trace elements becomes difficult with this small crystal spectrometer because of the confusion caused in the interpretation of a pulse height by the subsidiary peaks.

A parameter that measures the quality of the pulse-height distribution in terms of the production of a single large peak for a single gamma-ray energy is the photofraction. The photofraction is defined as the ratio of the area under the main peak to the entire area under a pulse-height distribution produced by a monoenergetic gamma ray.

Berger and Doggett [2] have calculated photofractions, and their results are summarized in Fig. 6. The abscissa scale is the gamma-ray

energy, and the ordinate scale is the photofraction. The curves apply to crystals of various sizes in the spectrometer. Note that at gamma-ray energies below 400 kev the crystal size is not too important, as the photofraction values for all the sizes are close to one. As one proceeds to higher energies, the crystal sizes must be made as large as 4 in. long and 2.5 inches in radius in order to maintain high photofraction values.

The difference in pulse-height distributions predicted for a large spectrometer (i.e., with a photofraction of 0.6) and a small spectrom-

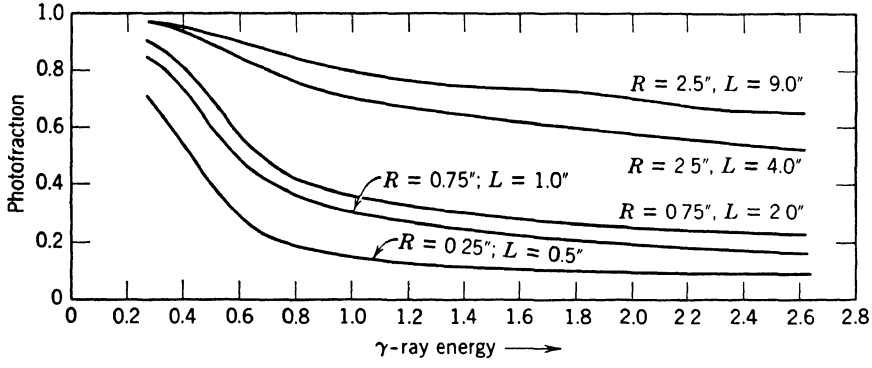


FIG. 6. Comparison of photofraction values versus gamma-ray energy for sodium iodide crystals whose dimensions are given on the curves.

eter (i.e., with a photofraction of 0.3) are shown in Figs. 7*a* and 7*b*. The distribution of Fig. 7*a* was predicted for a spectrometer with a 2.5-inch-radius by 4-inch-long crystal when the crystal is detecting three monoenergetic gamma rays from a source. The gamma-ray energies were 0.279, 0.661, and 1.17 Mev, and the intensities of these gamma rays were assumed to be proportional to their energies. For these gamma-ray energies and for the crystal size assumed, the photofraction values were larger than 0.6. The prediction was obtained directly from the data of Fig. 5. The result shows three well-defined peaks from which the identification of the numbers and relative intensities of the gamma rays could be made easily.

In contrast, the prediction for a spectrometer with a 0.25-inch-radius by 0.5-inch-long crystal is shown in Fig. 7*b*. The conditions of the gamma-ray source were assumed to be the same as those assumed in Fig. 7*a*. However, the "small crystal" distribution with photofraction values of the order of 0.3 shows subsidiary peaks that make the interpretation of the distribution difficult for any trace-analysis experiment

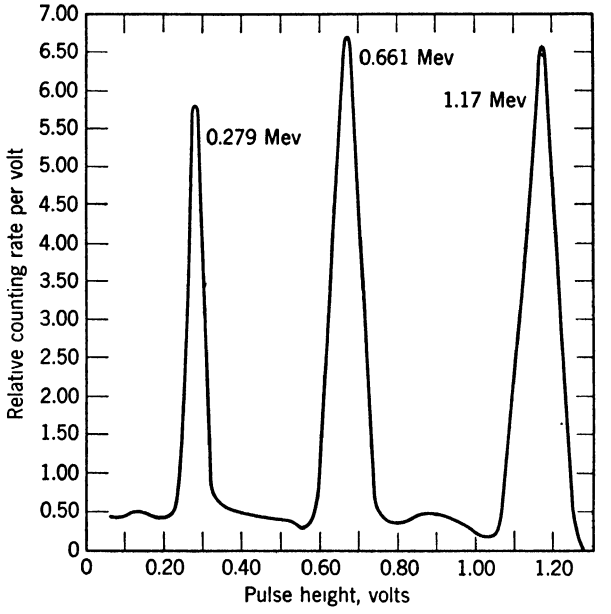


FIG. 7*a*. Prediction of the pulse-height distribution produced by 0.279-, 0.662-, and 1.17-Mev gamma rays in a spectrometer whose crystal dimensions are 2.5 in. radius by 4 in. long.

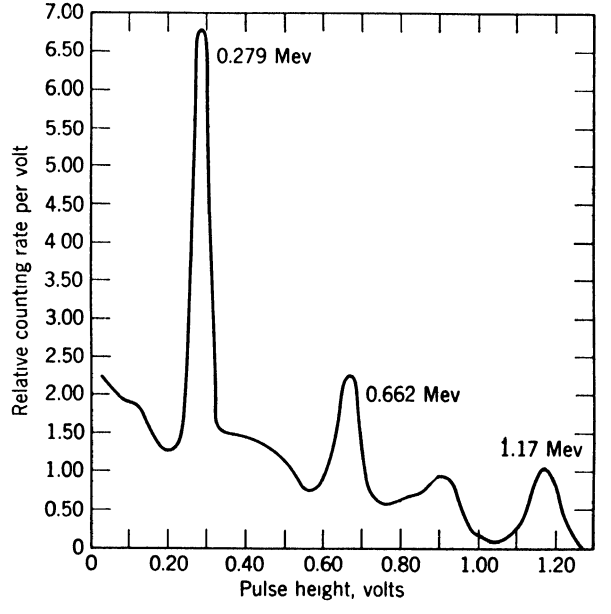


FIG. 7*b*. Same as (*a*) for a crystal whose dimensions are 0.25 in. radius by 0.5 in. long.

EXPERIMENTAL RESULTS WITH LARGE CRYSTAL SPECTROMETERS

The output pulse-height distributions predicted for a sodium iodide spectrometer (Figs. 5 and 7) were calculated on the basis of certain ideal experimental conditions. However, it would frequently be desirable to work with nonideal but practical conditions in order to

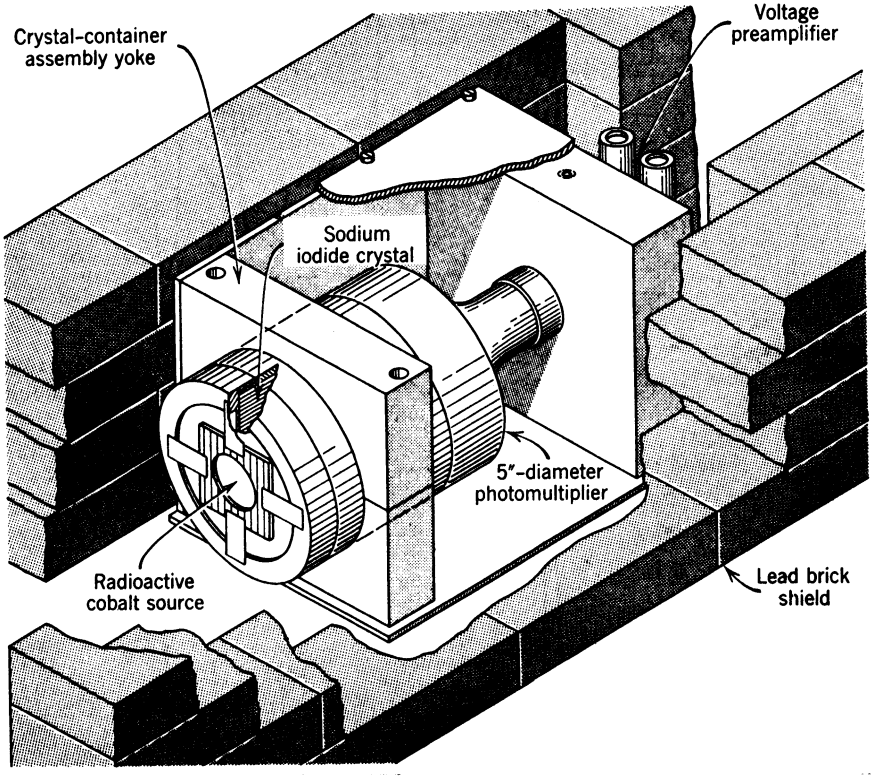


FIG. 8. A scintillation spectrometer containing a sodium iodide crystal whose dimensions are 2.5 in. radius by 4 in. long. A 5-in. diameter photomultiplier tube was used as the light detector. The thin evaporated Co^{60} source mounted in an aperture of an aluminum plate is shown taped to the end of the crystal container.

improve the sensitivity to radioactive impurities. To show the influences of these practical conditions, experimental pulse-height distributions were obtained with a 2.5-inch-radius by 4-inch-long crystal spectrometer and the two gamma rays from Co^{60}. The spectrometer that was used is shown in Fig. 8. The influence of gamma-ray source geometry, the presence of the host material, the natural background

in the crystal, and two crystals surrounding the source were studied.

Effects of Source Geometry. Limiting the size of the gamma-ray beam incident on the crystal can be accomplished as in Fig. 4 by a hole in a lead shielding wall. However, the gamma-ray detection sensitivity can be considerably improved by removing the defining aperture and by exposing the whole face of the spectrometer crystal to the gamma rays.

The influence on the shape of the pulse-height distribution of these two geometry conditions, one good and one poor, is shown in Fig. 9*a*. The source for the poor-geometry experiment was a thin evaporated source of Co^{60}, which was mounted directly on the face of the crystal. The pulse-height distribution for this "no-aperture" case shows two well-defined peaks with a photofraction value that is about half the value obtained with good geometry. However, the ratio of the sensitivities is approximately one hundred thousand. This large gain in gamma-ray detection efficiency at a sacrifice of some energy definition is desirable for some experiments in which small concentrations of a radioactive element are to be determined.

Effect of Host Material. The influence of the host material on the pulse-height distribution produced by the gamma rays from the impurity ‡ was tested by locating a cobalt source 3 inches from the end of the crystal spectrometer. The space between the source and crystal was filled with either a $2\frac{15}{16}$-inch-thick plate of Lucite or a $\frac{1}{8}$-inch-thick sheet of lead. The cobalt source was a thin evaporated source with a disintegration rate of about 200 per second and a weight of about 3×10^{-9} gram. The weight of cobalt impurity compared to the weight of Lucite or lead was approximately one part in 10^{11} (100,000 million). In spite of the very weak gamma-ray source, the two gamma rays are readily identifiable in the pulse-height distributions shown in Fig. 9*b* for all the conditions of no absorber, lead absorber, and Lucite absorber. The Lucite absorber reduces the prominence of the identifying peaks by about a factor of two from the no-absorber condition.

Crystal Background Rate. The background counting rates due to cosmic rays or natural radioactivities in the surroundings have been subtracted from the data of Fig. 9. The background contributions were small. Specifically, background counting rates in pulse-height intervals equal to the spectrometer peak width at a given energy were about 130 counts per minute [5] at 1.2 Mev (Co^{60} energy) and 0.090 Mev (Cd^{109} energy).

‡ Similar results are reported for the gamma rays from I^{131} in water (ref. 5*a*).

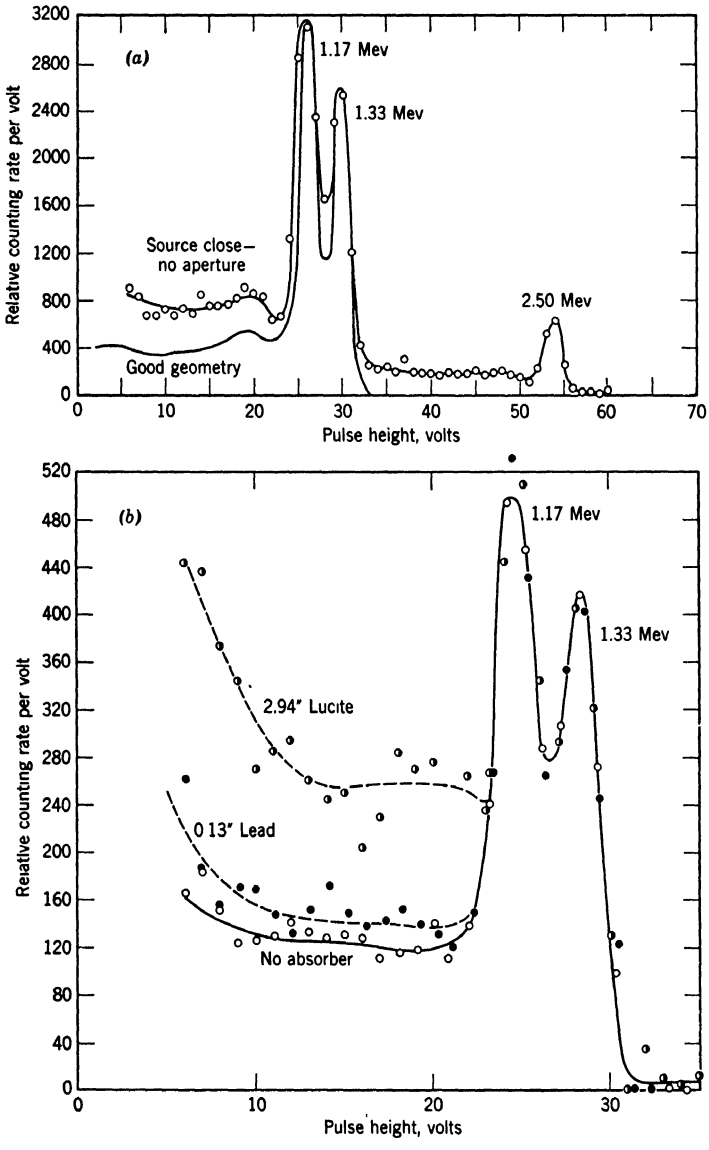
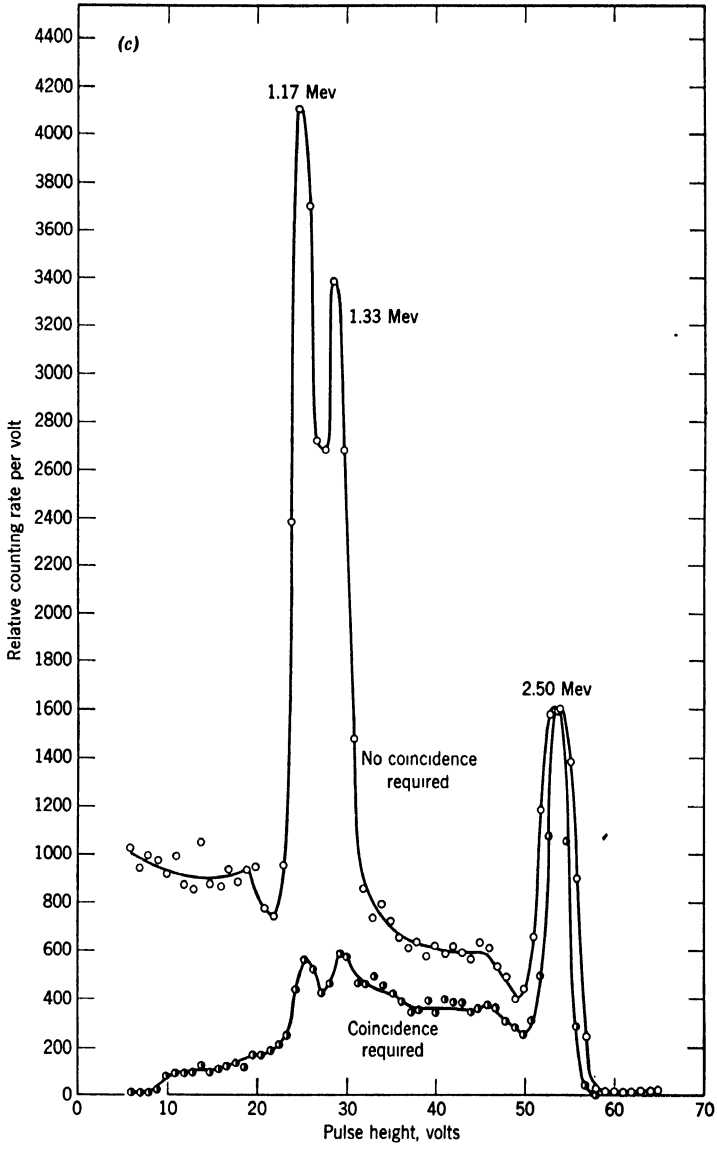


FIG. 9. Experimental pulse-height distributions obtained from the spectrometer of Fig. 8 with the Co^{60} gamma rays. The ordinate and abscissa scales have been normalized in the respective figures in order to illustrate differences in shape. These data were obtained from James Wyckoff. (*a*) Distributions showing the effects of source geometry. The pulses occurring at an apparent energy of 2.50 Mev result from the simultaneous detection of the two cobalt gamma rays. Very few



pulses at this apparent energy occur for the "good-geometry" case. (*b*) Distributions showing the influence of the presence of a host material between the gamma-ray source and spectrometer. (*c*) Distributions obtained with the source suspended between two spectrometers positioned close together. The influence of requiring a coincidence detection of gamma rays between the two spectrometers is shown in the lower curve.

Results with a Two-Crystal Spectrometer. The pulse-height distribution produced by a weak cobalt source effectively surrounded by sodium iodide was obtained with two spectrometers of the type shown in Fig. 8. The spectrometers were oriented with the ends of the crystal containers touching. The gamma-ray source was located between the two crystals.

Figure 9*c* is the pulse-height distribution obtained by simply adding the light pulse signals from the spectrometers. The top curve results when there is no coincidence required (i.e., no simultaneous detection of gamma rays in both crystals), whereas the bottom curve, marked "coincidence," required a coincidence of pulses in the two crystals before the addition pulse is recorded.

In comparing the two distributions in Fig. 9*c*, obtained with two crystals, with that of Fig. 9*a*, obtained with the source close to a single crystal, there seems to be little difference in the photofraction values for the 1.17- and 1.33-Mev peaks. However, the difference appears in the accentuation of the 2.50-Mev addition peak resulting from the simultaneous detection of the two cobalt gamma rays. Also almost all of the background counting rate has been eliminated by the requirement of a coincidence. For spectroscopy on very weak sources where natural backgrounds would mask the radioactivity of interest, the double-crystal and coincidence arrangement would be necessary.

SUMMARY

The factors affecting the one-to-one correspondence of peaks in a pulse-height distribution to the gamma-ray energies producing the distribution have been discussed. The points considered have included the effects of the spectrometer crystal size, spectrometer and trace material geometries, the thickness of the host material, and the background noise signals in the spectrometer.

The calculated and experimental results were intended to show:

1. The High Sensitivities Possible. The data for Co^{60} in Fig. 9*c* illustrated the sensitivity to cobalt impurities of one part in 100,000 million. The trace element could easily have been reduced by one thousand and still be detected and identified in this ideal example, in which there was no confusing gamma activity in the host material.

2. The Applicability of a Large Crystal Spectrometer to Studies Now Being Accomplished by Complicated Procedures. For example, the activation of a host material and its impurities in a uranium pile frequently requires radiochemical separations in order to eliminate the

confusion of beta and gamma activities in the host material. However, some of this work can be eliminated with a large crystal spectrometer with a good energy definition for the gamma rays. By using a beta-ray filter and detecting only gamma rays, the separate gamma rays from the host material and the impurities could be resolved.

As another example, some experimenters [12] have attempted to detect low-energy gamma rays in the presence of an intense high-energy line. They have used a thin crystal sodium iodide spectrometer in order to reduce the sensitivity to the high-energy gamma rays. However, depending on the gamma-ray energies involved, the use of a thick and effectively infinite crystal as described in this report can give greater relative sensitivities to the low- as compared to the high-energy gamma rays that can be obtained with a thin crystal. In the extreme example of a pulse-height distribution from the perfect spectrometer where distinct lines occur at each gamma-ray energy, this is obviously true.

3. Gamma-Ray Spectroscopy Is by No Means a Panacea for Trace Analyses. For example, not all chemical elements have radioactive isotopes whose decay properties are suitable. However, for those favored elements it is a technique whose benefits are worth further detailed study and application.

DISCUSSION

Discussion Leader
John Laughlin
Sloan-Kettering Institute

This spectroscopy technique which employs scintillating crystals and electronic pulse analysis is relatively new. Dr. Koch gave an excellent survey of how the technique works, and he has also told of some of his contributions toward the development of this technique.

I think two points should be emphasized. First, the very great sensitivity that is now available—of the order of 10^{-12}. Another aspect that cannot be ignored—the sample itself is not destroyed. The process of analysis in no way affects the sample during the analysis of the energies of the gamma rays emitted.

These electronic techniques are very versatile; they can be employed with other kinds of detectors: a proportional counter which has a higher resolution and higher sensitivity for very soft X rays. In

Chapter 17 Dr. Hall will describe his developments in this direction.

The gamma-ray spectroscope is a new technique that is beginning to have many diverse applications. I would like very briefly to mention just a few.

One application in which Dr. Hall, at the Sloan-Kettering Institute, has been involved, is the use of double labeling of compounds in the human body. For instance, if one wishes to examine the localization of a tumor antibody sera, and at the same time follow a normal serum, one could label them with two isotopes of iodine. The labeled elements would be chemically identical, but, since the isotopes have different atomic weights, the gamma-ray spectra are different. The use of a crystal, and a pulse-height discriminator, make it possible to take a biopsy from the tumor and determine the relative localization of the two different sera.

Another application is the use of the spectrometer for examining the purity of radioactive shipments, although the purity is very high on most samples, particularly those in production a long time. We sometimes use isotopes that are produced by rather experimental methods, and the spectrometer is very valuable in this connection in sorting out the presence of impurities.

Another application is in the scanning of patients and in the examining of the uptake and localization of isotopes in the body of the patient. Since the gamma rays emitted by iodine or other isotopes in the body suffer some scattering, the use of the pulse-height discriminator makes it possible to ignore the lower energy-scattered radiation and thus get more information on the actual localization of the isotope.

Other applications have been reported. One by Marinelli [13] at the Argonne National Laboratory involved the determination of the amount of radon in the human body by identification of members of the radium family by means of their gamma-ray energies. The amount of K^{40} naturally present was also determined.

These are just a few examples of the many different ways in which this technique will become increasingly important in biological and medical research.

QUESTIONS AND ANSWERS

A. A. SMALES: Before I make my point I would like to state that I am a confirmed gamma-ray spectrometer man. I have been using this device for at least two years and I like it, but I must pour some cold water on the

optimism of Dr. Koch at this time. He has idealized the situation—he admitted this.

In practice one would almost never get only the 10^{-9} or 10^{-12} gram of, for example, cobalt, as the active material in 10 or 20 grams of inactive matrix, because the matrix would have been irradiated, to make not only the cobalt radioactive but all the other elements in the matrix also.

Consider, for example, the sodium, present to as much as a few per cent in a mineral sample—and in biological material it is also quite high. This sodium will be activated to give a 2.76-Mev gamma ray, and so far as I can see, even with a large crystal, the "tail" of the gamma spectrum will possibly obliterate, and certainly prevent accurate measurement of, a small amount of Co^{60}. I am sure that, except in a few very favorable cases, this effect is going to lower the sensitivity that you mentioned, Dr. Koch.

It is that sort of cold water which I feel must be poured on any intense optimism at this stage.

H. W. Koch: I am sorry if I was overly optimistic. I agree wholeheartedly that one must qualify any optimism with the severe limitations that exist in the applications of gamma spectroscopy. For example, I mentioned that it is limited in the number of chemical elements to which it can be applied. Also it is limited because of the activation of the host material. However, for those materials for which it is particularly applicable, gamma-ray spectroscopy certainly shows great promise in providing high sensitivities to trace quantities of material.

G. H. Morrison (*Sylvania Electric Products*): I would like to pour some hot water back on the discussion. We have been using the technique of gamma-scintillation spectrometry in conjunction with neutron-activation analysis for the analysis of trace impurities in silicon, germanium, and other materials, and have found it to be an extremely useful method. Of course there are limitations, but I think that we as chemists realize that no one technique can be used for all analytical situations. Thus, in combination with emission spectroscopy, and other trace techniques, activation analysis–scintillation spectrometry is a valuable tool. It is true that the analysis of trace impurities in semiconductors may be a unique case in that one is analyzing a relatively pure material containing a number of impurities in roughly the same concentration range, thereby resulting in relatively simple gamma spectra. The major limitation to the application of this technique to trace analysis of other materials, however, is the possible interference of gamma radiation from certain matrix materials, thereby necessitating some preliminary chemical separation.

J. T. Horeczy (*Humble Oil & Refining Co.*): I would like to find out a little bit more about associated electronics. Who manufactures the amplifiers, pulse-height analyzer, rate meter, scaler, etc.?

H. W. Koch: The simplicity of the electronics equipment is one of the features of gamma-ray spectroscopy. The electronics consisted of a photomultiplier, a nonoverloading amplifier, a high-voltage supply, and a single-channel pulse-height analyzer. All of our equipment was home-made except for the photomultipliers and the analyzer. The amplifier was a nonoverloading amplifier in order to insure that a voltage pulse due to cosmic rays going through the large spectrometer crystals did not overload and blank out

the device. I would be glad to discuss later any further details regarding circuit details and possible commercial sources.

W. WAYNE MEINKE: I think it might be well to point out the difference in cost between it and the smaller one, and would you comment on the possible application?

H. W. KOCH: You asked two questions about the scintillator. First, the difference in cost between a large- and small-crystal sodium iodide spectrometer would be only in the cost of the crystal. This difference would be $1000 to $2000 for unpackaged crystals. We have packaged our own crystals.

The second question related to the use of liquid scintillators in place of sodium iodide. In the gamma-ray energy range below 2 Mev, which is of interest for trace-analysis work, liquid scintillators would be unsuitable because of the poor energy resolutions obtainable and because of the large physical dimensions necessary. Both objections are eliminated by the use of sodium iodide which provides a practical compact scintillator with relatively good energy definition for gamma rays.

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Mass Spectroscopy

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ABSTRACT Several mass-spectrometric methods for the determination of trace-element impurities have been developed. The three methods that have the greatest potentialities are: (1) the Dempster vacuum-spark method, (2) the ion-bombardment method, and (3) the isotopic-dilution method.

The first two methods have sensitivities of up to 0.01 ppm and give information on all elements in a single experiment. The third has sensitivities of up to 0.0001 ppm with high accuracy but is limited to a measurement of one or at most a few elements at a time. The use of these techniques to date has been severely limited by lack of adequate commercial mass-spectrometric equipment. As such equipment becomes available, the use of the mass spectrometer for trace-element analysis will become commonplace.

INTRODUCTION

The use of mass spectrometers for quantitative chemical analysis of gaseous samples and especially of hydrocarbon mixtures is well known.[1] Mass spectrometers capable of making such analyses have been available from commercial sources since 1940. The countries producing

such equipment commercially in 1955 include England, France, Germany, Italy, Russia, and the United States.

By contrast with gas analysis, the use of mass-spectroscopic methods for chemical analysis of nonvolatile materials (i.e., materials not volatile at room temperature) has been very limited. The limited use has been due in large part to a lack of commercially available equipment capable of handling such materials. Since 1954, however, several commercial companies have been experimenting with mass spectrometers capable of making such analysis with the intention of producing a usable commercial instrument. The equipment limitations are therefore being relieved, and routine analysis of nonvolatile materials should soon be a reality. It is the purpose of this chapter to point out the potentialities and limitations of the mass-spectroscopic methods of analysis with special emphasis on trace-element determination. One short section will outline extensions of the usual mass-spectrometric gas-analysis techniques to trace determination in gases. The major portion, however, will be devoted to discussion of trace determination in samples having negligible vapor pressure at room temperature.

There are four fundamentally different methods of trace-element analysis in solid materials which merit discussion:

1. The Dempster vacuum-spark method.
2. The ion-bombardment method.
3. The isotopic-dilution method.
4. The total-evaporation method.

The first of these methods is analogous to the optical-spectrographic method in that it analyzes for all elements present in a single experiment. It has the advantage over the optical method that it does not have the blind spots characteristic of the optical method. Within about one order of magnitude, all elements are detected with equal sensitivity. The ion-bombardment method is similar to the Dempster-spark method in that it analyzes for all elements in one experiment. The range of sensitivities, however, varies over a wider range than with the spark method. The last two methods, i.e., the isotopic-dilution and total-evaporation methods, are similar in that they both detect only one or at most only a few elements in one experiment. They do, however, have the advantage of much higher sensitivity than either of the former methods.

The four methods of approach to nonvolatile samples listed above are as distinct as infrared and ultraviolet spectroscopy: i.e., the sources are different, the analyzers are different, and the detectors are different. A laboratory set up for one method is not necessarily able to handle

the others. This review of nonvolatile sample methods is therefore a review of four distinct methods of trace-element analysis.

The four methods to be discussed differ mainly in the mechanism of producing a charged beam of ions characteristic of the sample to be analyzed: i.e., in the ion source. In fact, with the first two methods the type of mass-sensitive analyzer is completely determined by the source used. It should be clear, therefore, that the discussion to follow must center about ion sources.

DEMPSTER VACUUM-SPARK METHOD

The vacuum-spark ion source was first applied to mass spectroscopy by A. J. Dempster.[2] His purpose in applying this source in mass spectrometers was to find a source that would produce ions of elements difficult to ionize by any other method then known: i.e., a versatile ion source. The immediate consequence of his development was that the exact masses of many of the isotopes were determined for the first time. In addition, however, Dempster realized that this source, since it was versatile in that it ionized all elements with no "blind spots," would be useful for chemical analysis. During the war 1941–1945, Dempster, working on the classified Manhattan Project, applied the technique to the analysis of a variety of solid materials. There was such interest in his technique that he built three mass spectrographs using the vacuum-spark source for other Manhattan Project laboratories. Dempster's death prevented his continuing his work on the method, and only one short note describing his work appeared in the unclassified literature.[3] In this note he pointed out that 1 part in 10^7 of some trace elements was readily detectable with the technique.

The vacuum-spark method has been studied by several other groups who have made important contributions. Shaw and Rall[4] working in Dempster's laboratory constructed a Mattauch type of double-focusing mass spectrograph for use with the spark source. The use of the Mattauch arrangement is very desirable when photographic recording is to be used, since it records a large mass range, and hence a large number of elements, in a single exposure. R. H. Brittan[5] added an electronic pulsed RF oscillator in place of the Tesla coil used by Dempster. The use of such an oscillator increases the stability of the spark source and decreases the stray RF characteristic of Tesla coils. Improvements made by Gorman, Jones, and Hipple included the use of a ratio-recording electronic system to render the ion current

intensities measurable on a strip-chart recorder.[6] This system has been used only in recording compositions of alloys where the elements in question vary between 0.25 and 85%. There is, however, no fundamental reason why the ratio-recording system could not be applied to trace-element determination. N. B. Hannay has applied the vacuum-spark method to routine determinations of boron in silicon and antimony in germanium. In each case he attained sensitivities of one part in 10^7. To achieve this sensitivity, Hannay constructed a Mattauch type of double-focusing mass spectrograph, an improved-pulsed RF oscillator, and utilized photographic recording of the ion beam. Hannay's success in detecting boron and antimony in these semiconductors where the optical spectrograph failed is again clear proof of the power and value of the method.

Instrumentation. Figure 1 is one embodiment of the Dempster-vacuum spark. If the sample to be analyzed is a conductor, it is in-

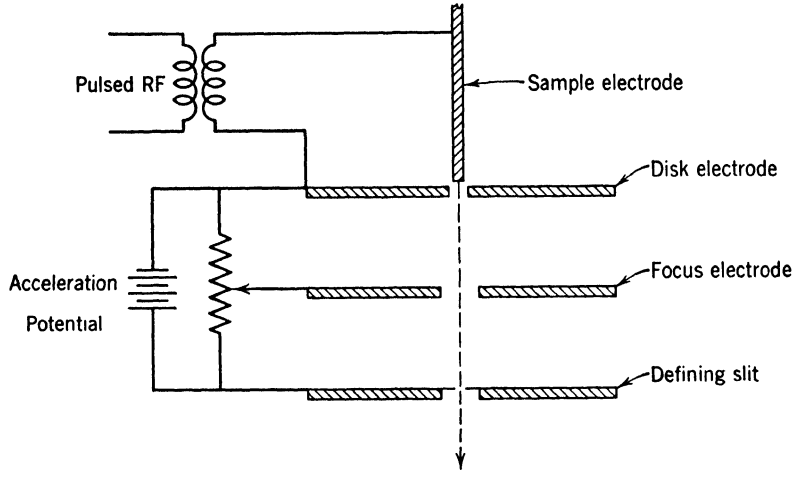

FIG. 1. Schematic diagram of the Dempster vacuum-spark ion source. A pulsed RF potential applied between the sample and disk electrodes produces electrical breakdown. A portion of the ions formed in the discharge are accelerated and focused by the focusing electrode through the defining slit into the mass-sensitive analyzer.

troduced at the sample point as a rod of about 0.040 inch diameter. Nonconducting materials are analyzed by packing them inside conducting tubes of about 0.040 inch diameter and placing them at the sample electrode position. Satisfactory container tubes are pure nickel and pure gold.

The vacuum spark is produced by placing a high RF potential

between the sample electrode and the disk. If the potential is sufficient to produce electrical breakdown, hot spots appear on the electrodes from which solid material is evaporated and sputtered into the space between the electrodes. The electrons present in the space resulting from the discharge produce ions by electron bombardment. Further ionization is produced by the impact of these ions against the electrodes.

The RF voltage placed on the electrodes must be pulsed since a continuous RF would melt down the electrode by simple IR heating. The usual pulsed supply produces an RF voltage of about 40,000 volts with an on time of about 100 μsec and a repetition rate of 60 pulses per second; i.e., the discharge is on less than 1% of the time, and the rest of the time the electrodes are cooling.

This description of the Dempster spark source is sufficient for us to point out the two major disadvantages of the ion source. They are:

1. Large spread in energy of ions.
2. Instability of ion emission.

The spread in energy results from the fact that ions are produced at all points between the two electrodes. The instability results from the fact that the source is on only 1% of the time, and the time of breakdown in any one pulse is variable, depending on the shape of the electrode.

The spread in energy in an ion beam produced in a spark source is of the order of 1000 ev. This inhomogeneity is of such magnitude that a single-focusing type of mass spectrometer is useless, since it *assumes* an ion beam homogeneous in energy.[9] This is the fact that has delayed use of the Dempster method of chemical analysis, since in 1954 all commercially available mass spectrometers were of the single-focusing type. The mass spectrometers capable of utilizing the vacuum-spark source are termed double focusing.[9] The most common of these machines combines as electrostatic deflection with a magnetic deflection. In 1955 the particular arrangement considered most satisfactory was the Mattauch arrangement. It consists of a 31°50′ radial electrostatic deflection followed by a 90° magnetic deflection as shown in Fig. 2.

The second disadvantage of the spark source, instability, has been solved by two techniques, by using a photographic plate as Dempster [3] and Hannay [8] have done, or by using a ratio-recording system in which the ratio of the resolved to unresolved ion beams are recorded rather than the magnitude of the resolved beam only.[6] With a ratio-record-

ing electrical detection system, the preference for the Mattauch type of instrument no longer applies, and any type of double-focusing mass spectrometer may be used.

A property of the spark source that has been little studied is its ability to give information on compounds existing in solid samples. Thus, though a sample may consist largely of SrO, the mass spectrum will show largely Sr^+ and O^+ ion currents.

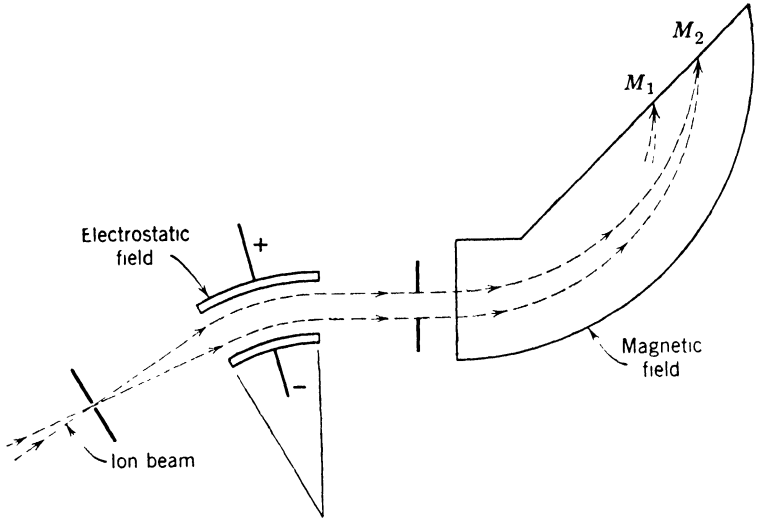

FIG. 2. Schematic diagram of the Mattauch type of double-focusing mass analyzer. The ion beam passes first through a radial electrostatic field of angle 31°50′, and then through a magnetic field where the beam is deflected through an angle of 90°. Different masses come to focus at different points along the exit to the field, as is illustrated by the beams M_1 and M_2.

A second property of the spark source is that ionization efficiencies for different elements vary by about one order. They are, however, not predictable from any simple rule.

For both of these reasons it is imperative to use calibration samples with the spark source. One can make a qualitative analysis without calibration but not a quantitative one.

Results. A vacuum-spark mass spectrum as recorded on a photographic plate is shown in Fig. 3. This figure gives the light element impurities present in a sample of uranium metal as taken from the work of Dempster.[3] This particular spectrum was obtained by maintaining the spark between two electrodes of the uranium in question rather than between an electrode and a disk as shown in Fig. 1. This

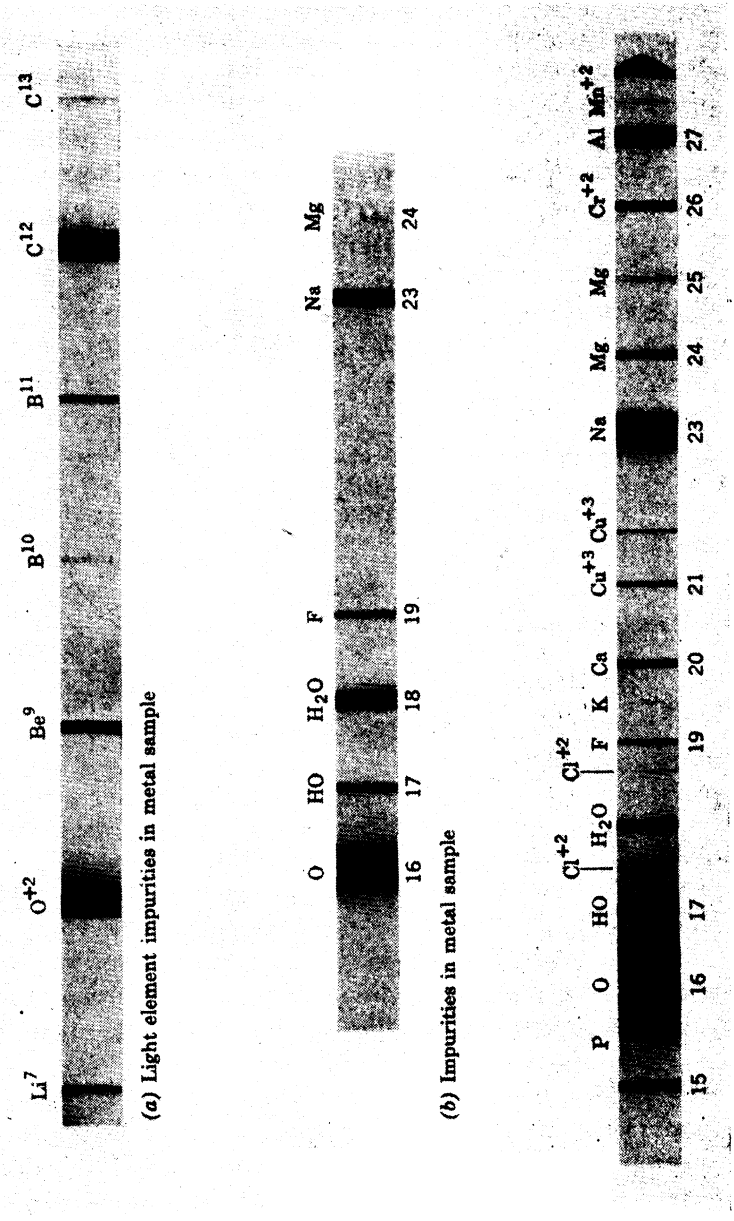

FIG. 3. Mass spectrum of the light-element impurities in a sample of pure uranium as observed using the vacuum-spark method of analysis.

is an alternative source-electrode arrangement which is often used but it has the disadvantage of requiring more accurate alignment. The spectrum of Fig. 3 was obtained during a 3-minute exposure, during which time about 10 mg of sample material was consumed. The strong line at mass 9 is complicated by triply charged ions of aluminum. From the figure it is apparent that trace impurities of 1 part in 10^7 are readily detected ($B^{10} = 3 \times 10^{-7}$ is ~10 times background). These data are in good agreement with those of Hannay and Ahearn,[8] where they detect one part in 10^7 of boron and antimony in silicon and germanium, respectively. There can be no question that the spark method is a trace-element method.

The absolute sensitivity of the spark method as determined from Fig. 3 is given in Table I. For the light-element impurities Dempster [3]

TABLE I

Impurity	Known Chemical Composition, ppm	Electrode Consumption, mg to Give Detectable Line	Wt. of Trace Element Just Detectable, g
Li^7	5	—	—
Be^9	0.02	2.0	0.4×10^{-10}
B^{10}	0.3	0.4	1.2×10^{-10}
C^{13}	4	0.2	8×10^{-10}
N^{14}	24	0.01	2.4×10^{-10}
O^{16}	40	0.002	0.8×10^{-10}
F^{19}	1.1	0.08	0.9×10^{-10}
Na^{23}	10	0.005	0.5×10^{-10}
Mg^{24}	14	0.025	3.5×10^{-10}

found that about 10^{13} atoms were necessary for detection. Hannay and Ahearn [8] report sample requirements of about 10^{11} atoms for a detectable effect. The latter work is for heavier elements than those studied by Dempster, which tends to decrease the difference between the two figures. The remaining discrepancy is probably related to the better vacuum techniques used by the latter workers and to different photographic techniques.

Dempster studied the effect of surface impurities. He found, as did Hannay and Ahearn, that surface impurities show up on the first exposure, but only very weakly on later exposures. The absolute sensitivity for surface films was studied by Hannay and Ahearn,[8] who found that about 10^{12} atoms were necessary to produce a detectable effect.

The ultimate sensitivity of the Dempster spark method is limited

by two factors. First, in trace-element determination there is always the question of background. If one continues to decrease the amount of carbon in a sample, one will find that there is a minimum below which the C^{12} ion current will no longer decrease. This occurs at the point where the C^+ peak is produced primarily from the hydrocarbon gases present in the residual vacuum and not from the carbon present in the sample. This background limitation is important at some mass positions and almost negligible at others. Thus the peaks at mass 10 and 11 in Fig. 3 almost undoubtedly result from the sample, whereas to prove that mass 12 is not due to background requires several exposures. For most elements the importance of background becomes less and less as one reduces the pressure. The use of modern vacuum techniques [10] should greatly improve the sensitivity and usefulness of the Dempster vacuum-spark method. The second limitation on ultimate sensitivity results from gas scattering, i.e., a small fraction of the major peak will be scattered by the residual gas to produce an ion current at the position where the impurity in question is to appear. This is the ultimate limitation in the B^{10} and B^{11} positions of Fig. 3. Again, as with background peaks, this limitation becomes less severe as the pressure is lowered. It is apparent that one important measure of the usefulness of a machine for trace analysis is ultimate pressure.

It is apparent from results already published that the spark method is a proved method of trace analysis for some elements. As more work is done, its general usefulness will become more apparent. Actual application to routine laboratory analysis awaits only the production of a satisfactory commercially available machine.

ION-BOMBARDMENT METHOD

When energetic ions strike surfaces, a variety of secondary effects take place. The best known of these effects is the production of secondary electrons. In addition to secondary electrons, however, secondary positive ions are also produced. These ions are characteristic of the target material. Thus ion bombardment may be used as a method of producing an ion beam characteristic of a solid sample, and hence of analysis of solid materials. Unfortunately, though the effect has been known for many years, it has not been extensively studied. Therefore only very general statements can be made regarding its use in trace-element determination.

The first attempt to produce secondary ions from high-energy elec-

tron bombardment was that of Dempster.[11] More recently Plumlee and Smith[12] have investigated electron bombardment more completely. From these experiments it is concluded that electron bombardment does give ions from electronic excitation of absorbed species on the surface but that it is useless for analyzing solid materials. This conclusion can be understood very simply by considering a collision of an electron and an atom. If an electron of 100,000 electron volts energy strikes a lead atom, conservation of energy and momentum shows that the lead atom recoils with a maximum energy of about 1 ev. So, if the lead atom were bound in a solid with a bonding energy of several electron volts, it would take an electron of several hundred thousand volts energy to release that atom from the surface. The cross section for such a collision would be nuclear rather than atomic in magnitude: i.e., about 10^{10} times smaller than the usual ionization cross sections involved in electron bombardment of gases. Thus, secondary ions produced by electron bombardment of solids are negligible.

The first study of ions produced under positive-ion bombardment was that of Smith.[13] A collisional calculation like that given above for electron bombardment shows that, even for the lightest ion H^+, the threshold for breaking the bonds of heavy elements is only tens of volts, not hundreds of thousands, as with electrons. Hence, with reasonable ion energies it appears quite possible that usable beams of secondary ions might be produced. An ion-bombardment source based on this principle has been described by Herzog and Viehboch.[14] Other variations have been described by Inghram and Hayden.[9]

The advantages of this type of source over the Dempster vacuum spark include the following: (1) The stability of the source is determined by the stability of the primary-ion beam; thus, since the primary beam may be produced by any of the stable types of ion sources, the secondary beam is stable and hence easily measurable. (2) The source is operating continuously, so that the difficulty with space-charge blow-up of the ion beam, which is serious with the pulsed spark source, is here greatly reduced. (3) The testing of the sample is almost nondestructive in comparison to the spark source which consumes several milligrams per minute. (4) The RF shielding problems characteristic of a spark source are not encountered.

The disadvantages of the source in comparison to the Dempster vacuum spark include: (1) The ionization efficiency for different elements varies over a larger factor than the spark source, making calibration imperative. (2) The problem of surface contamination is

much more serious since the surface is removed "layerwise"; thus the vacuum requirements are more stringent than with the Dempster spark.

Instrumentation. A diagram of the secondary-ion source used by Stanton and Inghram is given in Fig. 4. Though the energy spread in the ion beam produced by this source is much less than that produced by the Dempster spark source, it is still of the order of 100 ev, so that double-focusing mass spectrometers are a necessity. The instrument shown in Fig. 2 would be quite satisfactory. Stanton [15] has shown that the intensity of secondary Pb^+ ions produced by rare-gas bombarding ions is proportional to the energy of those ions in the energy range of 100 to 4000 ev, and that the major problem in the use of an ion-bombardment source is surface contamination. To overcome this problem three things may be done: (1) Increase the intensity of the primary-ion beam, (2) heat the sample being bombarded, and (3) use ultra-high-vacuum techniques.[10] Again as with the Dempster spark the major factor delaying application of the ion-bombardment method to chemical analysis is lack of adequate commercially available mass-spectrometric equipment.

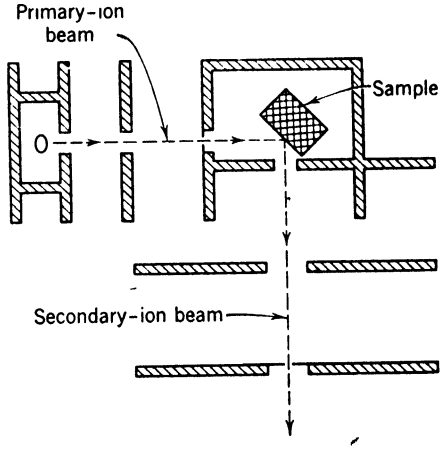

FIG. 4. Schematic diagram of an ion-bombardment ion source. The primary-ion beam is formed by electron bombardment of an appropriate gas (usually a rare gas). When this ion beam strikes the sample, it produces secondary ions characteristic of that sample. These secondary ions are formed into an ion beam by appropriate acceleration and collimation.

Results. The ion-bombardment method has not been applied specifically to trace-element determination, but extrapolation of experiments with metal targets indicate strongly that, if ultravacuum techniques are employed, the method will be a reliable trace-element method for several elements in concentrations of 1 ppm and below.

ISOTOPIC-DILUTION METHOD

The isotopic-dilution method of analysis is different from either the Dempster vacuum spark or ion bombardment in that it deter-

mines only one or at most a few elements at a time. It does, however, have the tremendous advantage of several orders of magnitude higher sensitivity. It has in fact been used to detect traces of one part in 10^{12} in two specific cases.

The isotopic-dilution method of chemical analysis was first proposed by G. Hevesy and has been used for a number of years by the biologists for the determination of the elements and compounds of hydrogen, carbon, and nitrogen.[16] The method has been limited in the past to these elements for which separated isotopes were available. Its power as a general trace-element method was not apparent from studies of these elements; however, as a result of the efforts of the Atomic Energy Commission in making available separated isotopes of many of the elements, the method has been extended, and its real power in trace-element determination demonstrated.

Inghram [17,18] has outlined the methods of trace analysis and has given typical examples of the isotopic-dilution technique as a tracer method. Only the procedure will be repeated here.

1. A weighed portion of the sample to be analyzed is dissolved in an appropriate solvent.

2. A weighed amount (usually an aliquot of a stock solution) of a separated isotope of the element in question is added to the solution of step 1.

3. The mixture of samples and tracer are processed through appropriate chemical mixing to insure that the tracer is properly equilibrated with the sample.

4. The element in question, now consisting of a mixture of normal and diluent isotope is extracted chemically (this step is optional, depending on the element in question and the sensitivity desired).

5. The change in isotopic composition of the element in question in the sample caused by addition of the tracer isotope is determined mass spectrometrically.

From the isotopic composition determined in step 5 and the known isotopic compositions of the same element in the sample (normal) and tracer the concentration of the trace element can be calculated from the equation

$$\text{Weight of element in sample} = K_{ij} \text{ weight of tracer added}$$

where

$$k = \frac{a_j^T}{a_j^S}\left[\frac{(a_i/a_j)^M - (a_i/a_j)^T}{(a_i/a_j)^S - (a_i/a_j)^M}\right]\frac{MW^S}{MW^T}$$

In this expression the superscript S refers to the original sample, T refers to the tracer, and M refers to the extracted mixture of tracer and sample. Further,

a_j = fractional abundance of isotope j

$\frac{a_i}{a_j}$ = ratio of two isotopes

MW^S = molecular weight of element in question

MW^T = molecular weight of tracer element

Thus, for example, the quantity a_j^T denotes the fractional abundance of isotope j in the tracer sample, and the quantity $(a_i/a_j)^M$ is the isotope ratio of isotopes i to j in the mixture. The molecular weight of the sample and tracer are of course different since their isotopic compositions are different. This equation can be much simplified if either the element or the tracer is monoisotopic or if there are non-overlapping peaks, in which case some a's are zero.

From consideration of the above procedure and method of calculation, some of the advantages of the isotopic-dilution technique are apparent. Perhaps the most important advantage of the method is that quantitative or known chemical recovery of the element in question is not necessary. Thus, if one loses 99% of one isotope of an element in chemical processing, one will also lose ~99% of the others with, neglecting contamination, no net effect on the calculation of the trace-element concentration.

In trace-element studies, serious troubles are encountered with contamination introduced from the reagents used to process the samples. This contamination is usually evaluated by processing two aliquots of the sample varying in size by a factor of two but retaining the same amounts of tracer and reagents. If there is no contamination arising from the reagents, the amount of the tracer element found in the two samples will differ by a factor of two. If there is contamination, it will differ by less than a factor of two, and from the deviation from the expected factor the actual amount of contamination can be determined, and hence eliminated from the final calculation.

To make an analysis by the isotope-dilution method, it is necessary that the element in question have at least two stable isotopes; i.e., the method depends upon isotopic ratios. Thus, at first thought it seems that only those elements having two or more naturally occurring iso-

topes can be handled by this method. This limitation is somewhat removed by the fact that a number of elements have long-lived isotopes which are stable as far as the mass spectrometer is concerned. Thus, for example, while cesium as it exists in nature has only one isotope, it can be analyzed by isotopic-dilution methods, using fission cesium as the tracer. Fission cesium consists of three isotopes, Cs^{133}, Cs^{135}, and Cs^{137}, all of about equal abundance. Table II summarizes

TABLE II

SCOPE OF ISOTOPIC-DILUTION METHOD OF CHEMICAL ANALYSIS

Elements Analyzable				Elements Not Analyzable
H	V	Pd	Gd	F
He	Cr	Ag	Dy	Na
Li	Fe	Cd	Er	Al
(Be)	Ni	In	Yb	P
B	Cu	Sn	Lu	Sc
C	Zn	Sb	Hf	Mn
N	Ga	Te	W	Co
O	Ge	(I)	Re	As
Ne	Se	Xe	Os	Y
Mg	Br	(Cs)	Ir	Cb
Si	Kr	Ba	Pt	Rh
S	Rb	La	Hg	Pr
Cl	Sr	Ce	Tl	Tb
A	Zr	Nd	Pb	Ho
Ca	Mo	(Pm)	Th	Tm
Ti	(Tc)	Sm	U	Ta
K	Ru	Eu	(Pu)	Au
	Total 68			Total 17

the scope of the isotopic-dilution method. The elements that are analyzable by the method are given in the columns at the left, and those that are not analyzable in the column at the right. Those tracers that are obtained from other than natural sources are included in brackets.

Instrumentation. The isotopic-dilution method of trace-element analysis is completely different from either the Dempster vacuum-spark or the ion-bombardment method in that no specific ion source is prescribed. Thus instead of requiring special machines as do the former methods, any type of mass spectrometer may be used, so long as it is capable of analyzing the element in question. At the present time, most workers doing isotopic-dilution analysis are using simple

single-focusing mass spectrometers,[9] a typical example of which is the sector machine shown in Fig. 5. From the instrument standpoint, therefore, this method is the least expensive of the methods so far discussed. As soon as versatile single-focusing machines become available, the technique should find extensive application.

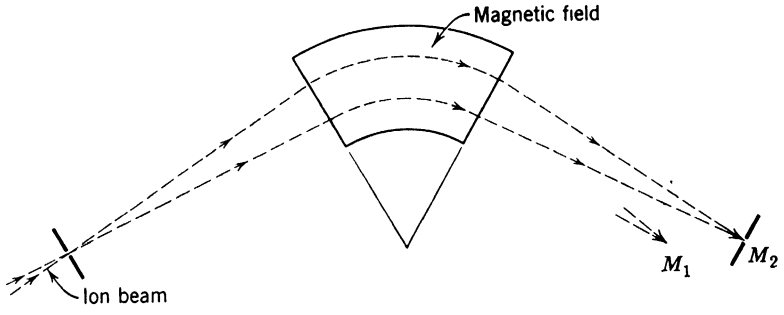

FIG. 5. Schematic diagram of the most commonly used type of single-focusing mass analyzer. Ions enter the magnetic field, are deflected through an angle of about 60°, and are brought to focus again at the point M_2. Different masses come to focus at different points, as is illustrated by the two focal points M_1 and M_2.

Results. The isotopic-dilution method of trace analysis has been used by the nuclear physicist and chemists for a variety of applications.[19,20] The technique has also been used extensively by geophysicists for trace-element and age determination of geologic materials.[21] The data of Table III, taken from the work of Tilton et al.,

TABLE III

URANIUM AND THORIUM CONTENT OF VARIOUS GEOLOGIC MATERIAL

Parts per million

Sample	Uranium	Thorium
Zircon	2650 ± 40	2180 ± 20
Sphene	303 ± 5	5375 ± 75
Perthite	0.22 ± 0.03	0.410 ± 0.008
Stone meteor	0.0054 ± 0.0002	—
Ammonium nitrate	0.000075 ± 0.000004	—

illustrates the determination of uranium in various geologic materials. The range and accuracy of the method is well illustrated by this table. The greatest sensitivity noted is for uranium in the reagent ammonium nitrate where a concentration of 75 parts in 10^{12} was determined with an error of only 4 parts in 10^{12}. It is obvious

from the relative errors that the mineral perthite is considerably more difficult to analyze than the nitrate. This results from the greater difficulty of chemical processing. The errors quoted are attributable to sample inhomogeneity and contamination; in no case do they represent a fundamental limitation of the method.

The requirement of a supply of separated isotopes to do isotopic-dilution analyses is a bottleneck only from the supply standpoint, not the cost. The tracer cost per geologic sample analyzed for lead by the techniques of Tilton et al.[21] is less than 1 cent per sample. As the demand for stable tracers increases, the bottleneck of supply will surely disappear.

The isotopic-dilution technique can be considered as a method of weighing impurities. The machines can detect as little as 10^{-12} gram of most elements. Thus it can detect an impurity as long as the total sample contains 10^{-12} gram of the impurity.

The isotopic-dilution method of trace-element analysis is already a proved method for a variety of problems[17] with sensitivities that in many cases far surpass other known analytical methods.

TOTAL-VAPORIZATION METHOD

The fourth method of trace-element analysis is the total-vaporization method. In this method the sample is placed in a small crucible inside the mass spectrometer in juxtaposition to the ion source. The peak corresponding to the element in question is then continuously monitored as the temperature of the crucible is raised. The concentration of the element in question is then proportional to the integrated peak height.

This method of analysis has been applied by Hickam to the determination of silver in copper and shown to have trace-element sensitivity.[22] More recently it has been applied by Honig to the determination of impurities in the semiconductor germanium.[23]

The limitations of the technique are again related to ultimate vacuum and background. As the temperature of the crucible is raised, the intensity of the background and scattered ions increases, giving an apparent impurity, even though none is actually present in the sample. The elimination of these effects requires the use of ultrahigh-vacuum techniques. An alternative method of decreasing the importance of background gases is to chop the molecular beam with a chopper and use a phase-sensitive electrometer such that it

responds only to the chopped signal and not to the other background gases.

The total-evaporation technique is almost useless for samples such as tungsten in copper, since it is difficult to get a container that will go high enough in temperature to evaporate the tungsten totally, without serious difficulties of other types being encountered. Similar difficulties are encountered with oxygen in many metals, since the tracer amount of oxygen may react with any of the various metals in the sample and be transported out as an unmonitored metal oxide rather than O_2.

One further difficulty is that some elements evaporate in pulses,[23] with special difficulties occurring at the time the sample melts. Most of these difficulties can be taken into account by running calibration samples to determine how a particular type of sample reacts chemically to total vaporization.

Instrumentation. Again, as with the Dempster vacuum-spark and the ion-bombardment methods, this method of analysis depends on a specific type of ion source. For this type of analysis, the ion source must include a crucible from which the sample can be vaporized into the ionization region. Ionization is usually accomplished by electron bombardment.[9] Thus, since the ion beam is homogeneous in energy, a single-focusing type of mass analyzer is adequate. Again single-focusing mass spectrometers are available commercially, but they are not satisfactory for use with a crucible-evaporation source. Thus routine use of the total-vaporization method must await the availability of suitable commercial equipment.

Result. Both Hickam and Honig report success in the use of the total-vaporization method as a method of trace analysis. The results of Hickam [22] on determination of silver in copper are given in Table IV. Within the accuracy of the data, there is no systematic error in the result given; the accuracy, however, is not at all comparable to that obtainable with any of the other three methods. Honig has measured the impurities in germanium by total vaporization. He has detected impurities of As, Pb, Sn, Fe, P, Sb, and Cd in the germanium. The complications in the method are illustrated by the fact that arsenic is observed as As_4^+ molecules, phosphorus as As_3P^+ and $As_2P_2^+$, etc., antimony as As_3Sb, As_2Sb_2 and even such cross-peaks as As_2PSb are observed. Such complications make use of the method difficult, though, when the observations are combined with known thermodynamic data, these peaks do give information on how

TABLE IV

TOTAL-VAPORIZATION DETERMINATION OF TRACE QUANTITIES OF SILVER IN COPPER

Known Composition, ppm	$\int I^+ dt$	Mass-Spectrometer Composition, ppm = $k\int I^+ dt$
3.7	25.5	4.8
8.6	28	5.3
8.7	40	7.6
17.4	109	20.7
34.3	165	31.4
34.4	200	38.0

Best fit k taken as 0.19.

the impurities are tied up chemically in the original sample. Honig calculates sample purities by assuming ionization cross sections, no surface reactions, accommodation coefficients, etc., and gets qualitative analyses of his germanium samples. It seems that these requirements are too severe and that calibration samples must be run. If this is done, there appears to be no reason why the total vaporization method cannot be used reliably for a variety of trace applications.

TRACE IMPURITIES IN GASES

The determination of the composition and purity of gaseous samples by mass-spectrometric methods is well known.[1] In rare gases, for example, impurities of 1 part in 10^5 are routinely determined using commercial equipment. The extension of gas techniques to much higher trace sensitivities will be a reality as soon as more satisfactory mass-spectrometric equipment becomes available.

The limitation of present machines is gas scattering and resolution. Thus an impurity of one part in 10^7 of krypton in xenon is undetectable with commercial equipment simply because the vacuum is not good enough. Wetherill,[24] using a modern machine, operating at pressures of 10^{-9} mm was able to measure adjacent isotopes to one part in 10^7; extrapolating to the difference between krypton and xenon, he could easily detect and measure one part in 10^8. An alternative method of eliminating scattered ions is to use a successive field first-order focusing type of mass spectrometer.[9] Such instruments operated with ultrahigh-vacuum techniques should have sensitivities for particular cases as high as one in 10^{10}.

ALTERNATE METHODS

There are a number of other possible methods of trace-element analysis but they have not been proved. One such method investigated by Muraca and Serfass [25] involves the use of a vacuum spark as a means of evaporating the sample into the ionization chamber of a standard single-focusing mass spectrometer. In such an evaporation element fractionation with time is negligible so that integration over time is eliminated. The sensitivity of the technique, however, is such that it cannot be considered a trace-element method.

One suggestion by Plumlee is to use the exploding-wire technique for evaporation. This technique, however, has yielded no results and is fraught with serious difficulties.

CONCLUSIONS

It has been demonstrated that the mass spectrometer is a proved instrument for trace-element analysis. The method in these early stages of development should not be applied to problems that can be solved by other standard methods. It should be reserved for those problems that cannot be solved by any other such technique.

The statement often made in this review should be repeated: "As soon as satisfactory mass-spectrometric equipment becomes available"; for it is this lack of equipment that has prevented extensive use of the various mass-spectrometric approaches to trace analysis. Each of the methods has been successfully used by those who build their own instruments.

QUESTIONS AND ANSWERS

W. D. COOKE: How much sensitivity is lost because of the necessity of using a double-focusing mass spectroscope with the spark source?

MARK G. INGHRAM: The sensitivity would be essentially zero without double focusing; i.e., the ion beams would not be resolved at all. Thus the double-focusing machine increases the sensitivity to trace-element determination. The things thrown away are ions of such wide energy spread that they would not appear at the proper mass position in the spectrum: i.e., those that would decrease the sensitivity for trace-element determination. Ion intensity is lost but not sensitivity.

W. D. COOKE: If one could get the whole beam, i.e., complete energy spectrum into a perfect electrostatic analyzer, how much higher current would you expect?

MARK G. INGHRAM: One would expect an increase in current of at least two orders of magnitude. This would result in a decrease in exposure time but should not change the sensitivity for trace-element analysis.

W. D. COOKE: Are there any matrix effects of which you know?

MARK G. INGHRAM: The answer to this question is quite different, depending on the method of trace analysis being used. In the total-evaporation method the matrix effects are very apparent. For example, if one is after the oxygen content of a platinum sample, one would find the oxygen evaporating as the compound BaO. Looking at the O_2^+ position in the latter case would show nothing, even though the sample contained large quantities of oxygen.

In contrast, there are almost no matrix effects in the isotopic-dilution method. The only precaution necessary is that the tracer be chemically equilibrated with the sample.

With the spark source one has to calibrate with samples of known composition. In so doing one finds that the sensitivities vary by about a factor of ten. This is a much smaller variation than that found with optical spectra; and so the matrix effects are correspondingly reduced.

A. A. SMALES: Would Dr. Inghram like to comment on the contamination problem?

MARK G. INGHRAM: The problem of contamination in trace analysis is, of course, an important limiting factor. In the isotopic-dilution method it is *the* limiting factor. For example, in the uranium analysis listed in Table III, in three cases the error tabulated is due to uncertainty in contamination. We always take contamination of our reagents into account by processing two samples of different size. For example, if we process two samples of meteorites, one of 10 grams and one of 5, and we add equal amounts of tracer to each, we could expect the absolute amount of impurity in the two samples to vary by a factor of two if no contamination is present in the reagents. On the other hand, we would expect the amount of impurity found to be equal if contamination is the only source of impurity. In those cases where the sample and half-sample results come out different from the factor of two, the contamination can be evaluated from the variation from the expected number.

The above method of evaluating contamination is perfectly reliable as long as contamination is introduced reproducibly: for example, from reagents. If the contamination is introduced erratically, for example, from dust, the above treatment does not apply. At the present time the highest sensitivity work on trace determination of uranium and lead is being done by C. Patterson at the California Institute of Technology. To minimize the erratic contamination problem he has constructed a special laboratory with special filters, special lead-free piping and paint, stainless-steel tables, etc. Obviously the contamination problem is important in the mass-spectrometric method of trace analysis as with almost all other methods.

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X-Ray Spectroscopy

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ABSTRACT X-ray spectroscopy is now a standard tool for analyzing metallurgical alloys and other industrial materials, improved methods are extending its range to include some biological materials. The principles and scopes of X-ray fluorescence analysis, with and without the use of a diffracting crystal, fluorescence analysis with electron-beam excitation, image-forming fluorescence analysis, and microradiography with monochromatic X-ray beams are reviewed.

INTRODUCTION

Scope of X-Ray Spectroscopy. The use of X rays for chemical-element analysis is rapidly growing. With the present commercial units,* one may analyze specimens for almost any element of atomic number higher than 20; errors are a few per cent or less for element concentrations of the order of one per cent or higher. This makes X-ray fluorescence a welcome tool for production control and prod-

* Commercial X-ray fluorescence analysis units are made in the United States by North American Philips Company, Mount Vernon, N. Y., General Electric Company, X-ray division, Milwaukee, Wis., and Applied Research Laboratories, Glendale, Calif. In Europe a major manufacturer is Philips, in the Netherlands.

uct development in the field of metallurgical alloys and in other fields where 1 to 10% "minor" constituents are important.

With careful use of the most modern commercial equipment, routine analysis has been extended down to concentrations of 10^{-4} (1 part in 10,000). In applications where the trace elements may be isolated and concentrated before measurement, the routine range extends down to about 1 μg of any particular trace element.

Engineering has not had time to catch up with the tremendous potential of X-ray methods, and these performance figures will be much improved in a few years. In addition, under some circumstances special instruments described below may perform analyses on amounts down to 10^{-11} gram and on specimen micro-areas down to a few square microns.

A more detailed picture of the capacity of X-ray analysis is given in Table II and Table IV.

There are several approaches to X-ray analysis; all share the following advantages and limitations:

Advantages:

1. X rays interact with elements as such; the measurement of a constituent element is independent of its state of chemical combination.

2. X-ray analysis is "nondestructive." Although specimens may suffer molecular changes, gross structure is not altered and constituents are not displaced during measurement.

3. The total measurement time is usually less than for most other methods. Where measurement is possible, the time will usually fall between a few minutes and an hour.

4. Specimen preparation is fairly simple. Often the specimen can be analyzed with no prior processing.

5. The X-ray absorption peak and emission wavelengths of the elements follow the sequence of the periodic table. The number of lines is much less than for optical spectra. Thus, the X-ray spectra of the elements form a simple, orderly pattern, with a unique spectrum for each element.

Disadvantages:

1. X-ray spectroscopy must use relatively "soft" (nonpenetrating) X rays. Therefore, analysis is limited to thin specimens, or to the top layers (about 10 to 100 microns deep) of thicker specimens.

2. For the thicker specimens, absorption effects and the excitation of one element by X rays from other elements in the specimen may necessitate elaborate calibration standards ("mock-up" mixtures) for quantitative work.

3. X-ray analysis equipment is complicated and expensive. The cost of commercial units is in the range of $15,000 to $30,000, and these cannot provide extreme "trace" analysis. For this is needed an apparatus specially designed for the problem at hand; such apparatus is liable to be costly in development time, and effort, if not in money.

Before reviewing the different approaches to X-ray analysis, we summarize here the basic principles underlying the method. The reader interested only in current applications should skip this summary; it should help the nonspecialist who wants to understand the subject.

Relationship between Ordinary and X-Ray Spectroscopy. As an analytical method, X-ray spectroscopy is quite analogous to conventional emission and absorption spectroscopy. Whereas the latter is based on the characteristic *optical* and near-optical lines of the elements, corresponding to the energy levels of *outer* electronic orbits, X-ray spectroscopy is based on the characteristic *X-ray* absorption and emission lines of the elements, corresponding to the energy levels of their *inner* electronic orbits.

In spite of this close relationship, the two fields use completely different instrumentation. In practice, X-ray spectroscopy is founded on the special separate field of X-ray physics.

Fundamentals of X-Ray Physics. *X-Ray Wavelength and Quantum Energies.* X rays, like all other electromagnetic radiation, possess some of the properties of wave phenomena and some of the properties of particles. For monochromatic radiation, wavelength and the energy of each radiation "particle" ("photon" or "quantum") are related by the equation

$$hc/\lambda = E \tag{1}$$

where h is Planck's constant, c is the speed of light, λ is the wavelength, and E is the photon energy. With numbers substituted for the universal constants h and c, the relation reduces simply to

$$E\lambda = 12.4 \tag{2}$$

with λ expressed in angstroms (10^{-8} cm) and E expressed in kiloelectron volts (kev, an energy unit equal to 1.6×10^{-9} erg). We shall be interested mainly in radiation with photon energies in the range 1 to 50 kev, corresponding to a wavelength range of about 12 to 0.2 A.

Production of X Rays. In an X-ray tube, electrons from the filament are accelerated by the tube voltage and then impinge on the

anode. While colliding with the atoms of the anode, an electron may give rise to X rays in either of two ways. The electron itself may radiate, as it undergoes deflection and energy loss. Alternatively, if it ejects an inner electron from an atom, an X-ray photon may be emitted by an outer electron of the ionized atom as it "drops" into the vacated inner level. In either case the energy of the emitted photon is simply equal to the energy loss of the radiating electron. But in the first case this energy loss may take any value up to the total energy of the incident electron, whereas in the second the loss must be the difference between two fixed energy levels characteristic of the atom. Hence X-ray tube spectra usually resemble Fig. 1, with a "white" spectrum produced directly by free-electron deflections, plus a "line" or lines with wavelengths characteristic of the anode material.

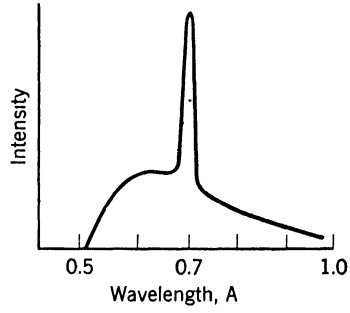

FIG. 1. X-ray tube spectrum, continuous plus characteristic line.

For a full general discussion of X-ray production, the reader may refer to Compton and Allison; [21] for the latest data on "white" spectrum production cf. Ehrlich,[28] Fuchs and Kulenkampff,[30] and Jennings; [32] and for modern work on the intensity of characteristic lines cf. Smick and Kirkpatrick [46] and Braxton et al.[14]

Scattering, Absorption, and Re-emission of X Rays. X rays interact with matter in three ways: scattering with no change in wavelength, scattering with increase in wavelength ("Compton scattering") and photoelectric absorption. For the wavelengths of interest in X-ray spectroscopy the most frequent process is photoelectric absorption, in which a photon is completely absorbed as it ejects an electron from an inner atomic orbit. This process has peak probability for photons that have just enough energy to eject an electron from a particular inner orbit; photons with slightly less energy cannot ionize this orbit and therefore have a much lower absorption probability. Hence photoelectric absorption curves take on the appearance of Fig. 2 (the curve for silver). The single peak in this figure corresponds to the energy of the innermost (K) electrons, and the group of three peaks corresponds to the energies of electrons in the next to innermost (L) shell.

Between peaks the data on photoelectric absorption can be matched fairly well by formulas of the form

$$\sigma = CZ^m\lambda^n \tag{3}$$

where σ is the photoelectric-absorption cross section, C is a constant, and Z is the atomic number of the absorbing element. Empirically m and n are found to be in the neighborhood of 4 and 3, respectively.

When photoelectric absorption occurs, an outer atomic electron must "jump" into the orbit of the ejected electron, emitting a "characteristic" X ray. This X ray is itself sometimes absorbed before it escapes the neighborhood of the parent atom; the escape probability, called the "fluorescent yield," varies from about 0.05 to almost 1, depending on the atom and energy levels involved. The characteristic X rays resulting from photoelectric absorption are referred to alternatively as the "fluorescent," or "secondary" radiation; the pattern of their wavelengths is identical with that of the emission spectra of the elements; and they provide the basis for almost all X-ray spectroscopy work.

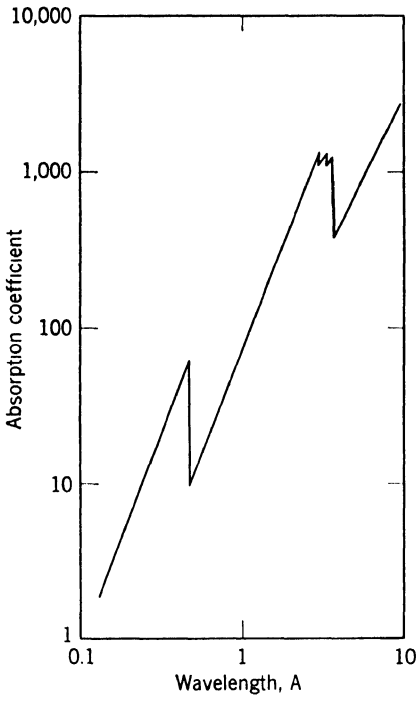

FIG. 2. Absorption curve for silver (log–log plot).

In absorption, an X ray removes an electron from an inner level to the *free state,* but with the corresponding emission an electron starts not from the free state but from another level. Therefore X-ray emission-quantum energies are somewhat less than the corresponding absorption-peak energies, leading to the important fact that elements are relatively *weak* absorbers of their own characteristic X rays.

A recent review of scattering and absorption with a data compilation, is given by White.[51] Victoreen [50] discusses empirical formulas for photoelectric absorption. Allen's table of absorption coefficients, as well as scattering coefficients, covering most elements, are given in Compton and Allison.[21] A new compilation of absorption-peak and emission-quantum energies is given by Fine and Hendee.[29]

Discrimination between X-Ray Lines. The highest resolution between emission lines is provided by diffracting crystals. The spacings between the planes of natural crystals are of the same order as the wavelengths of X-ray emission lines, leading to strong interference maxima in accordance with Bragg's law,

$$n\lambda = 2d \sin \theta \tag{4}$$

where d is the distance between crystal planes, θ is the angle between the diffracting plane and the incident radiation, and n, an integer, is the "order" of the diffraction. Most of the diffracted intensity goes into the first-order line ($n = 1$). Crystals now in use include the alkali halides (NaCl, LiF, KCl, KI, and KBr), quartz, gypsum, mica, aluminum, and ammonium dihydrogen phosphate. With these crystals diffraction work is feasible for elements from magnesium (atomic number $Z = 12$) to uranium ($Z = 92$). As Z increases, the K line wavelengths decrease, and at some Z value in the middle range (the exact value depending on the crystals available) the corresponding θ values become so small that the analyst must start to exploit the L emission lines instead.

Crystals easily resolve the K lines of adjacent elements. The L lines of adjacent elements are usually resolvable, but their pattern is much more complicated, and interferences occasionally occur. Other interferences sometimes occur, between a K line of one element and an L line of another, or involving the second-order diffraction of a line. But, for most problems encountered in practice, crystal resolution is good enough to preclude serious line interferences.

However, the use of crystals usually involves a severe loss of intensity. Although radiation satisfying equation 4 may be diffracted with high efficiency from a good crystal, for a monochromatic point source the diffracted intensity falls off almost to zero when θ is changed by a small fraction of a minute. Therefore, a flat crystal wastes most of the radiation falling on it, as does a curved crystal irradiated by an extended source. Only with a curved crystal and a "point" or "line" source is it possible to have the proper value of θ for all radiation hitting the crystal, and thus to diffract with high efficiency.

X-ray lines may also be distinguished by the use of filters. It is apparent from Fig. 2 that one can strongly select against the shorter of two close wavelengths if there is a filter material with an absorption peak between them. A further refinement is Ross's "balanced-filter" method, using two neighboring elements as filters, with thicknesses

adjusted so that their transmitted spectra coincide for all wavelengths except in the region between their absorption edges. By taking the difference between groups of data obtained with each filter alone, one obtains data pertaining to this intermediate region, often involving substantial contributions from a single element only.

Filters cannot compete with crystals in resolving power or for completeness of rejection of the unwanted lines; also a suitable filter system must be devised for each problem separately and is not always available. But, in situations where intensity difficulties rule out the use of crystals, filters may greatly enhance the resolution of noncrystal methods. This point will be enlarged on in a later section.

Discrimination between X-ray lines is provided also by proportional counters. An X-ray proportional counter consists of a cylindrical shell with a thin window to admit X rays, an insulated thin central wire mounted along the cylinder axis, and a gas filling. A voltage, in the range of 1 to 5 kv, is applied between shell and central wire. When a quantum is absorbed in the gas, the resulting chain of ionizations liberates an electric charge proportional to the absorbed energy, leading to a proportionate electric pulse on the central wire. By using an electronic pulse amplifier and a pulse-height selector, one may count pulses of any desired size, corresponding to particular X-ray lines.

Because of statistical fluctuations in the ionization process, impurities in the fill gas, defects in centering and uniformity of the wire, and occasional incomplete absorption of a quantum's energy, a counter cannot translate quantum energies perfectly. For weak quanta (a few kilo-electron volts or less) resolution is limited by the small number of ions per quantum, leading to large statistical fluctuations in this number. For high-energy quanta (above about 30 kev) many quanta are entirely undetected, or are incompletely absorbed (undergoing scattering instead of photoelectric absorption), so that the counter has low efficiency as well as poor resolution. Even in the optimum energy range between these extremes, counter resolution cannot be good enough to distinguish absolutely between the K lines of adjacent elements; the resolution obtainable in practice is shown in Fig. 3, illustrating the pulse-height spectra obtained with the K lines of adjacent elements. However, with its modest resolution the counter combines good detection efficiency. Used together with a diffracting crystal, it can eliminate interference between overlapping diffraction lines of different orders and quantum energies, and, used with filters,

it can substitute for a crystal where intensity is vital and maximum resolution is not needed.

For more information on crystals the reader may consult Peiser et al.[39] or Klug and Alexander,[34] the latter including a table of the characteristics of different crystal materials. Ross [43] analyzes quanti-

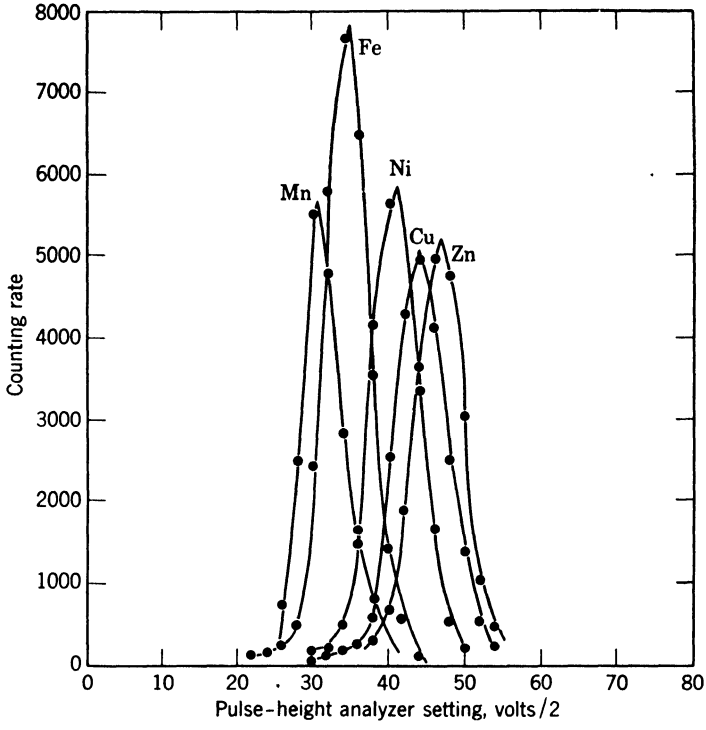

FIG. 3. Pulse spectra from adjacent elements. Fluorescent X rays: 30-mil beryllium window-brass tube, 90% A, 10% CH_4, 2100-volt battery pack, Zn 47 = 8.6 kev, Cu 13.5 = 7.98, Ni 40.5 = 7.43, Fe 34.5 = 6.32, Mn 30.5 = 5.58. (From Bernstein, Brewer, and Rubinson [6])

tatively the merits of filter systems and describes the balanced-filter method. Proportional counters are discussed in Curran and Craggs; [23] the resolution obtained in modern work is described by Bernstein et al.,[6] Miller,[37] and Rowland.[44]

Detection of X Rays. Photographic emulsion was the first X-ray detector. In X-ray spectroscopy, it is more time-consuming than other detectors and cannot be used for accurate quantitative work. It does have the advantage of simplicity, and is used in one of the new instruments described below.

The most usual detector in X-ray spectroscopy is the Geiger counter. This is similar to the proportional counter described above, but is operated with a higher ratio of voltage to gas pressure. Each detected quantum generates an electric discharge or "avalanche," with a pulse size of a few tenths of a volt or more. The pulses all have the

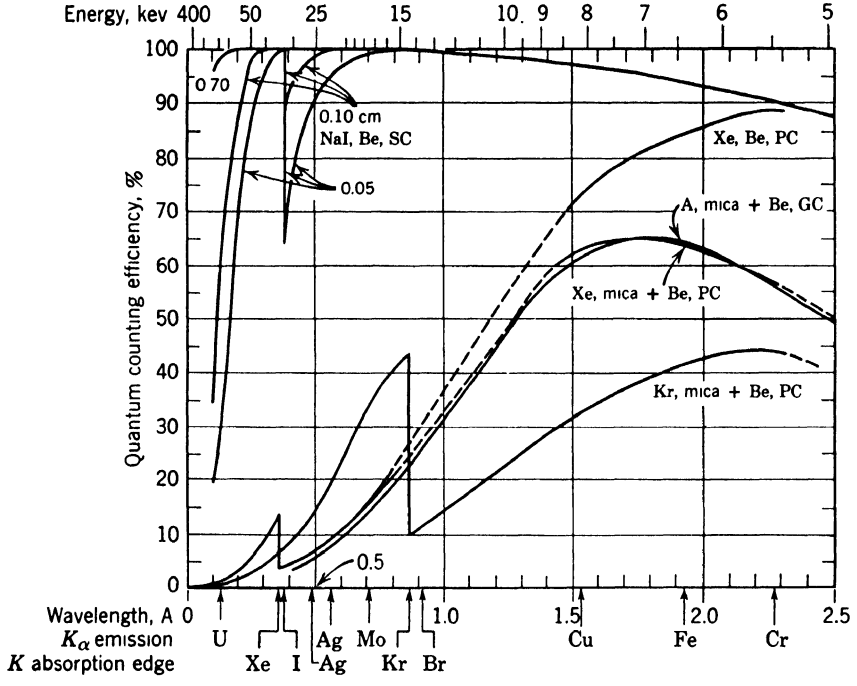

FIG. 4. Calculated counting efficiency of several detectors; SC scintillation counter, PC proportional counter, GC Geiger counter, argon, krypton and xenon gas fillings, beryllium and mica windows, sodium iodide scintillation crystal. (From Taylor and Parrish [49])

same height, regardless of the energy of the initiating quantum, so that the Geiger counter has no discriminating ability. It has the advantages of fairly good counting efficiency (in the neighborhood of 10 to 60%) and simple associated circuits, and the disadvantage of a long "dead time" following each pulse (a few tenths to 1 msec), so that quanta will be missed if the intensity is too high (above about 600 counts per second).

Proportional counters are as efficient as Geiger counters; in addition they have considerable discriminating ability, and a short dead time (around 1 μsec), permitting higher counting rates. Although they

require bulkier and somewhat more refined electronic circuits, they will probably replace Geiger counters in many applications.

Scintillation detectors consist of scintillators, which convert X-ray quanta into photons of visible light, coupled to photomultipliers, which convert the visible photons into photoelectrons and amplify the latter into a sizable electric pulse. The great merit of the scintillation detector is its detection efficiency of nearly unity for all the wavelengths encountered in X-ray spectroscopy up to wavelengths of about 3 or 4 A. However, except for the shorter wavelengths (less than about 0.4 A), the number of photoelectrons per quantum is so small that statistical fluctuations in this number preclude a discriminating ability comparable to that of the proportional counter.

The performances of Geiger, proportional, and scintillation counters as X-ray detectors are extensively analyzed by Taylor and Parrish.[49] Figure 4, taken from their paper, conveys most of the information of interest.

This brief review of X-ray physics should add meaning to the following section on modern methods of X-ray spectroscopy.

SPECIFIC METHODS OF X-RAY SPECTROSCOPY

Analysis of Fluorescent X Rays Using Diffracting Crystals. *Basic Arrangements.* In this section we discuss the most widespread method of X-ray spectroscopy, the only one available in commercial equipment. It utilizes a sealed-off thin-window X-ray tube which irradiates the specimen, an analyzing crystal which separates according to wavelength the fluorescent radiations coming from the specimen, and a detector responding to the radiation leaving the crystal at the proper angle. Crystal and detector are mounted so that the angle θ from the specimen onto the crystal face always equals the angle between the detector and the crystal face; this angle is adjustable so that the wavelengths of various elements are observable in succession, in accordance with equation 4.

In "scanning-type" apparatus, counting rates are plotted as the angle θ is "swept" over a wide range, resulting in data typified by the scan of an alloy shown in Fig. 5.

Figures 6, 7, and 8 illustrate the three basic variations of this method: flat crystal, curved transmitting crystal, and curved reflecting crystal.

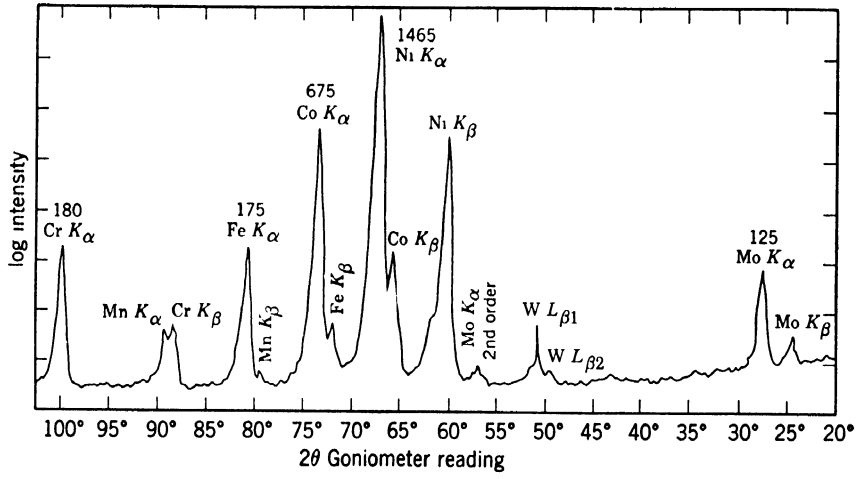

FIG. 5. Chart recording from an X-ray emission spectrograph for a typical high-temperature alloy. (From Liebhafsky [36])

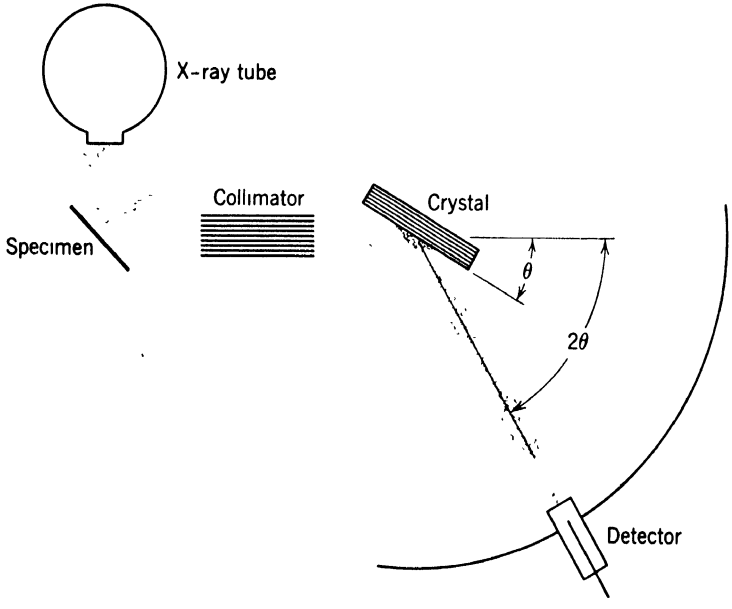

FIG. 6. Flat crystal, reflection method. Polychromatic fluorescent X radiation from specimen is collimated to parallel beam. Crystal analyzes beam by diffracting wavelengths, λ, at angles θ, according to Bragg equation. (From Birks, Brooks, and Friedman [7])

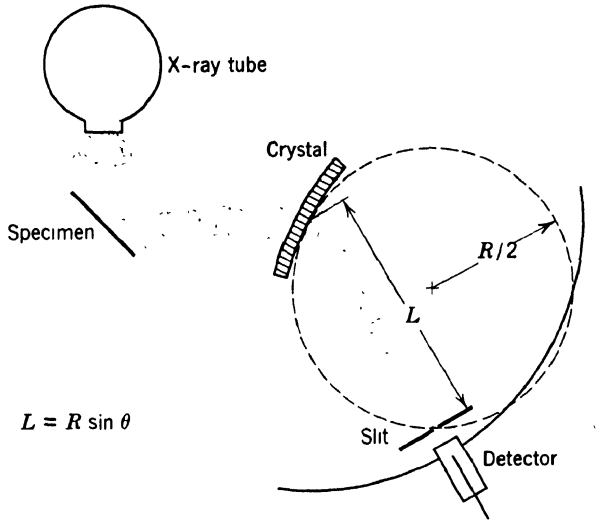

FIG. 7. Bent crystal, transmission method. Crystal, when set at an angle θ, selects radiation of wavelength λ traveling in proper direction so as to satisfy Bragg's equation and focuses it at slit in front of detector. Other wavelengths, if diffracted by some part of crystal, will not, in general, strike detector slit. (From Birks, Brooks, and Friedman [7])

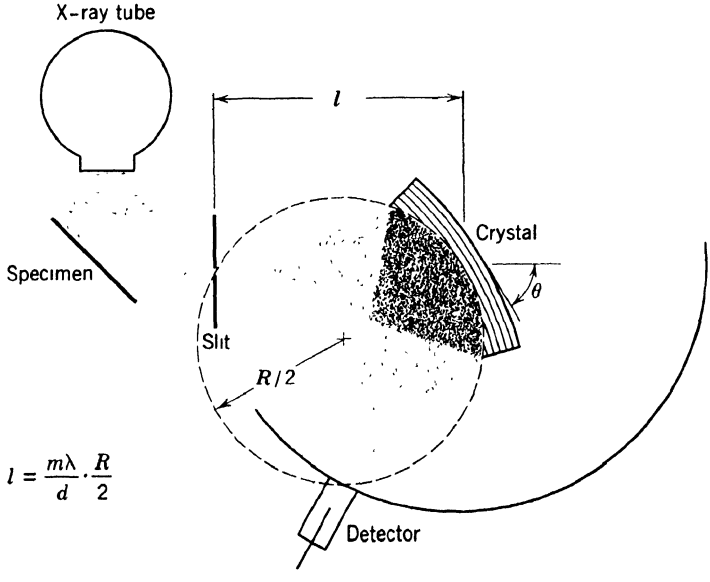

FIG. 8. Bent crystal, reflection method. Radiation of wavelength λ emerging from the effective point source on the focusing circle will be diffracted by crystal set at angle θ and focused near detector. Other wavelengths will not be diffracted. (From Birks, Brooks, and Friedman [7])

Minimum Amounts and Minimum Concentrations. The flat-crystal arrangement has a large advantage in mechanical simplicity and relative ease of crystal alignment.

Of the two curved-crystal systems, existing transmission types give higher resolution (and therefore better minimum measurable concentrations), whereas reflecting types give higher intensities (and therefore better minimum measurable absolute amounts). The curved crystals give higher resolution than the flat-crystal arrangement, but for large-area samples even the curved reflecting systems may not give much higher intensity than flat crystals when the latter are used with ideal collimation (i.e., slit systems rather than tubes).

The geometrical conditions for operation of the curved-crystal systems are compactly described by Birks et al.[7] and will not be repeated here. The same authors have compared the three arrangements quantitatively, with the results shown in Table I. In this table, how-

TABLE I

COMPARISON OF RESULTS WITH DIFFERENT ANALYZER TYPES [7]

Data Taken with Cu $K\alpha$ Radiation and Tungsten Target Tube; All Intensities Normalized to 50-kv 20-ma Operation

Method	Crystal	Order of Diffraction	Slit Size or Collimator	2θ, degrees	Intensity, counts/sec	Line Breadth 2θ, degrees	Relative * Resolution
1. Flat-crystal reflection	NaCl	1	1.8°; 4″ coll.	32–1	7,450	2	24
		1	0.45°; 16″ coll.	32	1,640	0.6	7.2
2. Curved-crystal transmission	Mica	1	0.3° divergence	62	2,100	0.6	3.3
3. Curved-crystal reflection	NaCl	1	0.25°	32	60,000	0.5	6.0
		2	0.25°	66	10,000	1.0	5.3
		2	0.06°	66	600	0.15	0.8

* Line breadth divided by $K\alpha_1 - K\alpha_2$ separation.

ever, the intensities shown for the flat system were not optimum, as tube-type collimation was used.

Speaking generally, the current commercial instruments can measure element concentrations down to about 10^{-3} or 10^{-4} (1 part in 1000 or 10,000) and element amounts down to the range 1 to 10 μg. Probably the lowest concentration measurement reported is a figure of about 10^{-6} for nickel in oil, reported by Hasler.[31]

New Instruments. In addition to the commercial units, two modern instruments built by Birks and his co-workers at the Naval Re-

search Laboratories merit description here. Birks and Brooks [11] describe a reflecting curved-crystal type with three crystal–detector systems mounted simultaneously, to cover a wide range of wavelengths. They stress the compactness of the unit, since the crystal focusing property makes collimators unnecessary. By extracting the trace elements from the samples before analysis and mounting the former on micro-areas or filaments, they have studied some 20 elements in iron, in concentrations down to parts per million and total amounts of the order of 1 to 10 μg, achieving sensitivities of the order of 10 to 40 counts per second per microgram.

A second new instrument of Birks and Brooks [12] is a "miniature fluorescent X-ray spectrograph," using photographic emulsion as a detector. The *thin* edge of a thin flat crystal (about 4 mils thick) is used to diffract the radiation from the specimen; hence each part of the film is at one definite angle with respect to the crystal, and no collimation is necessary. Exposure time is of the order of one hour, as compared with usual running times of a few minutes or less for all the instruments described above, but the device is extremely simple, economical, and compact, being interchangeable with a powder camera on most X-ray diffraction units. The low intensity from the small crystal area limits sensitivity, and ½% concentration (for Mn in Al) is the lowest yet reported for the arrangement.

Besides resolution and sensitivity, other matters of general interest are the range of measurable elements, measurements on small areas, and precautions necessary in specimen preparation.

Range of Elements. In most instruments, the X rays travel in air between the X-ray tube and the detector, and air absorption usually precludes analysis of elements lighter than titanium (element 22, with a $K\alpha$ line at 4.5 kev). However, helium atmosphere attachments are available for the commercial units, extending the range down roughly to sulfur ($Z = 16$). Davis and van Nordstrand [24] have reported that the helium attachment increased counting rate by a factor of about 14 for measurements of calcium in lubricating oils. Element range is extended still further by providing a vacuum path; Birks [8] built such a spectrograph for use from magnesium ($Z = 12$) to titanium. However, at $Z = 12$ special thin detector windows are necessary, and special thin X-ray tube windows are desirable to minimize loss of X rays in the windows.

Measurements of Small Areas. As shown in Fig. 8, cylindrical reflecting crystals have tremendous collecting power for the radiation from line-shaped specimens or small-area specimens properly located

on their focusing circles. Adler [2] considers his spectrograph (of the type of Birks [11]) suitable for measurements on areas down to about ½ mm in diameter; Hasler has quoted a somewhat lower figure for the similar Applied Research Laboratories commercial unit.

Specimen Preparation and "Matrix Effects." Except for specimens that are only a few microns thick, technique of specimen preparation is very important. For thicker specimens a significant fraction of the exciting beam is absorbed before traversing the full thickness; excitation of one element may be significantly increased by fluorescent rays from another element in the specimen, and some of the fluorescent rays of interest may be absorbed in the specimen. Because of these effects, referred to as "matrix effects," the measured intensity is not simply proportional to the amount or concentration of element present. The effects vary with particle sizes and physical states of the sample, so that it is vital that all specimens and calibration samples be prepared in the same way. Within this limitation, successful work has been done with ground or polished specimens, briquettes, powders of various mesh grades, glazed specimens, and liquid materials.

The usual way to correct for matrix effects is to prepare "working curves" from calibration samples of known composition and to read off the concentration of the element of interest in the unknown specimen from the appropriate curve. Mathematical analysis may reduce the requisite number of working curves (cf. Beattie and Brissey [5].) Another widespread method is the "internal reference standard"; to measure an element of atomic number Z one first adds to the specimen a known amount of an absent element of neighboring Z, preferably $Z \pm 1$, which will suffer almost identical matrix effects, and a linear standard is thus established. The internal standard method has been reviewed by Adler.[3]

Other, faster methods of matrix correction are under study, using a scattered beam as a correction monitor,[31] or a specimen segment as a filter between specimen and detector.[45] It is too early to evaluate these tactics. However, matrix effects diminish drastically as the concentrations of heavy constituents diminish, so that specimens with no heavy constituents above the trace level may require no matrix correction, and *dilution* of a specimen in a light material, where permissible, may eliminate the matrix problem.

For applications where inadequate resolution or sensitivity prevents direct examination of a specimen, the X-ray spectrograph may still provide quick reliable data after the trace elements are first removed from the specimen chemically, and then placed in the instrument in

concentrated form. For example, Edholm [27] has reported on the continuing work of Pfeiffer and Zemany at the Schenectady General Electric Laboratory, including the study of trace metals in various acids. After separation the metals are deposited in "spots" on ¼ mil Mylar film, which is studied with a flat-crystal spectrograph. In this way 0.03 μg of iron per gram of hydrofluoric acid, and 0.005 μg of manganese per gram of nitric acid were measured, with the concentration limit of the technique considered to be 0.001 μg of metal per gram of acid. Sensitivity was of the order of 20 counts per second per microgram of metal, and less than 1 μg could be measured conveniently.

The work of Birks and Brooks, with their curved-crystal spectrograph,[11] already described above, also relied on preconcentration. In this case, mercury-cathode electrolytic extraction was used, with the main interest centering on the extraction of niobium, hafnium, tantalum, thorium, and uranium from iron ores. At the same laboratory, Cavanagh [20] has measured the same elements separated in the same way from iron, by mounting them on Mylar and placing them in a flat-crystal spectrograph; he reports that 5 μg of these elements can be determined in the presence of each other to within 10% of the amount present; sensitivities were about 1 to 2 counts per second per microgram.

Detailed Résumé of Reported Measurements. Much of the work in this field has been reviewed by Liebhafsky,[36] in a general review of X-ray methods, and by Birks, Brooks, and Friedman,[7] in their review of fluorescent X-ray spectroscopy. We shall not redescribe this material, but in Table II we summarize mainly later work, representing the most advanced and potent technique. The accent in Table II is on trace measurements, and the huge field of higher concentration measurements is not represented there.

Comparison between Emission and X-Ray Fluorescent Spectroscopy. There are significant differences in the element ranges accessible to X-ray and to emission spectroscopy. For elements like sulfur, tellurium, chlorine, bromine, iodine, and many heavier elements—hafnium, tantalum, thorium and others—X-ray spectroscopy is better, whereas for elements of atomic number less than 16 X-ray spectroscopy is poor, at least at present.

Measurement time is low in both methods, and statistical and instrumental errors are usually not much different. However, emission spectroscopy in many problems extends to lower concentrations than can be reached with the commercial-type X-ray units.

TABLE II

RECENT MEASUREMENT REPORTS

Reference	Element	Matrix	Minimum Concentration, %	Accuracy, % Error	Minimum Amounts, μg	Sensitivity, counts/sec/μg	Running Time, min	Crystal Type
Adler [1]	Th	Rock	0.2				60	Flat reflecting
King [33]	Th	Rock	0.01					Flat reflecting
Campbell [16] *	U & Th	Ore	0.01	10			20	Flat reflecting
Campbell [17]	Ta	NbO_2	0.03					
			0.5	5			<10	Flat reflecting
Campbell [15]	Nb	Ores	0.01					
			0.1	0.03				Flat reflecting
	Ta		0.03					
Birks [8] †	S	Oil	0.5	0.03			5–10	Flat reflecting
Davis [24] ‡	Ba	Oils	0.05	2–3				
	Ca		0.05	2–3			~10	Flat reflecting
	Zn		0.005	1–2				
Dyroff [25]	Fe	Al–Si	0.1	3			15	Flat reflecting
	Ni		0.02					
	V		0.02					
Birks [9]	Fe	Blood		5			2–3	Flat reflecting
Birks [10]	U	Aqueous	0.01	5			½	Flat reflecting
Miller [38]	Sn	Brass	0.01					
	Cd	Brass	0.03					
	Mo	Steel	0.01				~1	Flat reflecting

Beacon [4]	Fe	Glass	0.009				
	Ag		0.02				Flat reflecting
	Sb		0.1				
	As	Glass	0.002				
	Se		0.002				
	Cd		0.01				Curved transmission
	Zn		1.0				
	Ni		14				
	Zr		0.005				
	Sb		0.02	10–20			Flat reflecting
			1.0	2			
Hasler [31]	Ni	Oil	10^{-4}			~2	Curved reflecting
Pish [40]	U & Th	Liquid	0.1			<30	Curved transmission
Lamb [35]	Pb	Gasoline	0.02	3		5–10	Curved transmission
Adler [2]	Zn				~0.1		Curved reflecting
Birks [11] §	Hf, Nb, Ta, Th, U	Originally iron			~1	~40	Curved reflecting
Cavanagh [20] §	Hf, Nb, Ta, Th, U	Originally iron		~10	~5	1–2	Flat reflecting
Edholm [27] §	Fe, Cr, Mn	Originally acid	10^{-7}		~1	~15	Flat reflecting

* Combined radiometric and spectrographic method.
† Vacuum path.
‡ Helium path.
§ Trace element chemically separated before measurement.

X-ray spectroscopy is nondestructive for the specimen, while emission spectroscopy consumes most specimens wholly or in part.

Analysis of Fluorescent X Rays with Image Formation. Figure 9 shows schematically a unique instrument built by von Hamos [31a] and called a "microanalyzer camera." *A* and *B* represent chemically different parts of a specimen, subjected to intense X radiation (labeled λ_1). With the crystal *C* located as shown, the fluorescent rays from the specimen will be reflected to form images of the specimen in accordance with the laws of geometrical optics. But in addition each

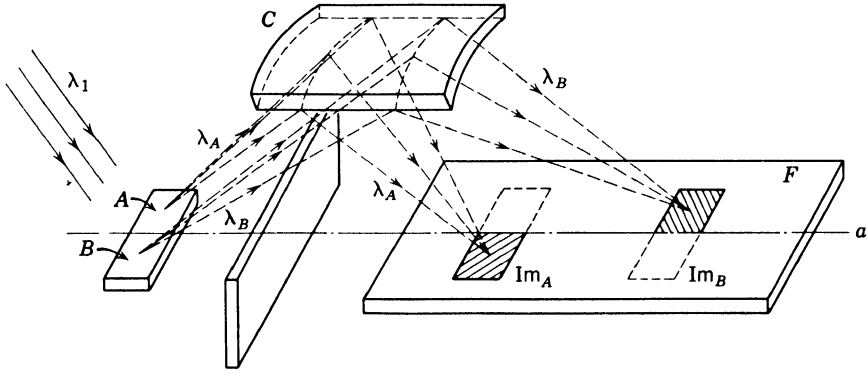

FIG. 9. One arrangement of the microanalyzer camera. (From von Hamos [31a])

element, having its own wavelength, must have it own angle of incidence and reflection in the crystal, as determined by equation 4. Thus one obtains on the film *F* a series of images of the specimen, each image representing the distribution of one element.

A very high intensity of X rays is needed at the specimen to avoid excessive exposure times, and therefore the instrument includes a special X-ray tube permitting the mounting of the specimen close to the tube anode.

Exposure times run of the order of one hour. Film is the optimum detector in this case, since the photographic image records simultaneously information about all parts of the specimen surface; a Geiger tube or similar detector operated with an aperture as small as the resolution of the film would take about an hour *at each point* to collect the same information. By calibrating the film and using a microdensitometer, one can convert the photographic densities into quantitative line intensities with an error of 10% or less.

The instrument can play a unique role in topographical microanalysis. Total specimen areas run 0.2 to 0.4 mm^2. The sensitivity

may be judged from the following datum: with 2×10^{-8} gram of copper concentrated on a surface of 0.01 mm^2, an exposure of 1 hour gives an emulsion density of 0.12 (a quite usable density) on Osray film. The spatial resolution obtainable depends on the grain size of the film, and the size of the densitometer probe spot, as well as on the geometry used and the quality of the crystal. But in general resolution of the order of 100 microns or somewhat better can be obtained.

The device is used generally on alloys, to explore the microdistribution of components in concentrations of 1% or more. In most alloys, such components in lower concentrations would presumably be obscured by background effects. However, lower concentrations of microcomponents could be investigated in lighter matrices, and the minimum measurable concentration would then have to be assessed case by case.

The microanalyzer camera is the only instrument giving a quantitative *picture* of the distribution of each element in a specimen.

Analysis of X Rays Excited by Electron Beams. In one of the earliest devices for X-ray analysis the specimens were placed inside a demountable X-ray tube in the anode position, and the characteristic X-ray lines were excited by electron bombardment. (Cf. Eddy and Laby.[26]) This approach yields very large intensities and high sensitivities, but demountable pumped-on X-ray tubes are inconvenient to use, and many specimens would be destroyed by the heat of the tube or by the electron beam, or else would interfere with tube operation by partial vaporization.

These difficulties have been largely overcome in a modern device built by Castaing and Guinier [18] and illustrated in Fig. 10. A slightly modified electron microscope focuses an electron beam on the surface of the specimen, the irradiated area being only 1 or 2 square microns. An optical microscope is provided so that one can see the area under analysis and choose an interesting field. Characteristic radiation from the specimen goes into a reflecting curved crystal spectrometer, and the rest of the instrument is similar to those described on page 466.

Because of the very small spot size, one obtains a very high surface density of excited characteristic radiation with the generation of almost no heat, and the curved crystal handles the radiation from the "point" source with a high efficiency. As pointed out by Cosslett,[22] for many materials heat dissipation as high as 10^7 watts per square centimeter can be tolerated in a 1-micron spot. Thus the limitations described above are substantially overcome.

It should be noted that the electron beam penetrates the specimens to a depth of only a fraction of a micron, so that the instrument carries out surface analysis, or else volume analysis for homogeneous material. Obviously, "matrix effects" occur, similar to those described on page

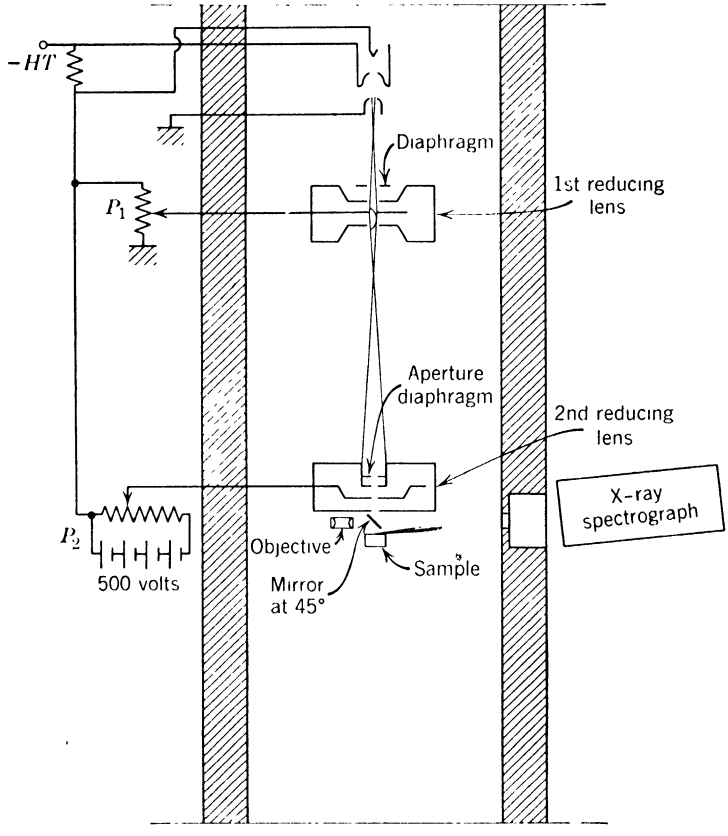

FIG. 10. Diagram of microanalyzer. (From Castaing and Guinier [18]) $-HT$ = minus high voltage.

472. In a recent paper Castaing and Descamps [19] have shown how to take account of matrix effects in quantitative work.

In metallographic alloys this instrument can measure the element content of a volume of about 1 cubic micron, with a surface area of a few square microns. The spatial resolution is about 2 microns. The minimum measurable amount of any one element is about 10^{-11} gram. Hence, a *trace* element in the minimum volume of 1 cubic micron could not be measured because there is not enough of the element present.

The instrument is used for studies of point-to-point variations in the composition of alloys, and with its high spatial resolution and high sensitivity it seems to be outstanding for this purpose. Figure 11 shows such an application: the point-to-point variation in zinc content in a diffusion region in a sample of brass.

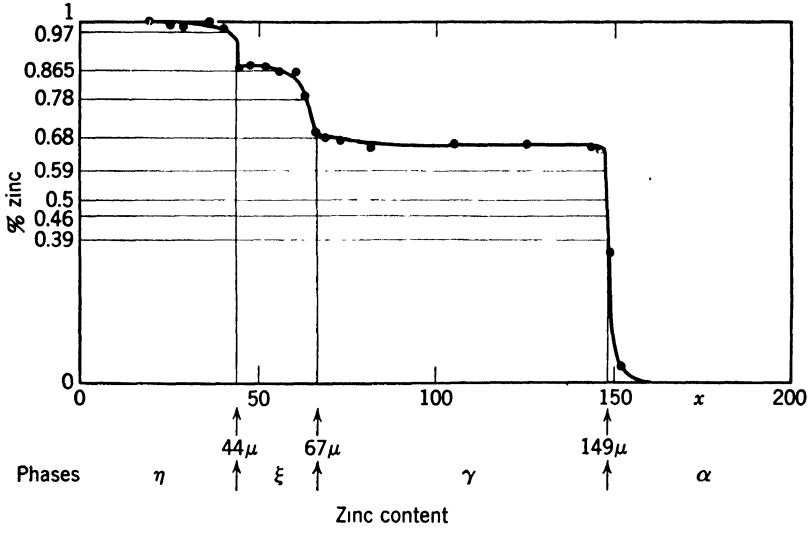

FIG. 11. Variation of composition of copper–zinc alloy in region of diffusion. (From Castaing and Guinier [18])

Analysis of Fluorescent X Rays with no Diffracting Crystals. The instrument described in this section was built for one purpose: the measurement of trace elements in individual biological tissue sections. The goal is to make the measurements on the very same sections studied by the pathologist under the microscope (sections about 10 microns thick and of the order of $\frac{1}{10}$ cm^2 in area).

The apparatus has been built by me and is now being tested. It has not yet been applied to biological materials, but estimates of its expected performance will be reviewed briefly here.

The elements of primary interest are the "micronutrients": copper, iron, and zinc [present in biological material in concentrations of 10^{-4} to 10^{-6} gram per gram (or cm^3) of tissue] and cobalt [in concentrations less than 10^{-6} and probably beyond our instrument's scope]. Sulfur, phosphorus, calcium, and magnesium (concentrations of the order of 10^{-2}) are also of interest, as well as many possible metabolites (manganese, aluminum, molybdenum, and others) and antimetabolites or poisons (nickel, arsenic, cadmium, and heavier

metals like lead). For elements in the last two groups, we can only hope that their concentrations are as high as, say, 10^{-6} in tissues where they play a biological role.

Since the specimens have a total volume of 10^{-3} to 10^{-4} cm^3, and the elements of main interest are in concentrations of 10^{-5} to 10^{-6}, each specimen will usually contain only 10^{-9} gram or less of the element of interest. Commercial-type units (page 467) cannot handle such small amounts. Castaing's microspot electron-beam analyzer (page 477) is unsuitable because of the shallow penetration of the electron beam and the relatively large surface area of the specimens.

But alongside the severe problems posed by low amounts and concentrations, biological tissue sections offer the analyst special simplifications. In any one problem, only a few trace elements are present in significant amounts, namely the micronutrients listed above and perhaps one or two other elements of special interest. The sections are so thin that "matrix effects" are unimportant—observed line intensities are directly proportional to the amounts present. And the matrix itself is very light, consisting mainly of carbon, nitrogen, and oxygen of a density around 0.2 gram per cubic centimeter for dried tissue sections, so that background intensities are very much less than in most specimens. These factors simplify the resolution problem so strongly that diffracting-crystal resolution may not be needed, while intensities and sensitivities are tremendously increased if a crystal is not used. This reasoning led to the construction of the apparatus diagrammed in Fig. 12.

The X-ray tube is a demountable home-made unit, with a ring-shaped filament and a saucer-shaped anode presenting several square inches of "target area." Efficient cooling makes feasible plate currents up to 500 ma at least. On the axis of the tube a secondary radiator is mounted, the choice of radiator material depending on the element of interest in the specimen. The output of the tube consists essentially of the characteristic radiation from the radiator, excited by the X rays from the anode. (Rogers [41,42] and Splettstosser and Seemann [47,48] have reported on the "purity" of the spectra produced in this way.)

The fluorescent radiation excited in the specimen by the tube output is detected in a nearby proportional counter, which is connected to pulse-height analysis circuits. For discrimination between different X-ray lines, one relies on the proportional counter, and on filters placed between specimen and counter and between tube and specimen.

Because diffraction is not used, all components may be kept close together, giving large solid angles and high intensities. In calibration tests to date, the arrangement has shown a sensitivity of about 10^{11} counts per second per gram of trace element for a tube current of 500 ma. This shows that, so far as *absolute amounts* are concerned, trace measurements in individual tissue sections seem feasible.

The remaining question mark involves minimum concentrations. By choosing a suitable radiator, one can avoid exciting fluorescence

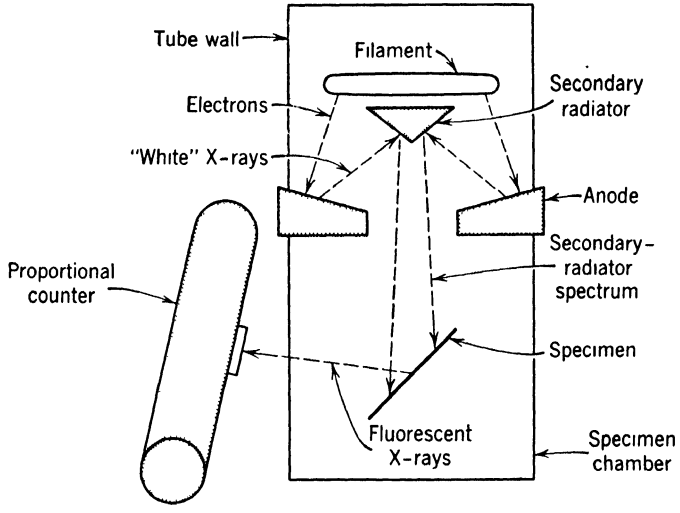

FIG. 12. Arrangement for fluorescent X-ray measurement in biological material (schematic).

in elements heavier than Z, the element of interest. The proportional counter can discriminate effectively against counting radiation from lighter elements, although in general a straightforward correction will be needed to correct for the contribution from element $(Z - 1)$. Minimum measurable concentrations will be limited primarily by the *electromagnetic scattering* (coherent plus incoherent) of the radiator's characteristic lines by the specimen as a whole, since the proportional counter cannot discriminate completely between these lines and the fluorescent lines from the element of interest.

However, after matching the radiator to the absorption peak of the element of interest, one can expect the fluorescent scattering cross section of the element of interest to exceed the electromagnetic scattering cross section of the tissue by a factor of about 10^3. The proportional

counter can add a discrimination factor of perhaps 10 or more. The remaining necessary resolution must be obtained by a filter placed between specimen and counter. Fortunately, in general element $(Z - 1)$ can serve as an excellent filter, as it will absorb radiation from element Z quite poorly and will absorb the radiator wavelength quite well. We expect that at some cost to intensity (a factor of 10 or less) filters will extend the minimum measurable concentrations *in biological tissues* to about 10^{-6}. (Even apart from their intensity reduction, filters cannot be exploited indefinitely because of the excitation of fluorescent radiation in the filter itself.)

Table III illustrates feasible choices for secondary radiators and for filters in the study of some elements of biological interest.

TABLE III

SUITABLE SECONDARY RADIATORS AND FILTERS FOR STUDY OF CERTAIN ELEMENTS OF INTEREST

Element of Interest	Absorption Edge, kev	Main * Emission Line	Secondary Radiator	Main * Emission Line	Filter †	Absorption Edge
Mn	6.54	5.90	Co	6.93	Cr	5.99
Fe	7.11	6.40	Cu	8.05	Mn	6.54
Co	7.71	6.93	Cu	8.05	Fe	7.11
Ni	8.33	7.48	Zn	8.64	Co	7.71
Cu	8.98	8.05	Pt	9.44	Ni	8.33
Zn	9.66	8.64	Au	9.71	Cu	8.98

* For platinum and gold the $L\alpha_1$ emission lines are listed; other emission line listings are the $K\alpha_1$ lines.

† The filter, placed between specimen and counter, is to suppress scattered secondary-radiator quanta and fluorescence excited in heavier elements by the $K\beta$ line of the secondary radiator. The secondary-radiator $K\beta$ line can be suppressed also by a filter between tube and specimen.

The instrument described here is obviously inconvenient. Each element of interest poses a separate problem in choices of radiators and filters and in possible background interferences, and the tube must be opened to change radiators. Many otherwise ideal radiator elements would vaporize in the heat of the tube, so that ideal radiator wavelengths are not always available. But no other apparatus offers the possibility of getting the same information about biological tissue sections.

Besides measurements on intact tissue sections, the apparatus offers very high sensitivity for the measurement of trace elements *after* they

have been chemically separated from their matrices. In such cases, the struggle to measure low concentrations is bypassed, and the full sensitivity of the device (with no filters) is available.

Microradiography. By taking a microradiograph with radiation matching an absorption peak of an element of interest, one can study the element's distribution in a thin specimen. Because the method is based on X-ray spectra, it comes under the heading of "X-ray spectroscopy," but as it is the subject of another chapter by Dr. Engström, we shall not discuss it extensively here.

One cannot measure "trace" concentrations by radiography, because trace constituents can contribute only negligibly to a specimen's total absorption. However, with fine-grained emulsions radiographs with one-micron resolution can be obtained; hence one may detect very small spots of high concentration of an element with low *average* concentration. In this way inclusion spots containing of the order of 10^{-12} gram of the element of interest may be localizable.

Chemical-Bond Analysis. Since X-ray spectra reflect the energy levels of inner atomic orbits and chemical forces arise mainly from the interactions of outer electrons, X-ray spectra are virtually unaffected by an element's state of chemical combination. However, chemical state does affect X-ray spectra slightly, producing small wavelength shifts and changes in the relative intensities of spectral lines, and these effects can be correlated with an element's valence. Thus, one can establish the valence of an element in a compound by comparing its X-ray spectrum with the spectra obtained for the same element in compounds where its valence is known.

Several examples of this method are listed in Liebhafsky's review.[36] In later work, Boehm et al.[13] established the triple valence of cobalt in vitamin B_{12} (a molecule containing 4.5% of cobalt by weight). Notably, the X-ray observations were fully nondestructive, to the extent of producing no observable effect on the biological activity of the irradiated material.

Comparative Table of Quantitative X-Ray Methods. Table IV indicates the scope of the different X-ray methods discussed above. The reader should keep in mind that the figures usually give optimum performance, which may not be available for all elements or all types of specimens. In some cases performance figures depend so strongly on particular circumstances that generalizations would be misleading; the corresponding spaces have been left blank.

TABLE IV

CAPABILITIES OF DIFFERENT APPROACHES TO X-RAY SPECTROSCOPY *

Method	Matrix	Measurable Elements	Minimum Amounts, g	Minimum Concentrations, % Weight	Minimum Volumes, cm^3	Instrument Status
Fluorescence, crystal analysis (p. 467)	Heavy	$13 \leqq Z \leqq 92$ †		0.01–0.001	$\sim 10^{-5}$	Commercial
	Light (oil, etc.)	$13 \leqq Z \leqq 92$		10^{-4}		
	None ‡	$13 \leqq Z \leqq 92$	$\sim 10^{-6}$			
Fluorescence, image formation by crystal (p. 476)	Heavy solid	$22 \leqq Z \leqq 92$	10^{-8}	1	10^{-8}	Custom-built
Electron excitation, crystal analysis (p. 477)	Heavy or light solid	$22 \leqq Z \leqq 92$	10^{-11}	1–10 §	10^{-12}	Custom-built ¶
Fluorescence, noncrystal analysis (p. 479)	Biological tissue section	$13 \leqq Z \leqq 92$	10^{-11}	10^{-4}? ‖		Custom-built
Microradiography (p. 483)	Biological tissue section	C, N, O, Ca (in bone), P, S	10^{-12}	1–10	10^{-11}	Custom-built

* The tabulated figures are optimum. It must not be assumed for any method that the performance figures are all attainable simultaneously in any given case, or that they apply to the full range of elements.

† Helium or vacuum path attachment needed for $Z < 22$.

‡ Trace element chemically separated before measurement.

§ With lower concentrations not enough *amount* of trace element would be in the electron microspot. Intrinsic concentration limit is of the order of 0.01%.

‖ This limit is not yet established experimentally.

¶ Commercial production of this instrument is now contemplated.

PROSPECTS FOR FUTURE DEVELOPMENTS

Major advances are possible in the X-ray tubes used for fluorescence spectroscopy. Almost all the tubes in current use were designed for X-ray diffraction work. We may expect tubes of higher power and shorter anode-to-specimen distances, increasing intensity at the specimens and reducing minimum measurable amounts by a factor of ten or more. Also, thin-window tubes are planned, to increase intensity and sensitivities in the atomic-number range aluminum (13) to titanium (22).

To a considerable extent, intensity can be traded for resolution, by reducing the openings of collimators and defining apertures. Thus more powerful tubes should lead generally to proportionate decreases in minimum measurable concentrations as well.

In more recent years there has been a steady appearance of new crystal materials with ever-higher reflecting powers, and presumably crystal intensity gains from a few up to tenfold are still to be harvested.

In the detector field, we may expect more widespread use of proportional and scintillation counters with pulse-height analysis circuits. This will improve resolution somewhat but cannot have a radical effect on performance figures. If there existed an X-ray detector like the proportional counter but with really good resolution, comparable to a crystal's, a tremendous advance would be scored, but there is no prospect for the creation of such a detector.

To sum up, future improvements of tenfold or better in intensity and resolution seem reasonable for the commercial-type units described on page 467. As for the special instruments (pages 470–471), models to date are essentially prototypes, and undoubtedly extensive engineering improvements are still to be exploited.

DISCUSSION

Discussion Leader
David C. Miller
North American Philips Company

I find it somewhat difficult to add much to Dr. Hall's excellent chapter since he has done such a careful and comprehensive job of covering the subject of X-ray spectroscopy as applied to the problem

of the detection of trace elements. I had originally planned to spend some time on discussing the capabilities of the commercial equipment available, but, in reviewing Dr. Hall's chapter, it seems that he has done quite a complete job on that score and there is really not a great deal more to be said.

However, since my interests are principally in the development of new instrumentation, I will take this opportunity to enlarge upon, and reiterate perhaps, some of the things that Dr. Hall mentioned in the last section of his chapter concerning the future developments to be expected in this rapidly expanding field.

It is obvious that we are making every effort to extend the sensitivity, increase the accuracy, and speed up the operation of the equipment, but, to be more specific on how these general specifications can be met, we must consider briefly the principal components of the fluorescent analysis equipment and try to forecast what changes they will undergo within the foreseeable future.

As Dr. Hall has already told you, the field of X-ray tubes is perhaps the most fertile one for improvement in this type of instrumentation. Most X-ray tubes which we have inherited for work with X-ray spectroscopy are converted either from medical X-ray tubes or from diffraction apparatus. It is obvious that increased sensitivity can be obtained through the successive application of more and more power, but there is a limit to which this is feasible in commercial equipment. The initial cost and operating costs of very large power supplies can be an important deterrent in the commercial use of X-ray spectrographic equipment. Recently we have introduced an X-ray tube operating up to 100 kv and up to 3½ kw on a full-wave rectified supply, but this seems to us to be about as far as we want to go in increasing the power of our exciting tubes until we have thoroughly explored every possible means for increasing the efficiency of these tubes.

A variety of methods of increasing the efficiency are still to be fully explored. It has been mentioned that the radiation in a band of two to three tenths of an angstrom shorter than the radiation to be excited provides most of the efficient excitation. Therefore, in most instances, the efficient excitation represents only a small fraction of the total radiation that is used. More flexibility in the choice of target materials may offer increased efficiency for the excitation of particular wavelengths. It may be possible to do this to a limiting extent with a sealed-off X-ray tube, but thought must also be given to the feasibility

of using the continuously pumped type of X-ray tube in which practically any kind of target could be used.

As Dr. Hall has also mentioned, more efficient excitation will result if the anode-to-specimen distance can be reduced, since we are dealing with square law relationships and even a small reduction in this distance will result in a substantial intensity gain. X-ray tubes in which the anode and the window are the same have been tried with indifferent success, but, if the cooling problem for the window target could be solved, this type of tube would be a substantial improvement over the type of X-ray tubes that are now generally used.

It is also possible that, if a continuously pumped X-ray tube were to be acceptable, certain types of samples could be mounted directly on the target of the X-ray tube and excited by primary excitation rather than by secondary radiation. This was the first FA method ever used, and, although it suffers from the disadvantage of having the white continuum as a background, there is some evidence that substantial gains in peak-to-background ratio could be achieved.

A third method of increasing X-ray tube efficiency is of principal application in the case of analyses requiring excitation of elements whose atomic numbers lie between number 12 which is magnesium and number 22 which is titanium. In this region, very substantial gains in sensitivity can be achieved through the use of thinner windows or no windows at all in the X-ray tube. Though it is now possible to obtain a counting rate of the order of 6000 counts per second of Al $K\alpha$ radiation from a pure aluminum sample with our standard commercial equipment, this is being done as a result of primary excitation from an X-ray tube whose window cuts off for all practical considerations all radiation of longer wavelength than 3.5 A. Since aluminum radiation is of the order of 8.3 A, we can see that the efficiency of excitation of aluminum radiation with this relatively hard primary excitation must of necessity be extremely low. If the cutoff edge of the X-ray tube window can be increased to 8 or 10 A, it should improve the sensitivity of the X-ray spectrographic method in the region around aluminum radiation by a factor of at least 100.

In addition to increasing the efficiency of the X-ray tube, we must also pay close attention to the purity of the resultant X radiation. Any impurities in the target or tube body may result, by scattering off the sample surface, in contaminating radiations which may lead us to a false assumption concerning the elements present in the sample itself. A great deal of work has been done on this problem, and the

latest X-ray tubes that we are providing commercially have strict specifications concerning the allowable levels of radiation impurities.

A second class of components, if it can so be called, which offers promise for increase in sensitivity for trace elements is the X-ray optical arrangement. One new arrangement is the use of curved-crystal optics. Dr. Hall has outlined the work being done with equipment of this sort, but I should like to add a few words. When a sample can be most conveniently prepared in cylindrical form where the diameter of the cylinder is of the order of a millimeter or less, there is enough evidence at hand to indicate that appreciable increases in intensity can be achieved using a curved-crystal in reflection over what could be achieved for the same tiny sample when viewed by conventional flat-crystal optics. The full achievement of the potential capacity of the curved-crystal optical system, however, faces several very important difficulties. First, the bending and grinding of the curved crystal is a precision job of the highest order and can be achieved with a select few types of crystals. The assessment of efficient operation of a curved-crystal instrument is difficult since, even with a poorly prepared crystal, a certain amount of focusing action will occur. If the ultimate sensitivity of this method is to be achieved, extreme care must be taken in preparing the crystal.

Second, the adjustment of the slit system is also a complicated and exacting job for the ultimate end performance. Unless great care is taken in the alignment of the slits and the crystal as an optical system, much of the potential advantage of the curved-crystal method is lost.

Third, the relative motion of the mechanical parts of the goniometer requires great ingenuity to avoid backlash and remain within the focusing tolerances.

Finally, it is probably pertinent to point out, as Dr. Hall has in his chapter, that Dr. Liebhafsky at the General Electric Research Laboratory, working with samples spread out on Mylar film, has achieved the same limits of detectability for small samples using flat-crystal optics as has been quoted in the literature by anyone using the curved-crystal methods.

In addition to the curved-crystal optics, there has been great interest in the use of nondispersive optics with energy-sensitive detectors. As has been previously mentioned, when the crystal analyzer can be left out of the system, we get a substantial increase in intensity, and, when a combination of filters, proportional detectors, and kilovolt discrimination can be applied to a problem, sensitivity substantially greater

than that now achieved with commercial instruments is quite feasible.

This leads us quite naturally to the third class of components which I wanted to mention: namely, the X-ray detectors. A new frontier in X-ray detection has been opened up by the proportional- and scintillation-counter detectors whose greater speed and energy-sensitive characteristics have already found many applications. The principal difficulty in the use of the proportional-type detector in a nondispersive setup is admittedly the problem of resolution. However, it is possible that the application of improved analytical techniques to the data obtained can be used to overcome this problem to some degree. As yet there are no detectors commercially available, to the best of my knowledge, which have been designed principally for work with nondispersive optics in the X-ray field, but I suspect that the situation may be remedied in the relatively near future.

In addition to the improvements that have been suggested in the conventional types of FA equipment, there are also with us the possibilities of the use of radioisotopes as exciting sources rather than X-ray tubes. For trace work over most of the energy spectrum, these sources, at the present time at least, do not seem to offer much competition to X-ray tubes. However, some work has been done, both by Dr. Friedman of NRL and Mr. Hendee of Philips Laboratories on the use of alpha particles to excite very soft radiations, again in the range of atomic numbers from 12 to 18, which have proved extremely interesting. Because the X-ray excitation is so inefficient at these soft wavelengths, and since the alpha particles, say from a polonium source, are very efficient exciters, reasonably sized polonium sources can excite, in air, aluminum radiation intensities comparable with that achieved from the best X-ray equipment now commercially available. A practical instrument using the alpha-particle source has not yet been achieved, but very careful attention is being paid to this possibility.

It also seems obvious, in listening to Dr. Hall's talk, that the marriage of X-ray equipment and electron-optical equipment is under way and that the results will be of considerable interest to all instrumentation people. The Castaing instrument described by Dr. Hall is a perfect example of the combination of X-ray and electron-optical techniques and provides a tool of great power for nondestructive analysis of very small areas. Similar instruments are being worked on in various places in the United States, and in the next few years I am sure we will see commercial instruments of this type available.

In conclusion, I fully agree with Dr. Hall's sentiments concerning

the degree of improvement in sensitivity and resolution that the X-ray method will achieve within the next few years and feel fairly sure that, like most technical forecasts, this will prove to be conservative when we look back upon it five years from now.

QUESTIONS AND ANSWERS

J. T. HORECZY (*Humble Oil & Refining Co.*): Dr. Hall, at the beginning of your talk, you mentioned someone was able to get one part of nickel in ten million. I would like to know who that was.

Also, I would like to know if the proportional-counting tubes are commercially available.

THEODORE HALL: That concentration figure was reported by Dr. Hasler of Applied Research Laboratories at the August 1955 Denver X-Ray Symposium. It is indeed remarkably low but I believe it is quoted correctly. The calibration curves in his paper show that concentrations in the range 10^{-6} to 10^{-7} should be measurable. Perhaps one of the people from Applied Research Laboratories in the audience could say more about this measurement.

W. E. DAVIS (*Applied Research Laboratories*): I think the figure is correct. I do not remember exactly. I want to point out that this is a special instrument designed for this purpose only.

THEODORE HALL: Was this the large ARL multiple-curved-crystal spectrometer that was used?

W. E. DAVIS: It could be considered as such but is special in that the sample is placed closer to the tube.

THEODORE HALL: As to the production of proportional counters, like many workers in the field, I have always made my own. But I think you can get counters suitable for soft X-ray work from North American Philips or General Electric.

There is one simplifying feature in making proportional counters for soft X-ray work. In general it is a difficult engineering job to make a good maximum-resolution counter. The thin central wire must be very uniform and precisely centered, and the gas filling must be quite free of contaminants.

But with soft X rays, where each quantum does not produce very many primary ion pairs, resolution is determined mainly by statistical fluctuations in the number of ion pairs per quantum, and the construction factors do not have to be so carefully controlled.

As to construction, a proportional counter consists essentially of a cylinder with an insulated wire down the middle and a gas filling.

J. T. HORECZY: Will you tell us about the best resolution you are getting now?

THEODORE HALL: Sixteen per cent full width at half height, which is somewhat better than the curves on the slides I showed, is the best resolution we are getting now.

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X-Ray Micrography

Analysis of elements by microtechniques utilizing absorption emission and scattering of X rays

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ABSTRACT Recent developments in microradiographic techniques permit the registration of X-ray absorption images of tissue and cell components less than one micron in diameter. The aim of the quantitative microradiographic procedures is to analyze as small a volume as possible in order to get analytical data on "homogeneous" functioning units. The type of radiation used in X-ray micrography can be varied in order to determine not only various elements but also certain compounds and total weight. When the samples, which have weight in the order of 10^{-10} to 10^{-14} gram, are analyzed, it is natural that the elements looked for must have a certain minimal concentration in order to be detected. This problem is discussed and weighed against the fact that, when we analyze small localized areas, it often happens that what is regarded as a trace element, judged from over-all analyses, in the very small localized areas has a relatively high concentration.

The last-mentioned aspect is also discussed from the point of view of preliminary experiments to use micro X-ray fluorescence and micro X-ray diffraction techniques.

INTRODUCTION

The interaction between X rays and materia, encompassing the phenomena of absorption, scattering, and the production of characteristic X rays can be predicted in a simple way. Analytical methods based on these phenomena, therefore, have found a wide application to various research fields. The present chapter aims to give some information about how such X-ray methods can be adapted to microanalysis of tissues and cells. Special interest is focused on the problem of the determination of elements of higher atomic numbers.

In biology and medicine the concept of a "trace metal" has been founded on analysis of relatively large pieces of tissues, and the concentration of a trace metal is usually given in parts per million or similar. The trend of modern micro- and histochemistry, however, is to analyze such small units of biological material that they can be considered homogeneous from a histophysiological point of view. One sometimes then finds that a trace metal occurs in a comparatively large concentration in a particular microscopic area, and as an extreme example we can consider the sample of molecular size, where the local concentration of the reacting substances of course is high.

This survey, far from complete, will indicate the possibilities of the application of micro X-ray methods to the analysis of elements other than H, C, N, and O in tissues. The analysis of C, N, and O by X-ray methods has been dealt with elsewhere, and the methods are still under development.

X-RAY ABSORPTIOMETRY FOR ELEMENTARY ANALYSIS ON THE MICRO- AND CYTOCHEMICAL LEVEL

When X-ray absorptiometry is applied to the determination of elements, the size of the characteristic absorption discontinuities is evaluated. The position in the X-ray absorption spectrum of the absorption discontinuities (absorption jumps or edges) is characteristic for each element and for practical purposes independent of the chemical combination of the element. It is true that the valence electrons influence the wavelength of the absorption edges to a minor extent and also that there is a fine structure on the sides of the edges. For practical analytical purposes, however, those effects can be neglected, and, for example, the position of the sulfur absorption edge can be

considered to have the same wavelength in elemental sulfur and in sulfur in sulfate or organic binding.

There are several absorption edges for each element, and the one with the shortest wavelength is called the K edge. At longer wave-

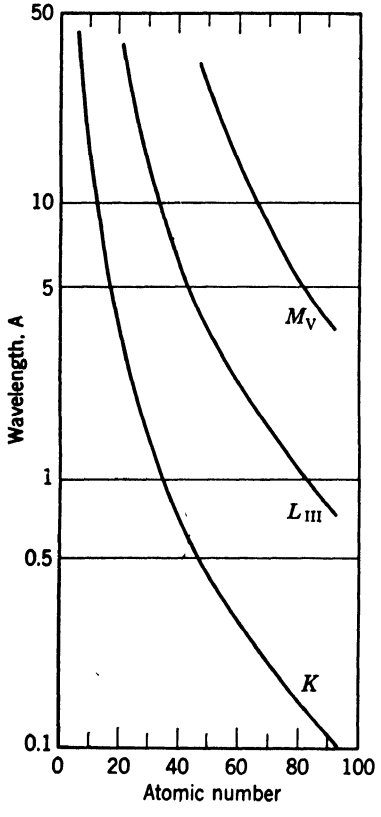

FIG. 1. The wavelengths of the K, L_{III}, and M_V absorption edges as function of wavelength.

lengths follow the three L edges (L_I, L_{II} and L_{III}) and at still longer wavelengths the five M edges, etc. Figure 1 shows diagrammatically the wavelengths for the K, L_{III}, and M_V edges as function of the atomic number of the elements. For example, the K edge for nitrogen (atomic number 7) has the wavelength 31.0 A, and that of iodine (atomic number 53), 0.37 A.

Suppose an element in the amount x grams per square centimeter is to be determined in a mixture of other elements, the total amount of which is q grams per square centimeter. Referring to Fig. 2, we

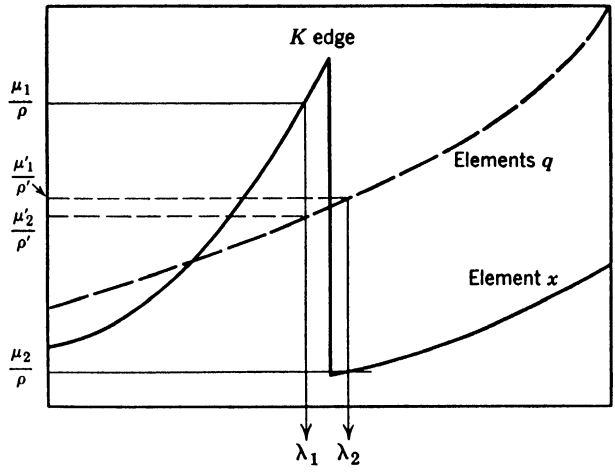

FIG. 2. Schematic representation of a K absorption edge. The symbols are found in equations 1*a* and 1*b*.

can write the absorption of monochromatic X rays on either side of the edge in the following way:

$$I_{11} = I_{01} \cdot \exp - \left(\frac{\mu_1}{\rho} \cdot x + \frac{\mu'_1}{\rho'} \cdot q \right) \tag{1a}$$

$$I_{12} = I_{02} \cdot \exp - \left(\frac{\mu_2}{\rho} \cdot x + \frac{\mu'_2}{\rho'} \cdot q \right) \tag{1b}$$

In these equations I_{01} and I_{02} are the intensities of the incident X rays, and I_{11} and I_{12} the intensities of the transmitted X rays. The mass-absorption coefficient is denoted by μ/ρ and has the dimension square centimeters per gram. Index 1 stands for the short-wavelength side of the edge, i.e., λ_1, and index 2 for the long wavelength side, λ_2. If the two X-ray beams are situated very close to the edge and we assume the same value of the incident intensities in both cases, we can write

$$\frac{I_{12}}{I_{11}} = \exp x \left(\frac{\mu_1}{\rho} - \frac{\mu_2}{\rho} \right) \tag{2}$$

as μ'_1/ρ' and μ'_2/ρ' for the foreign elements have the same values if the difference in wavelength between λ_1 and λ_2 is negligible. If very small amounts of the elements are to be determined, the difference between I_{11} and I_{12} is small, and we can write

$$V = x\left(\frac{\mu_1}{\rho} - \frac{\mu_2}{\rho}\right) \tag{2a}$$

where V is the fraction by which I_{12} exceeds I_{11}. (This approximation is valid only for differences between I_{11} and I_{12} that do not exceed 5%).

From equation 2*a*, an idea about the smallest detectable amount of an element with this technique can be obtained. If V is assumed to be 5%, the smallest detectable amount is shown in Fig. 3. It is evi-

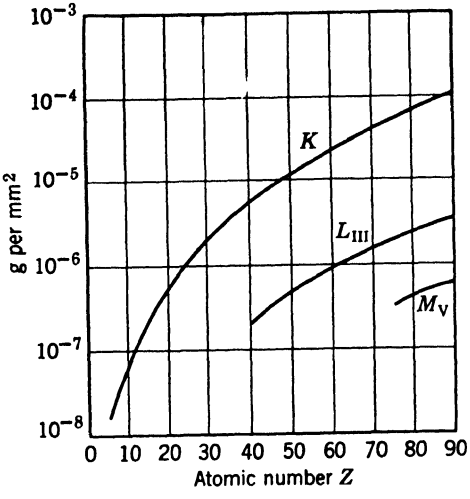

FIG. 3. The smallest detectable amount of an element as function of wavelength. (From Engström, 1946)

dent that the sensitivity of method increases with decreasing atomic number of the element to be determined. Since it is the amount per unit area of the particular element that sets the lower limit, it is also evident that, if a thick specimen is analyzed, it is possible to determine certain elements in relatively high dilution. This point is illustrated in Fig. 4. If the thickness of a biological sample is comparable to that used in histo- and cytochemistry, i.e., 1 to 10 microns, it is clear that the elements must be present in relatively large concentrations in order to be detected by this technique. In other words, for such small and thin samples the method cannot be characterized as a "trace method," although it is possible to determine as small amounts as 10^{-12} gram. Within this very small volume the element to be determined must occur in a relatively large concentration, and the elements of higher atomic numbers cannot be determined in such thin

sections if their concentrations do not approach that of their pure form.

In practice, the X-ray absorption method for elementary analysis can be conducted in two ways. The original method of *Glocker* and

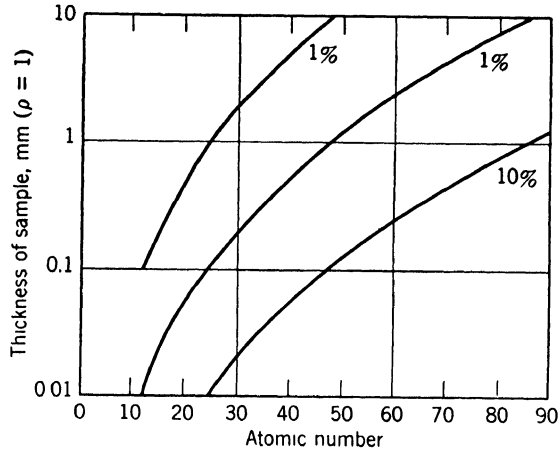

FIG. 4. The thickness of a biological sample of unit density required to fulfill the minimal detectable amount shown in Fig. 3 when the element is present in various concentrations.

Frohmayer which is illustrated in Fig. 5, utilized a continuous X-ray spectrum, the wavelengths of which covered the region of the particular absorption edge to be measured. After passing the sample, the

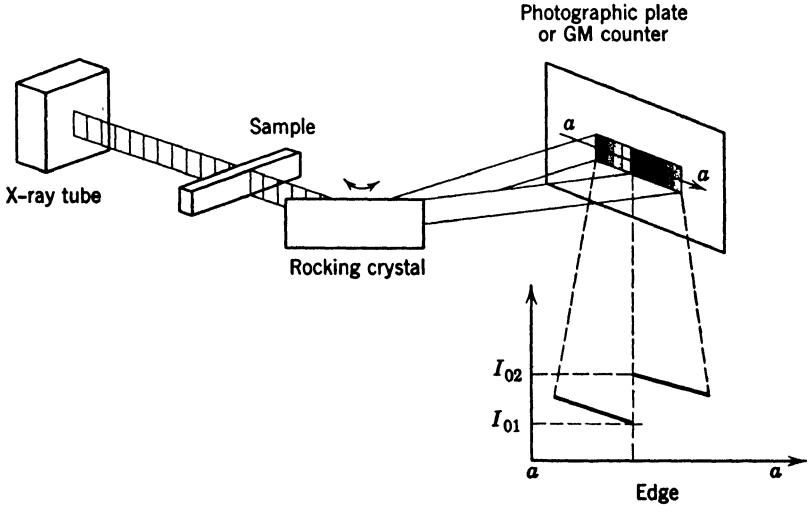

FIG. 5. X-ray absorptiometry of elements using a continuous X-ray spectrum.

intensity distribution in the X-ray spectrum is measured either photographic–photometrically or by a Geiger or scintillation counter. By this procedure the X-ray absorption can be measured or extrapolated very close to both sides of the absorption edge, and therefore equation 2 can be used for the evaluation of the experimental data.

The other modification of the X-ray absorption elementary analysis is to select monochromatic X rays, and choose the wavelengths as close to the absorption edge as possible. The calculation of the amount of the particular element to be determined (cf. Fig. 2) can be done from equations 1*a* and 1*b*, and we can write

$$x = \frac{\ln \dfrac{I_{01}}{I_{11}} - \left(\dfrac{\lambda_1}{\lambda_2}\right)^P \cdot \ln \dfrac{I_{02}}{I_{12}}}{\dfrac{\mu_1}{\rho} - \dfrac{\mu_2}{\rho}\left(\dfrac{\lambda_1}{\lambda_2}\right)^P} \tag{3}$$

The term $(\lambda_1/\lambda_2)^P$ is a correction factor necessary because the two wavelengths λ_1 and λ_2 generally are at a certain but small distance from the absorption edge. The exponent P generally has the value 3 or slightly less. It is seen that the closer to the edge the wavelengths of the measuring beams are situated the less important is the exact knowledge of the magnitude of the exponent P.

The standard error ϵ of x in equation 3 has been derived by Lindström,[17] and, if the denominator in equation 3 is termed $f(u)$, we get

$$\epsilon^2[f(\mu)]^2 = \left(\frac{dI_{01}}{I_{01}}\right)^2 + \left(\frac{dI_{11}}{I_{11}}\right)^2 + \left(\frac{\lambda_1}{\lambda_2}\right)^{2P}\left[\left(\frac{dI_{02}}{I_{02}}\right)^2 + \left(\frac{dI_{12}}{I_{12}}\right)^2\right] \tag{4}$$

where dI with proper index is the standard error of the respective X-ray intensity. If the coefficient of variation in the experimental determination of the X-ray intensities is $\pm 100r$, we get

$$\epsilon = \pm \frac{r\sqrt{2}}{f(\mu)} \sqrt{1 + \left(\frac{\lambda_1}{\lambda_2}\right)^{2P}} \tag{5}$$

The analogous expression for equation 2 then gets the form:

$$\epsilon = \pm \frac{r\sqrt{2}}{\mu_1/\rho - \mu_2/\rho} \tag{6}$$

The accuracy of the X-ray absorption method according to equation 4 thus depends not only on the accuracy by which four X-ray intensi-

ties can be measured but also on the accuracy by which the mass-absorption coefficients μ_1/ρ and μ_2/ρ is known.

The discussion presented above has been concerned with the determination of the minimal amount of the element per unit area. If we assume that the measuring area is 1 cm^2 and the determination concerns Zn with atomic number 30, the minimum detectable amount is about 2×10^{-4} g per cm^2. On the other hand, if we can measure the X-ray absorption within an area that is 1 square micron, i.e., 10^{-8} cm^2, we immediately see that the total amount is reduced to 2×10^{-12} gram. This is certainly a small total amount. To define such a small area in order to obtain histological or cytological localization, it is necessary to have thin samples; hence the concentration of the particular element must reach relatively high values. One can anticipate in certain instances, however, that what is generally regarded as a trace element, when a large volume of tissue is analyzed, occurs at high local concentrations in certain cells or part of cells, and in these instances the X-ray absorption method for elementary analysis of "trace metals" may prove useful.

It is now possible to register X-ray images of a sample with a resolution that is better than one micron. In the elementary analysis two microradiograms are taken with monochromatic X rays with wavelengths on either side of the absorption edge of the element to be determined. By comparing the X-ray transmission in equivalent structures (photographic–photometrically) the amount of the element can be determined, together with its localization in the tissue.

Various types of equipment have been constructed for X-ray absorptiometry, and Fig. 6 shows a simple X-ray spectrometer published by Engström [5] in 1946. This spectrometer used a plane crystal *C* to to get monochromatic X rays. The target of the X-ray tube *A* consisted of elements, the emission lines of which were desired for the analysis. Microcuvettes or holders for microradiography could be placed at *E* and *F* and either photographic or electric recording of the incident and transmitted X-ray intensities was possible. With this equipment small amounts of Zn, Fe, and I were determined. The measurements are performed at atmospheric pressure of air; hence X rays softer than about 2 A are strongly absorbed, which means that elements with an atomic number lower than 25 cannot be determined.

Recently Lindström [17] has improved the equipment for elementary analysis (Fig. 7). He used a curved crystal to obtain monochromatic radiation of a relatively high intensity, and a Geiger counter or a

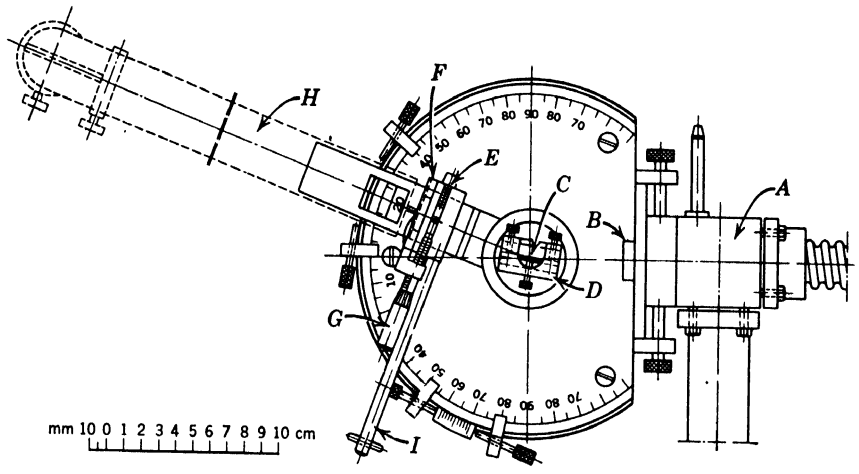

FIG. 6. X-ray spectrometer for the determination of elements with atomic number higher than 25. (From Engström, 1946)

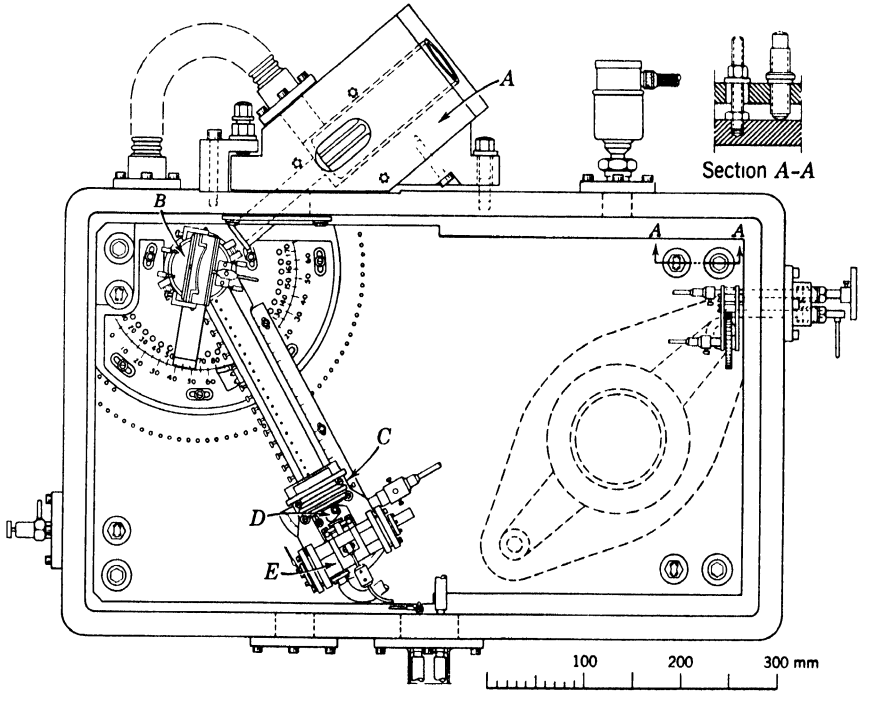

FIG. 7. Vacuum X-ray spectrometer according to Lindström (1955). The X rays from the tube *A* enter the vacuum spectrometer, and the curved crystal is situated at *B*. The sample and the devices for intensity measurements are at *C*, *D*, and *E*.

photographic film was used for the recording of the X-ray intensities. The whole spectrometer could be evacuated, thus permitting the determination of elements with low atomic numbers. For example, Lindström determined the amount of phosphorus in the nucleolus of an egg cell by this technique, although the accuracy was not the best.

The equipment described and used up to now is relatively complicated. It seems possible to simplify the apparatus considerably by the use of a proportional counter. A fine collimated X-ray beam (1 to 10 microns in width) from an X-ray tube run at a suitable potential is passed through the particular structure in the sample. The differential absorption in this beam, which will be due to the action of the absorption edges from the elements in the sample, is analyzed with an electron-energy discriminator, and the simple equation 2 can be used to evaluate the amount of element sought. There are also possibilities of introducing totally reflecting surfaces, in combination with constant potential on the X-ray tube to obtain relatively monochromatic X rays. The main advantage of the proportional-counter techniques is the tremendous gain in intensity obtained by the omission of the crystal for monochromatization.

MICRORADIOGRAPHIC DETERMINATION OF TRACES OF DUST PARTICLES

X-ray micrography with extremely soft X rays has found a very wide use in various fields of biological and medical research. There are three different forms of X-ray microscopy: (1) contact microradiography (CMR), (2) projection microradiography (PMR), and (3) true X-ray microscopy with lens systems.

The method of contact microradiography offers the simplest form of X-ray microscopy. The biological sample, in the form of a biological section or an otherwise prepared sample, is placed in contact with a fine-grained photographic emulsion and radiographed with soft X rays. The small X-ray image, the microradiogram, is viewed in an ordinary light microscope. The resolution depends primarily on the granularity of the photographic emulsion. Fine-grained emulsions such as Eastman Kodak spectroscopic plate 548 or 649 or Kodak maximum resolution plate have a resolving power better than 1000 lines per millimeter. It is easy to arrange the geometrical conditions in such a way that a resolution better than 1 micron can be obtained. In practice and dealing with objects exerting a good X-ray contrast, a resolution better than 0.5 micron has been reached. Ultimately the

resolution of this technique is the resolution of the light microscope used to observe the microradiograms.

The resolution of the projection-microradiographic technique is inherently very high as is also that of true X-ray microscopy with lenses. These two methods, however, are technically complicated,

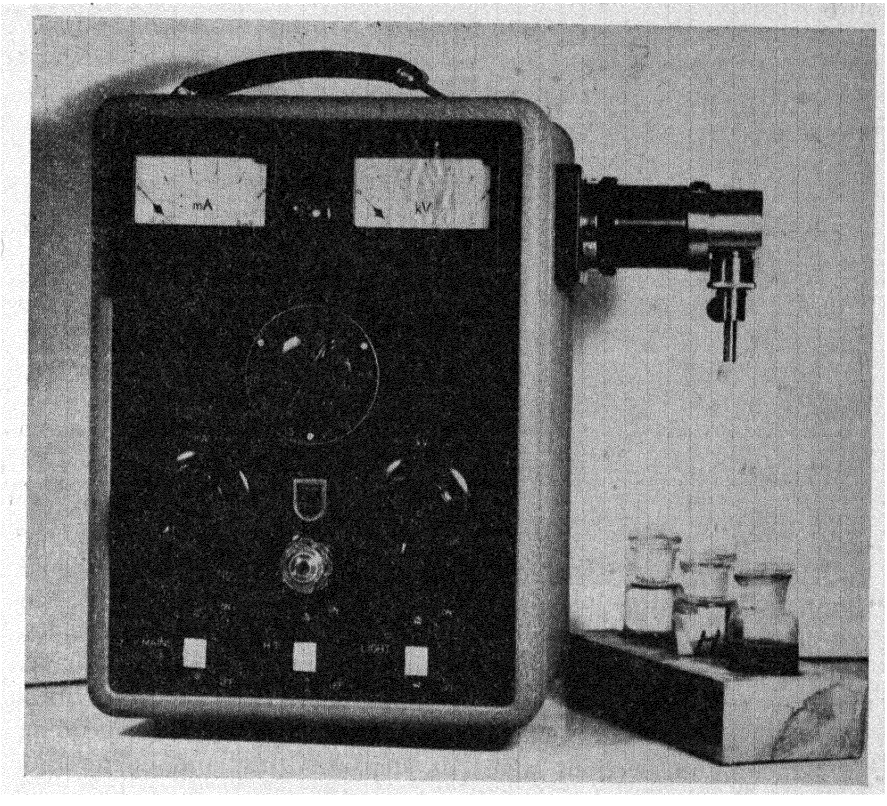

FIG. 8. Equipment for microradiography with soft X rays (Philips).

and at present the simplest and most convenient form of X-ray microscopy is the direct-contact microradiography.

Various types of experimental equipment have been constructed, and recently a simple model has been marketed (Fig. 8). This equipment for microradiography has a sealed-off miniature X-ray tube with a window of beryllium, only 50 microns in thickness. The tube can be energized with a potential variable between 1 and 5 kv (12 to 2.5 A). The focus of the X-ray tube is small, and therefore the target-to-film distance can be kept small, which is important from an intensity point of view as the fine-grained photographic emulsions are about

10,000 times less sensitive than ordinary X-ray film. The exposure times vary between 10 seconds and 15 minutes, depending on the potential on tube.

Small differences in X-ray density of adjacent structures can be detected. This means that, if the linear-absorption coefficient is slightly greater in one structure, the proper thickness of the sample that gives maximum X-ray contrast can be deduced. Suppose two adjacent structures have the linear-absorption coefficients μ_1 and μ_2. If the intensity of the incident X rays is I_0 and the intensities of the X rays transmitted by the two structures is I_1 and I_2, we can write

$$S = I_2 - I_1 = I_0 \cdot (e^{\mu_2 \cdot t} - e^{\mu_1 \cdot t}) \tag{7}$$

where t is the thickness of the sample. The thickness $t_{\max}$ which S has maximum is

$$t_{\max} = \frac{\ln (\mu_1/\mu_2)}{\mu_1 - \mu_2} \tag{8}*$$

If there are small differences between μ_1 and μ_2, we can write

$$t_{\max} = \frac{1}{\mu_2} \sim \frac{1}{\mu} \tag{8a}$$

The soft X-ray microradiography has been used to trace various dust particles introduced into the organism. In an investigation on the fate of inhalated dust of vanadium pentoxide, useful information was obtained about the number and localization of the dust particles. Rabbits inhaled air contaminated with dust of vanadium pentoxide, and after certain time intervals the animals were sacrificed, the lungs removed, and pieces of them fixed by freeze-drying. After embedding in paraffin, thin sections were cut, deparaffinized, and microradiographed. At the inspection of the microradiograms in the light microscope, the size and number of the dust particles localized to various histological structures in the lungs could be seen (Fig. 9). Large pieces of the lungs were ashed, and the amount of vanadium was determined spectroscopically; with this latter method the amount of vanadium found could be defined as occurring in trace quantities. The example has been chosen to show how simple microradiography in certain cases can be of use in the study of the localization of elements which by an over-all analysis occur in minute quantities. It should be emphasized that also with this technique it is sometimes

* Derived under the condition that it is possible to measure I_0 by a photographic procedure.

possible to differentiate among various elements occurring as minute particles, simply by taking several microradiograms at different potentials and applying the reasoning presented above and in connection with the X-ray absorption elementary analysis.

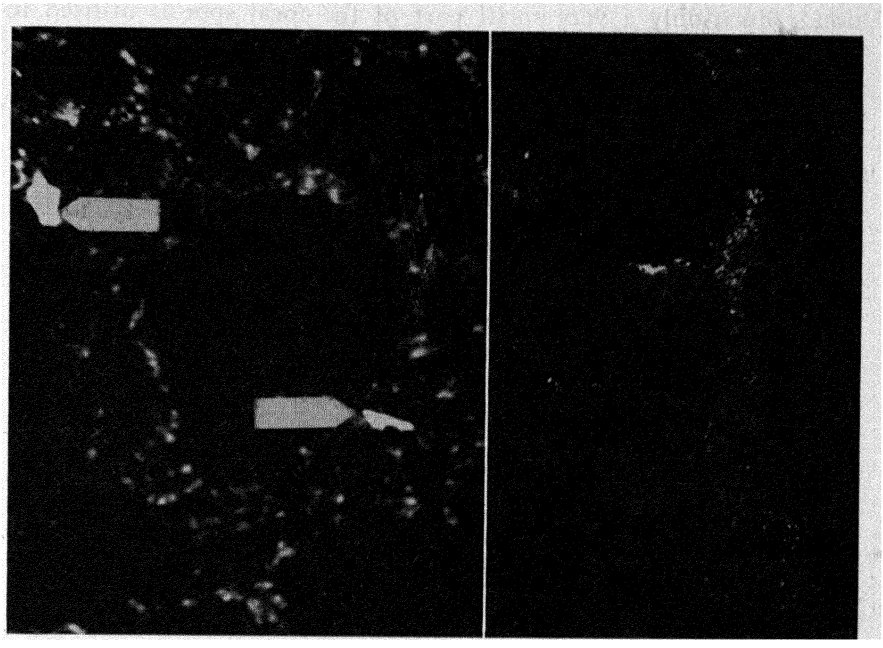

FIG. 9. *Left,* a microradiogram of a 5-micron-thick section of a lung from a rabbit which had inhaled dust of vanadium pentoxide. This picture was taken with 3 kv, and the cells of the lung tissue are seen. Two particles are seen in this picture and are indicated by arrows. Magnitude 230×. *Right,* a microradiogram of a 1-mm-thick section of a rabbit lung. The animal was exposed to lead oxide dust. This image was recorded with 30-kv X rays and a great number of particles are seen. At this high voltage and thick section the X-ray definition of the soft tissue itself is not good owing to overlapping. Magnitude 100×.

MICRO X-RAY DIFFRACTION

The size of the sample examined by conventional X-ray diffraction techniques is relatively large. In the field of histo- and cytochemistry microdiffraction methods will be of great use in identifying certain inclusions in tissues and cells. For this purpose it is necessary to record the diffraction patterns from areas as small as 1 to 10 microns in diameter of thin samples. The fine collimation thus is made necessary by the small size of the homogeneous sample.

If the X-ray beam from an ordinary diffraction tube is collimated to

a pencil 10 microns in diameter, only a small fraction of the focal spot in commonly used X-ray tubes is utilized. The apparent size of the focal spot in an ordinary diffraction tube is in the order of 1 mm square in one direction and perhaps 10×0.1 mm in the other direction. Since only a very small part of the focal spot is utilized in microdiffraction, the intensity is low, and the exposure times grow

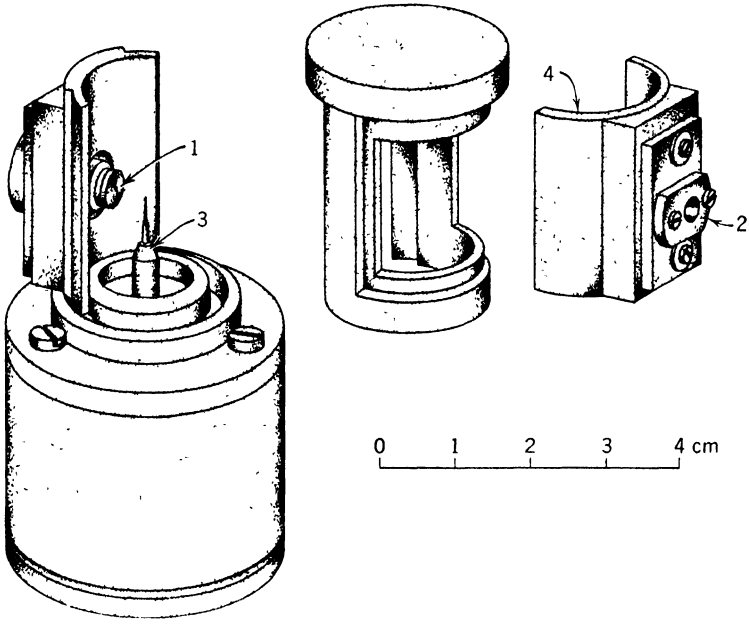

FIG. 10. Micro X-ray diffraction camera of 19 mm diameter. (1) Capillary collimator, (2) beam trap, (3) specimen, (4) photographic film. (From Carlström, 1954)

enormously long. The obvious way to solve this problem is to decrease the size of the focal spot of the X-ray tube, with the consequence that better conditions for heat convection will be present and a high specific load can be obtained. In the fine-focus tube constructed by Ehrenberg and Spear,[4] the electrons from the filament are focused by an electrostatic lens leading to a focal spot of 40 microns in diameter. The specific output of such a small focal spot is considerably higher than that in conventional X-ray tubes for diffraction work. Although this tube has been of very great use in microdiffraction studies, the above-mentioned demand on a diffraction technique capable of resolving structures 1 to 10 microns in size would require an X-ray tube with a focal spot of these dimensions. This can be obtained in the projection X-ray microscope designed by

Cosslett and Nixon [8] or the inverted use of the X-ray microscope as suggested by Kirkpatrick and Pattee.[14] These last two mentioned techniques seem to offer possibilities for X-ray beams about 1 micron in diameter and having a high intensity.

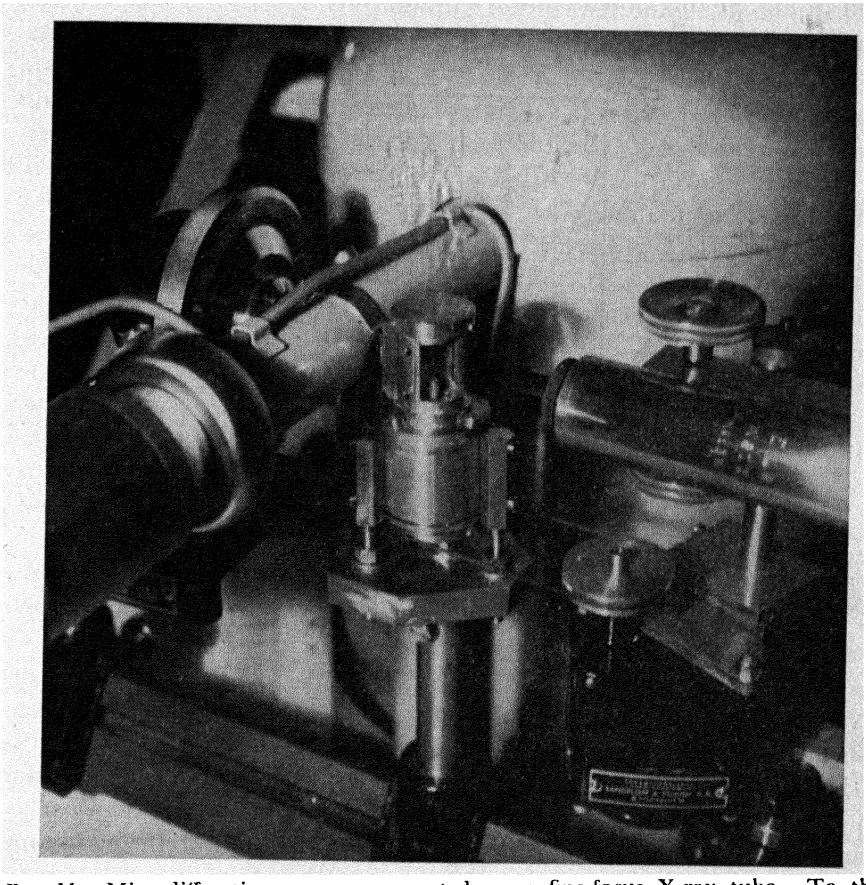

FIG. 11. Microdiffraction cameras mounted on a fine-focus X-ray tube. To the left a Chesley camera for flat film, and to the right the rotation camera shown in Fig. 10, with motor and microscope for the alignment of the specimen.

Several types of cameras for use in microdiffraction studies have been described. Owing to the high collimation of the narrow beam, the intensity is small, and therefore the sample-to-film distance is small in the microcameras, usually between 2 and 15 mm. It is natural that most cameras described such as the "tapering" microcamera of Kreger,[15] the "Mikrokonvergenzverfahren" of Kratky,[16] and the handy microcamera constructed by Chesley [2] all have flat

films, as this construction offers the simplest way of mounting and aligning the specimen and film. Carlström [1] has constructed a rotation microcamera which also can be adapted for work with a holder for a flat film. Figure 10 shows a diagram of the Carlström camera, and in Fig. 11 this camera and a Chesley microcamera are mounted to an Ehrenberg–Spear microfocus X-ray tube.

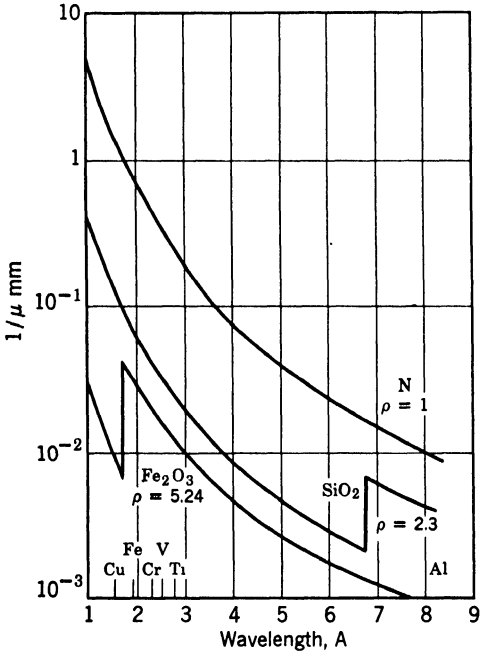

FIG. 12. The value of $1/\mu$ for some compounds as function of wavelength.

The most commonly used type of radiation in diffraction work is the copper $K\alpha$ line radiation (1.54 A). In order to obtain maximum diffraction from a sample, its thickness is chosen to $d = 1/\mu$, where μ is the linear-absorption coefficient (per centimeter) and d the thickness of sample (in centimeters). The numerical value of μ increases approximately with the third power of the wavelength and the fourth power of the atomic number of the element in the sample but changes abruptly at the absorption edges. The scattering from a sample, however, does not increase so rapidly with wavelength as the photoelectric absorption. For microdiffraction studies of many samples there is a considerable advantage to work with Cr $K\alpha$ instead of Cu $K\alpha$ radiation. As the samples are thin, from an intensity point of view it would be better to register the diffraction pattern with still

softer radiation than Cr $K\alpha$. To illustrate this point $1/\mu$ has been calculated for some elements of biological interest, and the results are shown in the diagram in Fig. 12. It is obvious that Al $K\alpha$ radiation is well suited to record the diffraction pattern from an organic sample of a thickness corresponding to one cell only. This radiation is excellent when one is looking for long spacings and the diffuse particle scatter as they occur in organic material. Inclusions containing *inorganic* compounds, such as various dust particles and microcrystals, have interplanar spacings at relatively short wavelengths and hence Al $K\alpha$ cannot be used in these instances. From Fig. 12 it is evident, however, that, for these small *inorganic* inclusions, Cu $K\alpha$ and especially Cr $K\alpha$ radiations are well suited.

Summing up this discussion, it is obvious that the equipments for microdiffraction now commercially available permit a great variety of diffraction studies, not only for the purpose of identification of substances but also for fine structure analysis on the histological level. At our laboratory, small inclusions weighing approximately 10^{-8} to 10^{-9} gram have been identified in scar tissues, and the nature of the microscopic stato- or otoliths, small calcified inclusions, etc. have all been successfully analyzed by microdiffraction procedures.

MICRO X-RAY FLUORESCENCE

The focusing X-ray image spectrograph proposed by von Hamos [10] gives opportunities of studying the X-ray emission spectra of small samples. The principle of the instrument is apparent from Fig. 13. Primary X rays of suitable wavelengths excite secondary X rays in the sample. The wavelength of the primary X rays must be shorter than

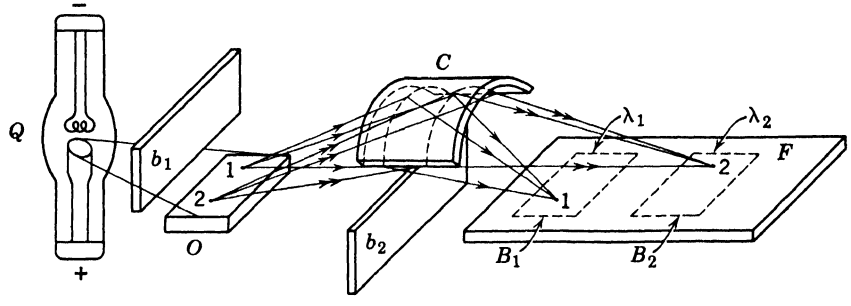

FIG. 13. The X-ray image spectrograph. The X-ray tube Q emits primary X rays which hit the sample O, the fluorescent X rays are focused by the curved crystal C, and the spectral images B_1 and B_2 are recorded on the film F. (From von Hamos and Engström [12])

the absorption edge to which the excited emission lines belong. The secondary X rays are focused by the cylindrically bent crystal, and on the photographic film there appears a series of topographic images of the sample. The plane of the sample and film must coincide with the rotational axis of the cylindrical crystal. The position of the image on the photographic plate depends on the wavelength of the secondary radiation and hence gives information about what element has emitted this radiation. The intensity of the image gives informa-

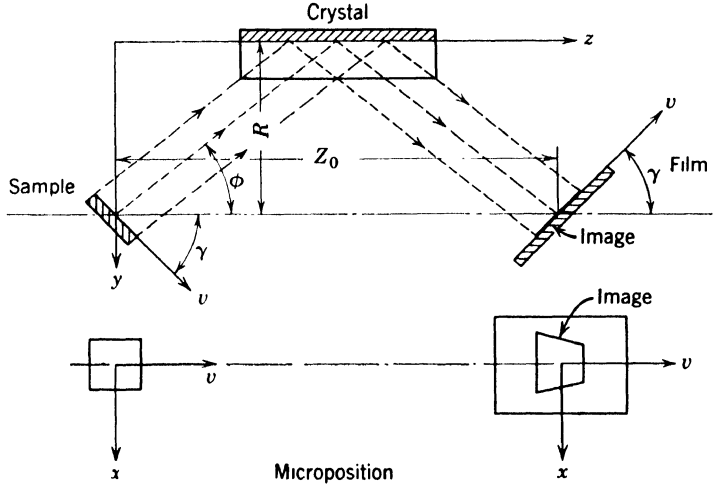

FIG. 14. X-ray microanalyzer in "microposition." The distortion of the spectral image is indicated. (Redrawn from von Hamos [11])

tion about the quantity of the element, and fluctuations of the intensity within the image give the topographic distribution of the element. This arrangement of the components in the microspectrograph is called "analysis position."

If one particular element in the sample is studied, it is not necessary to produce several spectral images also of other elements, and the spectrograph can be adjusted for the "microposition" shown in Fig. 14, where maximum resolution is obtained, although there is a distortion in the image. If the wavelength of the radiation emitted by the sample is λ and the grating constant of the crystal is d, the relative positions of sample crystal and film are given by the equation

$$Z_0 = 2R\sqrt{\left(\frac{2d}{\lambda}\right)^2 - 1} \qquad (9)$$

As mentioned above, the minimum error in focusing for the central parts of the image in the microposition is obtained when the planes of the object and film are perpendicular to the central rays: that is, $\gamma = 90° - \phi$ (cf. Fig. 14).

The quality of image formation is influenced by several factors such as the thickness of the X-ray emitting layer in the sample, the nature

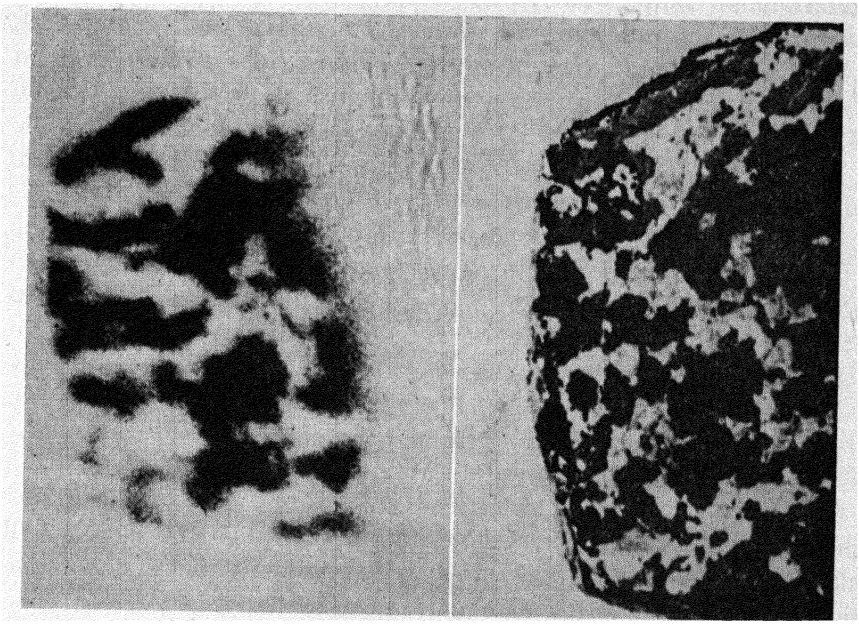

FIG. 15. (*Left*) X-ray emission image (Cu $K\alpha$) of an ore sample, and (*right*) sample photographed in ordinary light. (From von Hamos [11])

of the crystal surface, and aberrations due to the finite spectral width of the spectral lines: that is, "chromatic aberrations." The finite thickness of the photographic emulsion also can cause an error which, however, is minimized in the microposition. In order to minimize errors due to imperfections in the crystal surface, the crystal can be moved along the symmetry axis of the spectrograph during the exposure. Figure 15 shows the Cu $K\alpha$ image of a sample, indicating the distribution of copper, and for a comparison the sample is also photographed in ordinary light.

There are a number of difficulties involved in the quantitative determination of elements with the microfluorescence techniques. In the case of a thin homogeneous object containing a single element, the intensity of the secondary radiation will depend on the thickness

of the object, and the angle between the sample and incident and excited X rays. In multicomponent samples the prediction of the intensity conditions is very complex.

Suppose that the sample contains a single element and the intensity of the secondary X rays can be considered to be the sum of the contributions from a series of parallel layers. The calculation of von Hamos [11] shows what type of reasoning one has to apply to get quantitative information. The intensity of the primary radiation is I_1 and will be reduced by absorption and reach a certain depth in

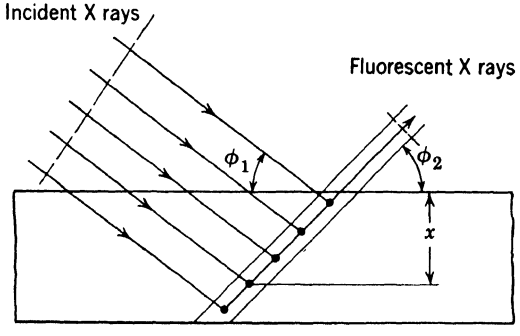

FIG. 16. To illustrate equation 10. (Modified from von Hamos [10])

the sample. The characteristic radiation excited in the sample will also be absorbed on its way out to the crystal. With the denomination in Fig. 16, the following equation can be derived:

$$I_2 = \frac{fI_1}{(\sin \phi_2/\sin \phi_1)\mu_1 + \mu_2} \cdot \left[1 - \exp - \left(\frac{\mu_1 \phi}{\sin \phi_1} + \frac{\mu_2 \rho}{\sin \phi_2}\right) x\right] \quad (10)$$

where ρ is the density of the sample and μ_1 and μ_2 are the absorption coefficients for λ_1 (primary) and λ_2 (secondary), respectively. It is seen that for solid samples of alloys, etc., containing several elements, the conditions become complicated. For biological purposes, however, where we can use this method to determine an element of high atomic number dispersed in the organic matrix which has a low atomic number the conditions will be somewhat simplified. The thickness of sample has to be small because in this case the absorption of the primary and secondary X rays can be neglected. Therefore the intensity of the secondary radiation will give a measure of the "surface density of the element" in question. The absolute amount will

be obtained by comparing the secondary intensity from the sample with that from a thick homogeneous sample of the element. The surface density δA of an element A thus is obtained:

$$\delta_A = \frac{I_{2A}}{I^P{}_{2SA}} \cdot \delta^P{}_{SA} \tag{11}$$

when $\delta^P{}_{SA}$ is the calculated surface density from the pure homogeneous specimen of the element, and I_2 with respective indices indicate the intensity of the secondary intensities in the two cases with sample and homogeneous element. By this technique, for example, the amount of iron and zinc could be determined in thin microtome sections of tissues (von Hamos and Engström [12]).

It ought to be emphasized that little work has been done up until now by the X-ray microanalyzer on the determination of small amounts of elements in biological tissues. In metals, as small volumes as 10^{-8} cm^3 have been successfully analyzed by von Hamos,[11] but the error in the determination was relatively large. The rapid and elegant development of the X-ray fluorescence analysis for industrial purposes has led to the construction of refined X-ray equipment, and it may be anticipated that, if these experiences are brought over to the field of micro X-ray fluorescence studies, techniques valuable for routine analysis in biology will be rapidly developed.

SUMMARY

The use of X-ray absorption techniques for the determination of elements is discussed in detail. Simple microradiography can be utilized to detect small inclusions of, for example, dust particles (metal oxides) in very small volumes of tissues. In general, the absorption techniques are applicable to very small volumes (masses of 10^{-12} gram, or less), but the concentration of the elements must be relatively large within these very minute samples. If larger specimens are analyzed, minute concentrations of heavy elements can be determined by the X-ray absorption techniques.

The applications of X-ray microdiffraction and the micro X-ray fluorescence analysis to biological samples is also considered. A resolution of about 10 microns is possible, and as small amounts as 10^{-8} gram can be analyzed. Also in this case the concentration of the elements must reach a certain minimal value.

DISCUSSION

Discussion Leader
Herman A. Liebhafsky
General Electric Company

I cannot tell you in 5 or 10 minutes why I consider the work of Dr. Engström and his colleagues to be as fascinating and as important as any analytical chemistry I know of in the world today. Perhaps

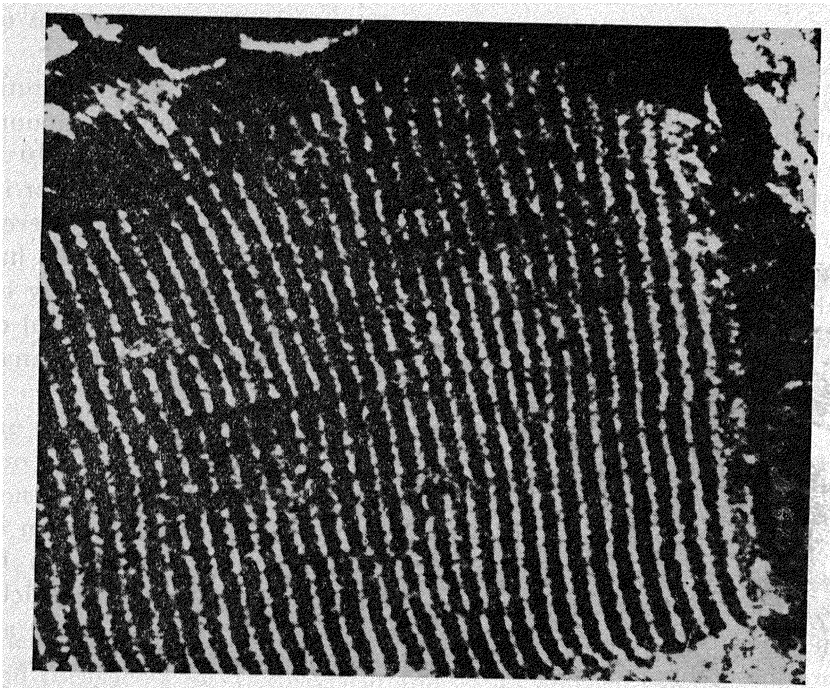

FIG. D1. Microradiogram, ×1500, of a 3-micron-thick section of a formalin-fixed striated muscle of *Chironomus;* 1.0-kv X-ray beam.[1]

an example can do this for me. Here is a microradiogram (Fig. D1) prepared by Dr. Lindström, a colleague of Dr. Engström's. Let me quote from Lindström's thesis: [17]

A 3-μ thick section of a striated muscle, registered at a voltage of 1.0 kv and secondarily magnified 1500 times. Anisotropic bands with a high total dry weight and a width of between 0.5 and 0.7 μ alternate with somewhat broader isotropic bands with a width of between 1.3 and 1.5 μ which have a lower dry weight. The ratio between the dry weights per unit volume in the anisotropic and isotropic bands was (1.41 $\pm$ 0.05): 1 in a series of 10

determinations, including the biologic variation. The photometric measurements were performed on areas with a size of about 1.5 μ^2 in order to resolve the periodic band structure.

Let us look at a few points of interest:

1. The size of sample is 3 microns thick and 1.5 square microns in area, making a volume of 4.5 (10^{-12}) cm^3. This explains why micromicrograms are the weight units. It also explains why the utmost in photographic technique is required in this work.

2. The detailed band structure of the muscle is revealed. The widths of the bands show the high degree of resolution necessary. You will agree, I think, that trace analysis gains in significance when variations in local concentration can be observed—here we observe significant variations in local weight.

3. The technique is governed by the following chain: Light elements require long-wavelength X rays; long-wavelength X rays require vacuum; vacuum requires dry samples.

4. The ratio between dry weights was found to be 1.41 ± 0.05. Our first reaction might be: How do they know? Nothing was weighed. Well, two assumptions are involved. First, it is assumed that the ultimate composition of the dark and white bands is the same. Second, it is assumed that nitrocellulose foils are reliable comparative standards if appropriate corrections are made. Both assumptions are closely realized. Note the high precision, which includes the natural variation.

I think it is remarkable that we can learn so much about the detailed structure of a tissue. But this is only a beginning. The full significance of such work becomes clear only when one remembers that it can be combined advantageously with other techniques. For example, Dr. Engström has explained how differential measurements of X-ray absorption across the edge of an element can be used to determine the element, this being the method of Glocker and Frohnmayer [9] subsequently modified by Engström and others. But to analyze for an element, we must weigh the sample. That is what Lindström did in the first slide. Suppose now that we wanted to know the sulfur contents of the black and white bands. In principle, we could apply the method of Glocker and Frohnmayer to this same sample, and we could get the per cent sulfur in each kind of band. Precision might not be all we want (is it ever?), but we could do the job in principle by methods developed in Dr. Engström's institute.

Let me show you very quickly some tentative conclusions that Dr. Lindström reached in examining a wide variety of samples.

Human Bone Tissue. Single Haversian systems differ significantly in calcium to phosphorus ratio.

Arterial Wall in Arteriosclerosis. Dense, homogeneous, calcified section of wall shows 3.3 times as much calcium as phosphorus.

Human Kidney Section. Homogeneous section of renal calculus contains 5.3 ± 0.2 $\mu\mu$g calcium per square micron.

Sea Urchin Egg. Corneal and spinous layers are roughly identical in sulfur content.

The principal impression I want to leave with you is that the powerful X-ray methods of Engström and his colleagues, when properly combined with other techniques, will give us valuable quantitative information about living tissues. This information is no less valuable because the percentages of the constituents analyzed for are much greater than those usually associated with traces. Owing to the high degree of localization attainable by the Engström methods, they reveal variations within samples so small that the absolute amounts of the elements being determined are certainly comparable with trace amounts. This high degree of localization makes observations by these methods unique.

Finally, I am sure that Dr. Engström will be glad to have me say a few kind words in behalf of X rays themselves. These kind words are simplicity, predictability, and reliability. Were these not characteristic attributes of X-ray methods, I, who know nothing about histology or cytology, should have found it difficult to comment on Dr. Engström's work.

QUESTION AND ANSWER

EDWARD C. DUNLAP (*E. I. du Pont de Nemours*): Could you give us some information concerning the methods of sample preparation? Is this normally done by freeze-drying?

ARNE ENGSTROM: Yes, but not by normal freeze-drying. The best procedure is to freeze sections of the material in a cryostat and then to dry it at a low temperature on the specimen holders.

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Neutron-Activation Analysis

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ABSTRACT The scope, advantages, and limitations of neutron-activation analysis in the field of trace-element determinations are briefly reviewed. Particular problems in which this technique has proved of value are discussed, including applications in geochemistry and in industrial pure materials: e.g., semiconductors.

Preparation of samples for irradiation, radiochemical separations, and recent instrumentation improvements are also described.

GENERAL PRINCIPLES

The availability of nuclear reactors associated with the development of atomic energy has made possible the extension of a technique, first used some twenty years ago by G. Hevesy,[1] into one of the most sensitive and specific methods available for the determination of minute quantities of a wide range of elements. Hevesy used a laboratory radium–beryllium neutron source to irradiate milligram quantities of one or two rare-earth elements. Dysprosium, for example, became radioactive with this treatment, and by measuring its radioactivity, using a Geiger counter, it was possible to determine the dysprosium

quantitatively, even when mixed with some of the other rare-earth elements which gave negligible radioactivity under similar conditions.

The essential basis of the method can be seen from this early application; i.e., the element to be determined is made radioactive, usually, but not necessarily, by slow neutron irradiation, and then this radioactivity, after suitable chemical separation if necessary, is a measure of the mass of the element originally present. Because of the great sensitivity of present methods of measuring activity, because of the almost specific characteristics of individual radionuclides, e.g., decay period and energy of emitted radiation, and because nuclear reactors are now available giving neutron fluxes greater by factors of 10^8 or more than those used by Hevesy, this method of radioactivation or, more simply, activation, offers very great attractions.

Let us consider what happens when an element is made radioactive by placing it in a homogeneous flux of energetic charged particles or neutrons. The rate of growth of the number of radioactive atoms N^*, with time, is given by

$$dN^*/dt = f\sigma N - \lambda N^* \tag{1}$$

This on integration over the period of irradiation, becomes

$$N^* = f\sigma N(1 - e^{-\lambda t})/\lambda \tag{2}$$

where f is the flux of bombarding particles in units of particles per square centimeter per second, σ is the isotopic cross section for the nuclear reaction in units of square centimeters per target atom (and might therefore be considered as the "effective area" of the atom for the nuclear reaction), N is the number of target atoms and λ is the radioactive decay constant for the radionuclide formed, being connected with the half-life T by the relation $\lambda = 0.693/T$.

The amount of radioactivity A_t, in disintegrations per second, exhibited by the atoms N^* produced up to a time t is given by the expression

$$A_t = \lambda N^* = f\sigma N(1 - e^{-\lambda t}) \tag{3}$$

$$= f\sigma N(1 - e^{-0.693t/T}) \tag{4}$$

where t and T are expressed in the same units.

So far, it has been assumed that the element is mono-isotopic; however, considering a weight W grams of an element of atomic weight

M, and if θ is the abundance of the particular isotope (of the element under consideration) giving rise to the activity, then we have

$$A_t = 6 \times 10^{23} f\sigma\theta W(1 - e^{-0.693t/T})/M \tag{5}$$

After the irradiation is stopped, the activity formed will of course decay with its characteristic half-life, so that, at a time d (in the same units as t and T) after the cessation of the irradiation, the activity becomes

$$A = 6 \times 10^{23} f\sigma\theta W(1 - e^{-0.693t/T})(e^{-0.693d/T})/M \tag{6}$$

From this relationship, then, it can be seen that high activity (and therefore high sensitivity) is obtained for a given mass if the flux and the activation cross section are high. In addition, other things being equal, sensitivity is greater for lower-atomic-weight elements and for those with high relative abundance of the isotope concerned. The half-life of the radionuclide does not control the inherent sensitivity of the method, except insofar as it becomes a practical limitation, either for long-lived emitters on the time of irradiation, or for short-lived emitters on the time between the irradiation and the actual measurement of radioactivity.

The character of the radiations emitted by the radionuclide formed also affects the sensitivity of the method, and must be considered in any assessment of its potentialities. The detailed study of the overall efficiency of detection devices for the various kinds of rays and particles emitted by radionuclides covers a very wide field, however, and cannot be made here. However, many of the practical applications of the method will be covered by saying that, for a radionuclide emitting beta particles of maximum energy >0.5 mev, it is generally possible to count at least 10% of the total distintegrations using a normal end-window Geiger–Müller tube.

From what has been said so far, it can be seen, provided that the magnitude of the flux, the activation cross section for the nuclear reaction, and the half-life of the radionuclide formed are known, that a determination of the absolute disintegration rate should then enable the calculation to be made of the absolute mass of the constituent being determined. However, accurate knowledge of the flux and cross section and accurate determination of the absolute disintegration rate are not usually possible, but in practice these difficulties are avoided by using a comparative procedure: i.e., simultaneous irradiation of samples with standards containing a known proportion of the element being determined.

PRACTICAL STEPS

The practical steps in an analysis by radioactivation are, then, as follows:

1. Irradiate weighed quantities of the sample and standard in suitable containers for a sufficient time to give adequate radioactivity in the element to be determined.
2. After irradiation, dissolve the samples and standards and add a known weight of the element being determined as carrier for the small quantity that has been irradiated.
3. Treat the solution in such a way that the carrier and the element in the sample are in the same chemical form.
4. Carry out chemical separations to isolate the element (or a suitable compound) free from all other radionuclides: i.e., until it is radiochemically pure.
5. Determine the chemical "yield" by any conventional method. The fact that this step is included makes it unnecessary for the chemical separation in step 4 to be quantitative, since the final measured activity will be corrected for losses, using the chemical yield figure.
6. Compare the radioactivity of sample and standard under identical counting conditions, making correction if necessary for self-absorption, dead-time losses, decay, etc.
7. Check the radiochemical purity of the isolated active compound by measuring (*a*) the rate of decay, from which the half-life may be obtained, (*b*) the activity through different thicknesses of aluminum, from which an estimate of the energy of the rays or particles emitted may be made, and (*c*) the gamma energy, using, for example, a scintillation spectrometer.

In certain favorable cases, steps 2 to 5 may be omitted.

Then the mass of X, the constituent to be determined, is obtained as follows:

$$\frac{\text{Mass of } X \text{ in unknown}}{\text{Mass of } X \text{ in standard}} = \frac{\text{Total activity from element } X \text{ in unknown}}{\text{Total activity from element } X \text{ in standard}}$$

ACTIVATION SOURCE

All the foregoing has dealt quite generally with any method of activating the sample, whether it be by neutrons, charged particles or gamma rays and irrespective of the source of these particles or rays. Particle accelerators such as cyclotrons offer good sensitivity as a high

flux of bombarding particles can be obtained, and in fact such machines have been used for activation analysis for trace-element determinations (see ref. 2 for examples). There are some disadvantages in this type of activation, however, as there is generally a limitation on the type and size of sample material for irradiation because of the heat to be dissipated and the small area of the beam. In general, the activation cross sections using fast neutrons or charged particles are lower than those for slow neutrons, while a further difficulty with high-energy particles is the possible multiplicity of nuclear reactions which may occur simultaneously. As facilities for this type of irradiation cannot be regarded as generally available, it is not intended to deal further with them here, but they should be borne in mind as possibly being of value in certain cases.

The most generally useful and available activating source in England and in the United States, and also in some European countries now, is the nuclear reactor, with its abundant flux of (mainly) slow neutrons and the relative simplicity with which irradiations can be carried out, even by persons not connected with atomic-energy projects. The advantage of slow neutrons as the activating particles is that generally only one nuclear reaction takes place, i.e., the n,γ reaction, and the cross section for this reaction may be quite high.

SENSITIVITY AND RANGE OF ELEMENTS

Meinke [3] has listed the usable neutron flux from a number of sources, and his table is reproduced with the addition of details for the Harwell pile as Table I.

TABLE I

NEUTRON SOURCES AND USABLE FLUX

Neutrons per square centimeter per second

Ra–Be 25 mg		10^2–10^3
Sb–Be 1 curie		10^3–10^4
Van de Graaf		10^7–5×10^9
Cyclotron		10^8–10^9
Reactors		
Oak Ridge	X-10	5×10^{11}
Oak Ridge	LITR	10^{13}
Brookhaven	BNL	2×10^{12}
Chalk River	NRX	2×10^{13}
Idaho	MTR	2×10^{14}
Harwell	BEPO	10^{12}

Using the value for the Harwell pile, 10^{12} neutrons per square centimeter per second, potentialities of the neutron activation method can be calculated from equation 6. Substituting $1\mu g$ for W, using units of barns (1 barn = 10^{-24} cm^2) for σ, and, if we express A in disintegrations per minute, equation 6 reduces to

$$A = 3.6 \times 10^7 \frac{\sigma\theta}{M}(1 - e^{-0.693t/T})(e^{-0.693d/T}) \qquad (7)$$

The term $(1 - e^{-0.693t/T})$ has been called the "saturation factor" and has a value of ½ for an irradiation time equal to one half-life of the radionuclide produced, and approaches a value of 1 for irradiation times of more than about 7 half-lives. Similarly, the term $(e^{-0.693d/T})$ can be called the decay factor and has a value of ½ for a decay time of one half-life period. However, it is convenient to be able to remember a simple formula for calculating the sensitivity of the radioactivation method for a wide range of elements, and equation 7 can be reduced to

$$A = 3.6 \times 10^7 \frac{\sigma\theta}{M} \qquad (8)$$

if we assume an irradiation time of several half-lives and no decay period. All that is needed in conjunction with equation 7 or 8 is a chart of the nuclides such as that published by the General Electric Company.* A complete list of the nuclides with cross sections, nuclear reactions for formation, etc., is available, and kept up-to-date by issue of supplements, in the *U. S. National Bureau of Standards Circular 499.*

As an example of the application of this simple relationship, we can consider the determination of the rare-earth element: dysprosium. Referring to the chart of nuclides, we find that Dy^{165} with a half-life of 140 minutes can be made by an n,γ reaction on Dy^{164}, the fractional isotopic abundance of the latter isotope being 0.282 and the cross section for formation being 2800 barns. Since the chemical atomic weight of dysprosium is 162.5, the application of the simple relationship in equation 8 shows that the activity due to Dy^{165} from 1 μg of the natural element being irradiated for about 12 hours in a flux of 10^{12} neutrons per square centimeter per second would be

* Available without charge from Department 6-221, Schenectady 5, N. Y. This chart is rather a large document, and I have found it an advantage to have a small photostatic copy prepared, 18 by 9 in. approximately, which can be carried around and referred to with the assistance of a small pocket lens.

$$A = 3.6 \times 10^7 \times \frac{0.282 \times 2800}{162.5} \text{ disintegrations per minute}$$

$$= 1.7 \times 10^8 \text{ disintegrations per minute}$$

Now Dy^{165} emits both β particles (maximum energy 1.2 Mev) and gamma rays, so that it is relatively easily counted using a conventional end-window GM tube. Let us assume that 10% of the total disintegrations can be counted. This means that we can get 1.7×10^7 counts per minute from 1μg of dysprosium. If we consider the limit of detection as about 8 counts per minute since this will just about double the normal background of a shielded end-window GM tube, then it can be seen that as little as about 5×10^{-13} gram of dysprosium should be detectable immediately after irradiation. This example is, of course, highly idealized, since in practice a sample will almost certainly contain other radioelements after irradiation, and chemical separations will be necessary. The time taken for these will mean that some sensitivity is lost. Nevertheless, it will be appreciated that there is immense potential sensitivity available when it is remembered that the limiting sensitivity of the best spectrographic or absorptiometric methods is about 10^{-8} or 10^{-9} gram. On the other hand, it should be stressed that radioactivation analysis cannot be used to determine all elements, since the radionuclide formed must (1) be sufficiently long-lived so that it can be made ready for counting and (2) emit rays or particles that are measurable. It does seem that the determination of some 70 of the elements should be possible by this method, although in general the very light elements are unsuitable because of the short half-life nuclides formed and the low sensitivity obtainable. To give some idea of the range of application of the method, Table II lists in decreasing order the theoretical sensitivity attainable for a number of elements. Sensitivity is defined for purposes of Table II as that weight of element in grams which on irradiation to saturation in a flux of 10^{12} slow neutrons per square centimeter per second gives an activity of 100 disintegrations per minute of the radionuclide mentioned, but it must be emphasized that the practical achievement of these sensitivities needs a good deal of work.

Nevertheless these are not wildly optimistic values, except for those elements giving rise to long-lived radionuclides, such as cobalt where clearly irradiation for a period of 5 years is not practicable. In arsenic, for example, it has been demonstrated[4] that amounts approaching 10^{-10} gram can be determined.

TABLE II

SENSITIVITY FOR SOME ELEMENTS BY NEUTRON ACTIVATION

Element	Radionuclide Formed	Half-Life	Activation Cross Section, barns	Abundance of Parent Nuclide, %	Sensitivity, g
Dy	Dy^{165}	140 min	2,800	28.2	6×10^{-13}
Eu	Eu^{152}	9.3 hr	1,000	47.8	9×10^{-13}
Ir	Ir^{192}	70 days	740	38.5	2×10^{-12}
Rh	Rh^{104}	44 sec	138	100	2×10^{-12}
Tm	Tm^{170}	127 days	120	100	4×10^{-12}
In	In^{116}	54 min	52	95.8	5×10^{-12}
Lu	Lu^{177}	6.8 days	3,600	2.6	5×10^{-12}
Co	Co^{60}	5.3 yr	34	100	5×10^{-12}
Au	Au^{198}	2.7 days	95	100	6×10^{-12}
Ag	Ag^{110}	24 sec	85	48.6	7×10^{-12}
Ir	Ir^{194}	19 hr	130	61.5	7×10^{-12}
Ho	Ho^{166}	27.2 hr	67	100	7×10^{-12}
Sm	Sm^{153}	47 hr	200	26.8	8×10^{-12}
Tb	Tb^{160}	74 days	55	100	8×10^{-12}
Re	Re^{188}	18.9 hr	74	62.9	1×10^{-11}
Sc	Sc^{46}	85 days	12	100	1×10^{-11}
Cs	Cs^{134}	2.3 yr	26	100	1×10^{-11}
Mn	Mn^{56}	2.6 hr	12	100	1×10^{-11}
Ta	Ta^{182}	117 days	21	100	2×10^{-11}
Ag	Ag^{108}	2.3 min	34	51.4	2×10^{-11}
Lu	Lu^{176}	3.7 hr	20	97.4	3×10^{-11}
V	V^{52}	3.7 min	4.8	99.8	3×10^{-11}
Pr	Pr^{142}	19.3 hr	11	100	4×10^{-11}
La	La^{140}	40 hr	9	99.9	4×10^{-11}
As	As^{76}	26.8 hr	4	100	5×10^{-11}
Br	Br^{80}	18 min	8.5	50.6	5×10^{-11}
Cu	Cu^{64}	12.9 hr	4.3	69	6×10^{-11}
I	I^{128}	25 min	6.1	100	6×10^{-11}
Na	Na^{24}	14.8 hr	0.48	100	1×10^{-10}

Although all the examples above are calculated for a flux of 10^{12} neutrons per square centimeter per second, it must not be forgotten that even higher fluxes are becoming available, and the use of reactors similar to the materials testing reactor at Idaho should permit sensitivities about one hundred times better than those quoted.

EQUIPMENT

Laboratory Facilities. When a sample is irradiated with slow neutrons, *all* the elements in that sample become radioactive to a greater or lesser degree. Therefore, although the element being determined

may be giving only a few hundred disintegrations per minute, the other components of the sample may be extremely radioactive, as can be calculated from equation 8 above. Thus the chemical separations mentioned at item 4 in "Practical Steps" must be carried out with due regard to the hazards of handling radioactive sources. Since most university and industrial centers are equipped with "hot labs" for the use of radioactive tracers, many applications of the activation method can be conducted in these existing facilities.

On occasions, however, particular types of sample may become so active that special precautions have to be taken, at least in the initial steps. Some mineral samples, for example, may require the initial treatment to be carried out in a good fume cupboard, or even in a dry box, but it is normally possible quickly to separate the element being determined, from the bulk of the radioactive residue. The further chemical treatment can then be carried out at much lower activity levels, the more active residue being retained behind lead shielding for decay to take place before ultimate disposal. Clearly some initial radiochemical training and knowledge of health physics procedures are essential before undertaking radioactivation analysis, but, given this, a good number of applications are possible in normal laboratories without structural alterations.

Counting Facilities. As mentioned earlier, it is impossible in a short chapter such as this to give a detailed description of all the radiometric equipment of possible application to detecting and measuring the rays or particles emitted by a radioactive material. Again the radiochemical training mentioned earlier will provide an introduction to this subject. The amount of radiometric equipment needed for many applications of activation analysis is quite small. A good deal can be done with an end-window GM counter with its associated equipment of EHT supply, probe unit, and scaler. The total cost of such equipment in England need be no more than two or three hundred pounds, a sum that is quite modest by comparison with the cost of many important analytical instruments.

For more ambitious use of this method, greater complexity of equipment becomes necessary. For example, in checking the radiochemical purity of several samples simultaneously, some form of automatic turntable and printing mechanism is desirable, though not essential. For counting low-energy beta particles, special types of counter are necessary to obtain maximum sensitivity, while for counting gamma rays a scintillation counter is desirable. In some cases which will be illustrated later, a scintillation spectrometer is valuable.

LIMITATIONS

Like any other method of analysis, activation has its limitations, and the would-be user should be aware of them. One purpose of this symposium is to explore the limitations of the several methods as well as their advantages. Frequently in publications these two items are not afforded equal treatment. It is hoped that this will not be said of this particular chapter.

Elements for Which Neutron Activation Is Not Suitable. Although, as mentioned earlier, almost all elements become radioactive to some extent after neutron irradiation, the half-life of some of the radionuclides formed is too short to allow for removal of the sample from the pile followed by the necessary chemical separation and counting. Elements in this category include He, Li, B, N, O, F, and Ne, with half-lives of seconds or less, and the normal activation techniques are not, therefore, readily applicable to these elements.

To a lesser degree Al, Mg, Ti, V, and Nb, with half-lives of a few minutes, are similar. Some elements give, on neutron activation, only very long-lived radionuclides so that sensitivity is very low for their determination, e.g., Be and C, while H and Pb have such low-activation cross sections that determination by activation is scarcely worth considering.

In some of the above cases specialized treatment may provide a method where none appears to be feasible at first sight. Thus, Osmond and Smales [5] have determined oxygen (in beryllium) by irradiating the sample mixed with a lithium salt, when a combintion of nuclear reactions takes place, giving F^{18}, a 112-minute half-life radionuclide, in amounts proportional to the oxygen. It would seem possible to extend this to a lithium determination if desired. Also, it must be remembered that the cross sections for n,α and n,p reactions are often quite high for these lower-atomic-number elements, and this fact may sometimes be of use. Finally, other types of irradiation may be more favorable than that by neutrons in some cases: e.g., Be using the γ,n reaction [6] and C using the d,n reaction.[7]

Physical Limitations. There are certain physical limitations that must be borne in mind in considering irradiations in a nuclear reactor. Heat is being produced by the latter as well as neutrons, and therefore samples must be of such a character that they can withstand the particular temperature to which they are to be exposed and must be enclosed in such a manner that there is no danger of contaminating

the reactor or other people's samples. There is almost certainly some limitation in the over-all size of sample that can be irradiated. With these considerations in mind samples of crystalline or metallic solids are preferable, and these should be sealed in polythene (see below) or silica and placed in a screw-topped cylindrical aluminium can. For the Harwell pile, normal sizes of can are 2.5 and 30 ml volume with external dimensions of 1.9 cm diameter by 2.03 cm length, and 3.05 by 8.13 cm, respectively. Larger sizes can be dealt with,[8] at a lower neutron flux, up to a limit of 60 by 60 by 90 cm.

Liquids must normally be sealed in silica ampoules to fit into one of the standard-size cans, and these ampoules should have been pressure-tested before irradiation by being heated to 100° C for 24 hours. However, in special cases, irradiation of liquids in silica containers, open to the atmosphere, has been possible: for example, 10-ml lots of sea water have been irradiated in this manner.[9]

Chemical Limitations. Samples put into a nuclear reactor will be bombarded both by gamma rays and neutrons. Structural damage and decomposition by processes under the general title of radiation chemistry must, therefore, be considered. Silica, for example, after a few days' irradiation becomes almost black, so that such a simple thing as marking of samples must allow for this effect. Polythene becomes brittle with a tendency to crack, after only a few days' irradiation, and, therefore, long irradiations of samples must not be carried out in such material. Many substances partially decompose on irradiation, some with evolution of gas, and the pressure build up of such gas can cause breakage even of sealed containers which have been heat-tested. In spite of these difficulties, many reactor irradiations have been carried out so that the would-be user of the activation method should not be discouraged by this list.

As will be recalled, under item 4 of "Practical Steps," chemical separations of the element being determined are usually carried out. These separations must be really efficient, as the success of the method depends on the fact that the isolated material, used for the final activity measurement, should contain no significant amount of radioactivity due to any other element, and this is not so much a limitation as an essential requirement (except in special cases: e.g., where gamma spectrometry can be used; see later). This achievement of "radiochemical purity" is, in practice, not too difficult, particularly when it is remembered that the repetition of steps and the use of non-

quantitative separations are possible. "Scavenging" procedures are also useful whereby a number of unwanted radioelements are removed in one treatment, without significantly affecting the element being determined. A very useful compilation of radiochemical separation schemes which could be of value in activation analysis has been made by Meinke.[10] It should be pointed out that any significant radiochemical impurity in the final product is usually brought to light by the decay and energy measurements and thus a very good check on the success or otherwise of the chemistry is obtained from these.

A further important point in considering the chemical limitations is one mentioned briefly under item 3 of "Practical Steps." This was to treat the samples so that the irradiated and the carrier element are brought into the same chemical form. Clearly, correction for losses in the chemical separations can only be made if the carrier and the irradiated element behave quantitatively alike, and they will do this only if they are in the same chemical form. Thus in making a determination of arsenic in biological material,[11] the arsenic in the samples might be present as AsO_2^-, AsO_3^-, As^{+3}, As^{+5}, or as an organic compound, while the carrier solution contained arsenic as AsO_2^-. Clearly it was essential to mineralize the organic material and then oxidize under alkaline conditions so that all arsenic, both radioactive and inactive, was present as arsenate before proceeding to separations. It must also be noted that the mineralization of organic matter, as with *any* step prior to the radioelement and carrier being put into the same chemical form, *must* be carried out quantitatively: i.e., without loss of arsenic.

Nuclear Limitations. The first nuclear limitation to consider is that the samples must not have such a high neutron absorption as seriously to affect the neutron flux in their neighborhood during irradiation. It is clear that a sufficient amount of, say, cadmium, if put into the reactor for irradiation, could easily stop the chain reaction which keeps the pile working—in exactly the same way that the control rods are used. It is unlikely that this situation would arise in practice, but even small amounts of such substances cause difficulty in an analysis because of "self-shielding." Briefly, this means that, in a sample of finite thickness, the neutron flux becomes smaller toward the center than at the outside, owing to absorption of neutrons by the sample, and, if this does not happen or happens to a different degree in the standard, then obviously the analysis will be in error.

The effect of this self-shielding can be approximately calculated from the relationship

$$f = f_0 e^{-nx\sigma}$$

where f = flux in particles per square centimeter per second at a distance x cm from the surface

σ = absorption cross section of the material, in square centimeters, for neutrons of the particular energy range available at the site of irradiation

n = number of atoms per cubic centimeter for the material

The absorption cross section of the elements for *thermal* neutrons, is given in the nuclide chart mentioned previously, but it must be stressed that this may be misleading, as in most nuclear reactors the neutron spectrum at the site of irradiation covers a fairly wide energy band, and at some energies "resonance" absorption occurs, where the value of σ can be much higher than for thermal neutrons. A list of the variation of neutron cross sections with neutron energy for most elements is now available.[11a]. If self-shielding is likely to be serious, it can usually be overcome by diluting the sample or standard with a material of relatively low cross section such as alumina, magnesia, or even water.

The major nuclear limitation is, unlike the two already mentioned, a real interference and arises from the fact that, in addition to the n,γ reaction which is the main one to take place with a sample in the reactor, it is possible for other nuclear reactions to occur even with slow neutrons, as well as with the fast neutrons and gamma rays which are present. This means in practice the possibility that the radionuclide which is being used as a measure of the mass of a given element may in fact be formed from quite a different element. Taking one example, the determination of arsenic by reactor irradiation depends on the formation of As^{76} from the mono-isotopic As^{75} by the nuclear reaction:

$$_{33}As^{75} + {}_0n^1 \rightarrow {}_{33}As^{76}$$

However, it is possible for As^{76} to be formed by several other reactions starting with elements *other than arsenic:*

(i) $_{34}Se^{76} + {}_0n^1 \rightarrow {}_{33}As^{76} + {}_1H^1$ (proton)

(ii) $_{35}Br^{79} + {}_0n^1 \rightarrow {}_{33}As^{76} + {}_2He^4$ (α particle)

(iii) $_{32}Ge^{74} + {}_0n^1 \rightarrow {}_{32}Ge^{75} \rightarrow {}_{33}As^{75} + {}_{-1}e^0$ (β particle)

$$\downarrow +{}_0n^1 \rightarrow {}_{33}As^{76}$$

(iv) $_{34}Se^{76} + \gamma \rightarrow {}_{33}As^{75} + {}_1H^1$

$$\downarrow +{}_0n^1$$

$$_{33}As^{76}$$

(v) $_{34}Se^{77} + \gamma \rightarrow {}_{33}As^{76} + {}_1H^1$

Of less importance, but still to be considered, is the formation of radionuclides of the element being determined other than the one being used for the determination. Again considering arsenic, although the radionuclide $_{33}As^{77}$ cannot be formed in significant amounts by neutron irradiation of arsenic (a double neutron reaction on arsenic is possible but with low yields compared with those for the primary neutron reaction to give As^{76}), it can be made by the following reactions.

(vi) $_{32}Ge^{76} + {}_0n^1 \rightarrow {}_{33}As^{77} + {}_{-1}e^0$

(vii) $_{34}Se^{77} + {}_0n^1 \rightarrow {}_{33}As^{77} + {}_1H^1$

(viii) $_{92}U + {}_0n^1 \rightarrow$ fission products including $_{33}As^{77}$

(ix) $_{90}Th + {}_0n^1 \rightarrow$ fission products including $_{33}As^{77}$

Although in this particular example it is a relatively simple matter to differentiate between As^{76} and As^{77} because of the great difference in maximum energy of the respective β particles, nevertheless some sensitivity is lost thereby. Other cases may be more or less complicated, and each element must be considered in detail. It will be noticed that, apart from the fission products arising from the naturally occurring heavy elements, the nuclear complications mentioned arise from elements adjacent in the periodic table to the element being determined. In general, because of the low value for the cross sections of the n,p; n,α; and γ,p reactions compared with those for n,γ reactions, these limitations only become apparent in the determination of a trace element in the presence of macroquantities of the adjacent elements, and the case of arsenic in germanium has been discussed by Smales and Pate.[4] It should be added that a practical study of such reactions has usually to be made on materials of remarkable purity; published cross sections have frequently overlooked the presence of small amounts of the element concerned occurring as an impurity

and giving rise, by the n,γ reaction to significant amounts of the radionuclide under study.

It may be possible to overcome these nuclear limitations by a partial or total separation of the element to be determined, before irradiation. However, such preliminary separation of minute quantities, although probably feasible for gaseous elements, will almost certainly be difficult for other elements at the very low level which makes it necessary, and in any case much of the advantage of the radioactivation method disappears if preliminary separation is necessary. Normally the n,p and n,α reactions are due to fast neutrons, and it may be possible to avoid this limitation, with some loss of sensitivity unfortunately, by carrying out irradiations in the "thermal column" of the reactor where the ratio of fast-to-slow neutrons is lower, possibly by a factor of 10^3 to 10^4, than in the normal irradiation positions. Seyfang and Smales [12] used this technique to avoid the fast-neutron fission of U^{238} in their determination of U^{235} by activation.

CONTAMINATION PROBLEMS

Theoretically the activation method should not suffer from contamination problems. Once the sample has been irradiated, the determination of an element depends on the measurement of a *radioactive* isotope of that element. Any contamination of the sample by reagents, etc. (unless gross, when it could affect the "chemical yield"), will be completely immaterial as the reagents will only contain the *inactive* element concerned. This fact is indeed one of the greatest advantages of the activation method; nevertheless contamination *can* occur in the laboratories regularly using activation methods and must be seriously considered.

Contamination of Sample before Irradiation. This kind of contamination can occur in any method. The laboratory regularly determining traces of lead, for example, will take steps to keep for this purpose one laboratory lead-free, and therefore prohibits as far as possible in that laboratory any significant quantities of lead compounds. In using the activation method, however, the radiochemical separations involve the use of "carrier" quantities of the element being determined. Thus the *radiochemical* laboratory is normally "saturated" with the element being determined at any one time—a position quite different from the normal trace-element laboratory. It is imperative, therefore, that samples for analysis be kept away from the radiochemical laboratory, and weighing and packing of samples, in-

deed any treatment of them prior to the sealing of the samples in their polythene or silica ampoules, must be carried out in a separate room *other than* the laboratory in which the subsequent radiochemical separations will be performed. Similarly the analyst should wash, and change to some degree, depending on the level being determined, before proceeding from the radiochemical laboratory to the sample preparation and weighing room. Clearly also a gravimetric determination of chemical yield, the usual method, involving as it does weighable quantities of the element concerned, must be carried out in a different balance room from that in which the samples are weighed out before irradiation. Small dry boxes can be of assistance sometimes where space is a difficulty, but the problem must be appreciated.

Surface contamination of a sample is, of course, readily overcome by dissolving the surface layer and discarding it. Such a step is applicable both to activation and conventional methods.

Contamination after Irradiation. As pointed out earlier, the only contamination that is significant after irradiation involves the pickup of *radioactive* material. If the latter is due to radionuclides of some element other than the one being determined, then this fact will make itself apparent when the radiochemical purity is checked. If, however, the contamination is by a radionuclide of the element concerned, then clearly this will give rise to an error in the results, and such contamination must be avoided. With radionuclides of half-life less than, say, 24 hours, the possibility of pickup in succeeding experiments is relatively small as, even where washing of equipment is imperfect, the activity decays by at least a factor two for each 24 hours that elapse. It is as if, in a conventional laboratory, concerned, say, with trace-lead determinations, a current of air swept through the laboratory and gradually cleaned up all equipment *and reagents* from lead! With radionuclides of increasing half-life, the decay factor becomes less valuable. Fortunately, however, it is still possible very readily to monitor equipment, apparatus, and reagent solutions, etc., before starting a particular experiment, in order to make sure that significant contamination is absent. This is possible because of the sensitive methods of detecting radioactivity that are available. Using the same basis for comparison as before, i.e., with the conventional lead determination, it is as if there were an instrument available that could be put near to a beaker or reagent solution which would immediately indicate that 1 μg of lead was present!

It seems reasonable to say, then, that, before any set of determinations by radioactivation, one can make certain that there is no signifi-

cant radiochemical contamination of the laboratory. Nevertheless, contamination *within* a set of determinations *can* occur, and, although this would have to be by carelessness, it is worth considering the problem and how it is best avoided. As described earlier, the samples are irradiated together with standards. It is rarely possible to get standards that contain the element required at the level being determined in the sample. Much more likely is it that the level in the standard may be several orders of ten greater than that in the sample (e.g., in the determination of 0.1 ppm of arsenic, the standard might be 10.00 mg of arsenious oxide). If at the dissolution or any subsequent stage even small quantities of the standard should be allowed to enter the sample, the result on the latter will be completely vitiated. To avoid this, the standards should be left sealed in their container until all the radiochemical separations, and even a preliminary activity measurement, for the samples have been performed. The general aim is to avoid cross-contamination, first of all by careful work and second by handling, in any one batch of experiments, *similar* activity levels of the particular element concerned.

ACCURACY AND PRECISION

There have been rather few papers published in which the *accuracy* of the activation method has been adequately demonstrated. The reason for this is easy to understand, as the determination of accuracy requires that the method be applied to samples whose content of the element concerned is known with *complete* confidence. It is rare that such samples are available at the trace-element level. Some papers have given estimates of accuracy as $\pm 2\%$; others up to $\pm 10\%$. Even the value of $\pm 10\%$, if true, would be adequate in many cases of trace-element determinations.

Some results to be quoted later in this chapter may help to give some justification for thinking that in favorable cases the accuracy of the activation method need be no worse than $\pm 10\%$. There is much better documented evidence on the *precision* attainable by the activation method. Perhaps the most useful paper in this connection is one by A. P. Seyfang [13] on the determination of U^{235}. Taking seven weighed portions of pure U_3O_8, of natural isotopic composition, Seyfang determined the U^{235} content with a coefficient of variation of only $\pm 0.53\%$, of which $\pm 0.44\%$ was the expected precision of the counting only. It would certainly seem that there is no reason to expect, in activation analysis, a precision very much worse than that

actually associated with the statistics of counting. The same paper incidentally gives some figures showing the accuracy of the activation method, as results are compared with mass-spectrometer determinations.

APPLICATIONS

The neutron-activation method has been used in several different fields of trace-element determinations. It is not the intention here to give a complete bibliography, as this is better dealt with elsewhere (see, for example, the biennial reviews on nucleonics in the journal *Analytical Chemistry*).

Instead, some of the more recent work at Harwell on this subject will be discussed, dealing with the applications under the two general headings of geochemical and industrial interest. It must not be thought that these are the only fields of application—the work of Tobias and Dunn [14] and Smales and Pate [11] on trace elements in biological material disproves that.

The early work of Brown and Goldberg,[15,16] who determined gallium, palladium, gold, and rhenium in iron meteorites, still serves as an excellent application of the activation method to the geochemical field, and it was perhaps a little disappointing to find, in an otherwise excellent textbook on nuclear geology,[17] no discussion of the activation method, although other nuclear techniques of analysis were dealt with.

More recently, methods for determining nickel, copper, and cobalt by radioactivation have been developed by my colleagues and me, and applied to a wide range of geological samples. In this case the three elements were determined on the same irradiated specimen by using a composite radiochemical separation scheme. The half-lives of the radionuclides used are 2½ hours, 12.8 hours, and 5.3 years, respectively, and this made it possible to deal adequately with the successive radiochemical separations in that order. Only one set of counting equipment was needed, and in fact an automatic eight-position turntable, designed and built by the Electronics Division of AERE, was used in conjunction with a printing mechanism so that decay curves could be obtained automatically on eight samples at a time.

Determinations of the three elements were made on specimens of marine sediments, oceanic rocks, and meteorites in an attempt to shed some light on the possible meteoritic origin of the nickel in the marine sediments.[18] A summary of the results for the three ratios involving nickel, cobalt, and copper are given in Table III, from which it was concluded that there was little significant contribution of meteoritic

TABLE III

RATIOS INVOLVING NICKEL, COPPER, AND COBALT

Material	Number of Samples	Ni:Co Average Ratios	Ni:Co Range	Ni:Cu Average Ratios	Ni:Cu Range	Cu:Co Average Ratios	Cu:Co Range
Red clay	11	3.1	1.6–7.1	0.85	0.4–1.3	3.7	1.3–5.7
Globigerina ooze	33	4.1	1.8–7.0	0.77	0.4–1.6	5.4	2.0–11.0
Manganese nodules	7	4.3	1.4–6.5	1.2	0.4–2.4	4.3	0.6–7.0
Oceanic rocks	9	2.3	1.4–4.3	1.6	0.2–3.1	2.7	0.7–6.9
Average for igneous rocks		3.5		1.1		3.0	
Meteorites (stones and stony irons)	9	22.7	12–52	100	75–119	0.24	0.2–0.4
Average for meteorites		13.1		92		0.14	

material to these sediments. Because of the sensitivity of the activation method, it was possible to determine all three elements at the level of a few parts per million and upwards, with adequate precision on only a few milligrams of sample, which was available only in rather limited amounts for the marine sediments and meteorites.

The same methods have been applied [19] to two rock samples, a granite and a diabase, which have had extensive circulation and which might well prove eventually to be of value as "standard" samples in the sense described earlier under the heading "accuracy." At the present stage, however, information is being built up which might assist in fixing the true trace-element content, and it is in this spirit that the values obtained by activation are given here. The figures are compared with the "recommended" values of Ahrens [20] (mainly spectrographic determinations) in Table IV.

TABLE IV

RESULTS FOR NICKEL, COPPER, AND COBALT IN GRANITE G_1 AND DIABASE W_1

	G_1			W_1		
	Ni	Cu	Co	Ni	Cu	Co
	parts per million			parts per million		
Recommended values (Ahrens) [20]	(5)	11	(4)	90	110	36
Activation values	1.0	9.4	2.1	73.5	112	53
	1.4	9.5	2.5	75.1	109.8	45.4
			2.2	70.5		48.1

In an attempt to measure the *accuracy* of these determinations of nickel, copper, and cobalt, standard steels were analyzed. It is appreciated that the level in the steels is somewhat higher than one would

wish, but at least it is better to know the accuracy at that level than not at all. The results are given in Table V.

TABLE V

DETERMINATIONS ON BRITISH CHEMICAL STANDARD STEELS

		Ni %		Cu %		Co %	
	Sample	BCS Figure	Activation	BCS Figure	Activation	BCS Figure	Activation
149	Pure iron					0.010	0.011
260	Pure iron	0.009	0.010				
212	Alloy steel	0.04	0.033	0.10	0.109	0.02	0.021
218	Carbon steel	0.17	0.178	0.16	0.157	0.01	0.014
258	Low-alloy steel	0.048	0.048	0.185	0.188		
241	Alloy steel			0.15	0.155		
220	Alloy steel			0.14	0.140		

Other applications of geochemical interest have been the determination of rubidium and cesium in sea water,[21] the determined levels being 0.12 and 0.005 ppm, respectively, and the determination of minute amounts of thorium in minerals.[22] One of the aims of this method, like that of an earlier one for uranium,[23] was to make possible age determinations on isolated individual minerals from rocks.

Turning to industrial applications, there is at present very great interest in trace-element determination in pure metals. In atomic-energy work, for example, a current interest is in the use of alkali metals as liquid coolants in reactors. Any impurity element that would give rise to a long-lived radionuclide, when used in a reactor, would be a nuisance. Activation methods have been developed [24] for several of the possible impurities, and a typical analysis of a sodium–potassium alloy showed the following results in parts per million:

Mercury <0.5, Chromium <0.2, Rubidium 77, Cesium 0.15,

Silver <0.02, Antimony <0.001, Strontium <0.5, Cobalt 0.02

Another field of pure materials of great current industrial interest is semiconductors. Since the first papers on the determination of arsenic in *germanium*,[4,25] activation has also been used for other impurities in that element.[26,27] Silicon is now coming into prominence as a semiconductor material, and an excellent article on the determination of impurities in silicon, by activation followed by gamma spectrometry,[28] has appeared quite recently. This technique of gamma spectrometry is a valuable addition to the armory of the

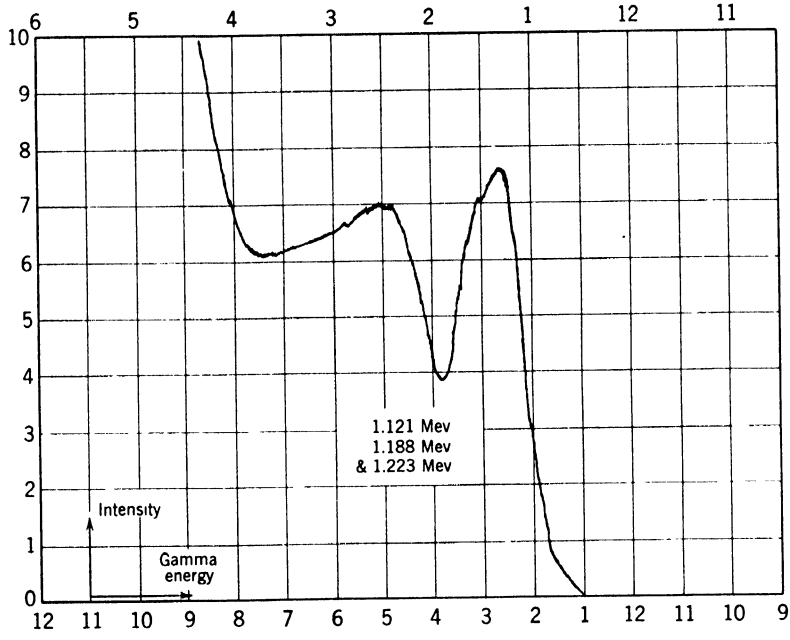

FIG. 1. High-energy gamma spectrum of Ta^{182}.

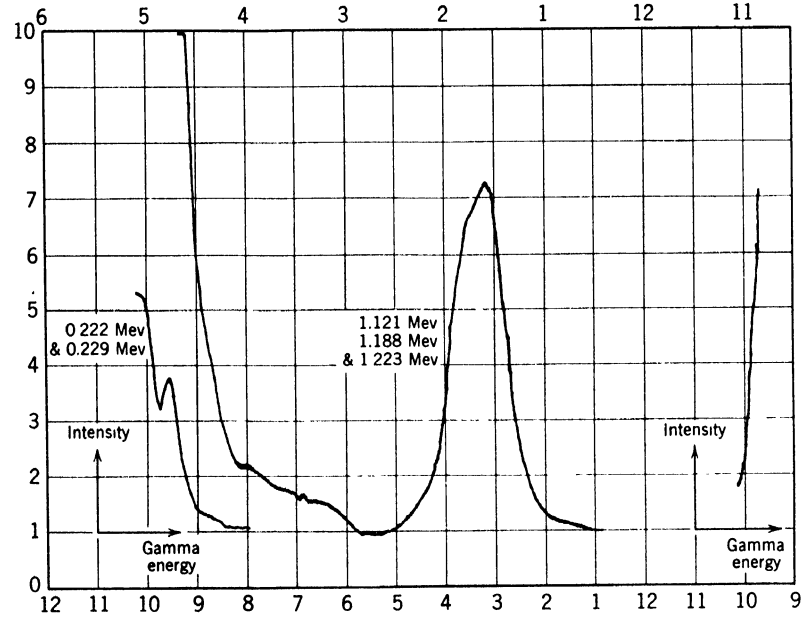

FIG. 2. High-energy gamma spectrum of Ta^{182} (compensated for Compton continuum).

radiochemist, as it is sometimes possible to avoid, or at least simplify, the radiochemical separations needed after activation, as already pointed out for tantalum [29] and sodium.[21] In the silicon work, Morrison and Cosgrove [28] determined zinc, arsenic, tungsten, iron, sodium, potassium, and tantalum, in some cases at levels of less than 1 ppm,

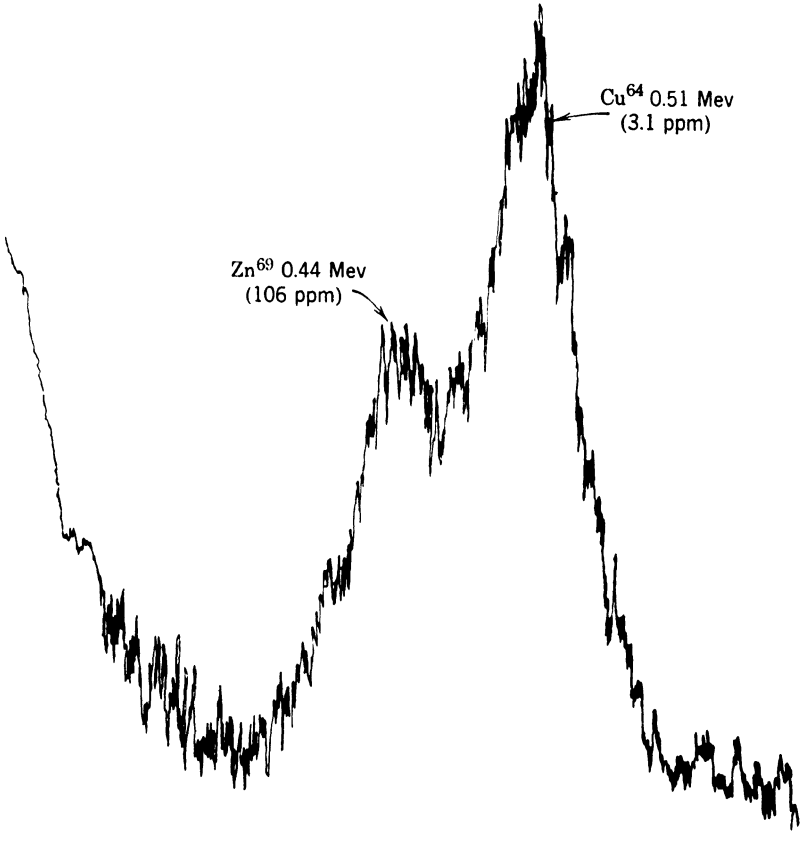

FIG. 3. Double-crystal gamma spectrum of irradiated silicon.

using a sodium iodide crystal scintillation spectrometer. An improved version of the scintillation spectrometer has been described by Peirson,[30] using two detecting crystals, one of sodium iodide and one of anthracene, in a device that allows for automatic subtraction of that part of the gamma spectrum due to Compton scattering. The net result is that a radionuclide emitting a single gamma ray gives a spectrum consisting of a *single* peak (photopeak) instead of the more complicated spectrum obtained on the conventional scintillation spectrometer. The spectrum for Ta^{182} on an instrument of the latter

type can be seen in Fig. 1, while that for the same sample on the Peirson instrument is shown in Fig. 2. The greater simplicity of the latter is readily apparent, and it will be appreciated how much more significant this is when a *mixture* of radionuclides is being measured. Figure 3 shows the gamma spectrum on the Peirson spectrometer of an irradiated silicon sample, and the photopeaks due to Cu^{64} and Zn^{69} can be seen. By comparison with standards of pure copper and zinc, irradiated with the samples, the copper content was determined as 3.1 ppm and the zinc as 106 ppm. Although Morrison and Cosgrove were able to get excellent sensitivity by the gamma-spectrometry technique, the *fullest* sensitivity of the activation method necessitates radiochemical separations followed by beta counting, because much smaller amounts of radioactivity can be measured this way.

CONCLUSIONS

The limitations of the activation method have already been explored in some detail but it is my opinion that the applications will grow steadily as analytical chemists become aware of its advantages and as training in elementary radiochemistry becomes more widespread. The techniques are quite easily learned, the cost of equipment is not outrageous by modern analytical standards, the cost of irradiation per sample is quite small, very few special laboratory modifications are required, and any health hazard can be kept well under control at the levels required for most applications.

It seems quite clear that the study of trace elements in many fields will be assisted by the application of the activation method, and its advantages can be summarized:

1. Very high potential sensitivity.
2. Specificity—the element being determined is measured using a radionuclide whose identity can be confirmed by decay and energy measurements.
3. Freedom from "reagent-blank" difficulties.
4. Nonquantitative separation methods can be used because a "chemical-yield" determination is made.
5. Chemical operations with submicrogram quantities are avoided, by the use of carriers.

Nevertheless it is only *one* of the possible methods available to the analyst, and certainly in our laboratory, when a new analytical problem occurs, the various approaches are considered impartially and the

most suitable one (or possibly a combination) chosen. As an example, emission spectroscopy has very great advantages where a *range* of elements has to be determined speedily, but the *standards* used in the emission spectroscopy work may well have been provided by activation analysis.

It is the balanced judgment of the experienced analyst, choosing from the remarkable range of possibilities now open to him, that is necessary to establish the chemical *facts* on which future research work in many fields will grow.

DISCUSSION

Discussion Leader
Erik Odeblad
Karolinska Medikokirurgiska Institutet
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After this excellent lecture I am going to say a few words on the possibility of performing activation analysis with charged positive particles.

There are several types of charged particles which can be accelerated in the cyclotron or obtained from natural sources. We have the proton, the neutron, the alpha particle, and the triton, and they can all induce nuclear reactions, and accordingly be used for activation purposes.

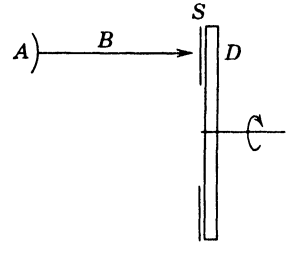

FIG. D1. Activation of sample by means of alpha particles *A* or cyclotron beam *B*. *D* is the rotating disk, and *S* the sample.

I have had the opportunity of working with these kinds of particles. This work was started in collaboration with Dr. Cornelius A. Tobias of the Donner Laboratory in Berkeley. I have continued the work in Stockholm. What we do is to use natural α particles or the deflected beam *B* from a cyclotron (Fig. D1). We place a specimen at *S*. Actually the specimens move in front of the beam on a rotating disk *D* carrying the specimens (including standard specimens). This arrangement makes the irradiation more homogeneous. The beam can also be deflected magnetically to insure homogeneous irradiation. All kinds of nuclei in the sample can be

activated. Another thing that complicates the method very much is that one type of nuclei often gives rise to four or more different radioisotopes.

It is necessary to look just a little into the relationship between the cross section of nuclear reactions and the energy in the incident particles in the beam (Fig. D2). Here the energy is plotted on the abscissa and the cross section on the ordinate. Owing to the potential barrier of the nucleus, the cross section becomes appreciable at C in Fig. D2, rises to a maximum M and tends to decrease, R.

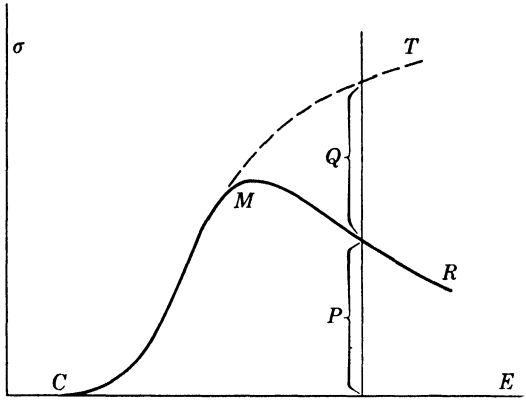

FIG. D2. Relationship between cross section of nuclear reactions and beam energy.

P is the particular nuclear reaction we are interested in. The other part Q of total cross section, up to T, is shared with other nuclear reactions. When looking for any particular nuclear reaction P, it is good to choose a working point, where we have small competition Q of radionuclide formation.

If particles are entering a thick specimen, they finally lose their energy. It is then necessary to introduce a correction for different energy loss when comparing standard and unknown sample. In histologic sections, differences in energy loss can be neglected, but, when thick samples are used, for example, tissue ash in quantities of one gram or so, the correction must be introduced.

N, P, and Fe are elements that can be easily detected with this technique. For the first two elements it seems quite certain that amounts of 10^{-12} gram can be recorded using a cyclotron. Be and Al can be detected in amounts of 10^{-3} to 10^{-6} gram using natural alpha sources.

These small recorded amounts of N, P, and Fe were detected photographically and were present in small tissue volumes, which means that their concentration is not necessarily small.

Figures D3 through D5 show reproductions of autoradiographs. Figures D3 and D4 are autoradiographic pictures of deuteron-activated sections of spleen and ovary 10 days after activation, and one can see that the activation is very homogeneous, depending on the position of sample in the beam. (The cyclotrons are often unstable both

FIG. D3. Autoradiograph of deuteron-activated spleen.

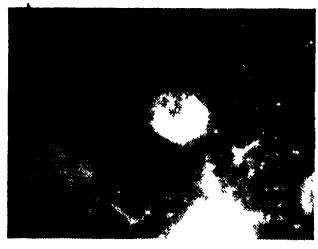

FIG. D4. Autoradiograph of deuteron-activated ovary.

FIG. D5. Autoradiograph of deuteron-activated iron in a mouse kidney.

in energy and in direction of the outgoing beam.) Figures D3 and D4 show the presence of phosphorus in tissues activated by reaction $P^{31}(d,p)P^{32}$. Figure D4 shows activation of a fingerprint, illustrating contamination hazards. Figure D5 is one of the few examples I can present on the detection of iron with the activation–autoradiography method. The section is from a kidney of a mouse 4 months after deuteron activation. The reaction was $Fe^{56}(d,2n)Co^{56}$. In this case as in most others, the sections were mounted on thin aluminium foils. It is very difficult to establish the exact nature of the induced activities in this case and in other cases where the induced activity is very

low. For that purpose we had to operate a scintillation spectrometer during several hours, up to 24 hours, to get the scintillation spectrum of the induced activity.

QUESTIONS AND ANSWERS

ISABEL TIPTON (*University of Tennessee*): Dr. Smales mentioned the need for standard samples in analyzing trace-material distribution, a subject close to my heart. We have given some thought to tissue standards, and we have not found any answer at all. If anyone has any suggestion as to what we can use for universal standards to submit to various laboratories for analysis, I would appreciate very much if he would get in touch with me.

ERIK ODEBLAD: The question of standards is important, but difficult to solve, I believe. I assume you would like to have some standards, to be the same for the various laboratories. Parts of a universal standard could be distributed among the various institutions. One part for Brookhaven, and another part for some other institution, and so on.

I may mention that we have tried various types of standards in our laboratory, and for the charged-particle activation of phosphorus we have used gelatin containing sodium phosphate as a standard for our autoradiographic work. For iron, we really have no standard as yet.

W. WAYNE MEINKE: I am sure that many of the people in the audience are listening to this talk about high sensitivity with activation analysis and are saying: But we do not have a reactor; we cannot go to Oak Ridge for irradiation, and therefore we must forget about this method. This may be true at the present time, but I would like to mention that during the past year or so, since the Atomic Energy Act has been changed, there has been a veritable "explosion" in this field of reactor development. There are now a number of research reactors being built, and within the next year or two there will be more than a dozen in operation. At the University of Michigan we are building a research reactor that should start functioning in the near future.

The point is: The analyst should work with the engineering group during the planning of the reactor. I know from our experience at Michigan that several years of planning went into our building and reactor. We will have facilities, though, whereby we will be able to receive a sample delivered directly into a chemistry hood 6 seconds after it has come from the reactor. If we had brought up our plans for this pneumatic tube apparatus after the engineering plans had been completed, we would not have gotten anywhere and would probably have had to carry the sample from the reactor to the laboratory.

Those people here who represent the companies that are getting into the reactor field should help early in the planning stage, so that they can make use of the neutrons available for analysis.

A. A. SMALES: A question was asked about the possibility of determining boron by neutron activation. I would like to say that we have done a little work on this, and we have what I would call a "potentially useless" method!

The α-particle tracks are obtained by irradiating 6×10^{-9} gram of boron, in contact with a nuclear emulsion, in a neutron flux of 5×10^{9} neutrons per square centimeter per second. The boron was in the form of a small drop of a dilute aqueous solution evaporated to dryness so that the total area covered was about 1 mm^2. It is possible to obtain a calibration graph by plotting the number of alpha tracks against mass of boron irradiated, in the range 10^{-8} to 10^{-9} gram of boron.

The "useless" part of this is that it can only be done if there is virtually no solid matter present with the boron. The determination is beautiful for boron in distilled water, but in practice one never wants to do this!

CORNELIUS A. TOBIAS: Dr. Kruger of the University of Illinois has developed radioautography analysis to determine the distribution of boron-containing compounds in brain tumors of animals. He obtained α-particle radiographs which may be superimposed on histological sections of the specimen. At the present time, this seems to be the only reliable method of evaluating B^{10} distribution in biological specimens.

BERT L. VALLEE: Do you believe that the National Bureau of Standards sample could be used as a standard in activation analysis?

A. A. SMALES: On that point I think that the main difficulty facing Dr. Tipton, Dr. Vallee, and others is that we do not have standard samples containing the elements at the low level at which we are interested. Generally speaking, the concentrations are too high in the National Bureau of Standards samples. We would prefer something with elements of interest at the level of parts per 10^8, I think, and the sample must be absolutely homogeneous.

ERIK ODEBLAD: Dr. Meinke has used low-level activation with neutrons, and I want to point out further that it is not necessary always to use piles and cyclotrons for activation. One can use, for example, a 1-curie polonium preparation, which radiates α particles and should allow the determination of beryllium at the level of about 1 μg by the $Be^9(\alpha,n\gamma)C^{12}$ reaction.

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Microbiological Techniques

Inorganic ions

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ABSTRACT All micro-organisms require certain metal ions for growth. If a culture medium complete with respect to all nutritional requirements except a single essential metal ion is prepared, growth in such a medium is proportional, within limits, to the concentration of that ion supplied. Since appropriate micro-organisms grow rapidly and reproducibly, and growth or suitable by-products of growth can be measured readily, such growth responses provide potential methods of high sensitivity and specificity for each of the physiologically important trace metals. Past efforts to devise such methods are reviewed, and the experimental difficulties in their application are discussed. Complications arise chiefly from sparing effects of one metal ion on the requirement for another, from antagonistic effects observed between certain related metal ions, and from the occurrence of the metal ions in various biological products as chelates of varied stability, and consequently of varied availability to the test organisms. Recognition of these difficulties provides means for their circumvention, and, where other methods are unsatisfactory or nonexistent, microbiological procedures may provide useful alternative methods.

* Formerly with Biochemical Institute, University of Texas, and Clayton Foundation for Research, Austin, Texas. Dr. Snell was unable to attend the symposium. His talk was presented by Dr. Alvin Nason, whose own paper and discussion appear at the end of this chapter.

INTRODUCTION

The requirement of micro-organisms for inorganic nutrients was revealed by the earliest studies of microbial nutrition. Pasteur, for example, observed that growth of yeast was enhanced by addition of yeast ash to his defined medium. Subsequent investigators readily demonstrated requirements for ions such as K^+, Mg^{+2} and PO_4^{-3}, which are required by some micro-organisms in *comparatively* large amounts. Identification of metal ions required in smaller amounts—the trace or micronutrient elements—was delayed by the lack of techniques for removing traces of these ions from culture media. It is not certain today that we know *all* of the micronutrient elements required by any living organism, and the number of micro-organisms for which detailed information concerning inorganic requirements is available is small indeed.

Much of the early work dealing with inorganic requirements of micro-organisms has been reviewed elsewhere: yeast,[1,2] fungi,[3–5] bacteria,[6] algae.[7,8] A listing of metallic ions shown to be required for one or another microbial species is given in Table I. Requirements for inorganic nonmetallic substances and anions such as PO_4^{-3} (required by all living organisms) and SiO_2 (required for growth of diatoms) are not included. It is certain that the list of Table I does not include all the metal ions that will eventually be found necessary. For ex-

TABLE I

METAL ION REQUIREMENTS OF MICRO-ORGANISMS

Metal Ion	Frequency of Demonstrated Requirement	Illustrative Organism Requiring	Reference
K^+	All organisms investigated *	Lactic acid bacteria	(9)
Mg^{+2}	Very high	*Aerobacter aerogenes*	(10)
Mn^{+2}	High	*Lactobacillus arabinosus*	(9)
Fe	High	*Pseudomonas aeruginosa*	(11)
Zn^{+2}	Moderately high	*Streptomyces griseus*	(12)
Cu^{+2}	Low	*Saccharomyces cerevisia*	(13)
Ca^{+2}	Low	*Chlorella*	(14)
Mo	Low	*Chlorella*	(14)
Na^+	Low	*Chroococcus*	(15)
V	Very low	*Scenedesmus obliquus*	(16)
Ga	Very low	*Aspergillus niger*	(17)

* In a few organisms, Rb completely replaces K; Rb spares K in all organisms studied (see text).

ample, a direct requirement of any micro-organism for Co^{+2} has not been demonstrated.* Many algae and bacteria, however, require externally added supplies of the cobalt-containing vitamin B_{12}. Closely related organisms synthesize this vitamin and undoubtedly require it in their metabolic processes, and hence must require a source of cobalt. So minute is the requirement, however, that the amount supplied as an impurity in spectroscopic iron, when salts of the latter are added to supply the minimum requirement for iron, would exceed by several times the cobalt requirement for growth.[18] Similarly, boron is required by many higher plants, but has not yet been demonstrated as a requirement of micro-organisms. In general, those metal ions most frequently found essential for micro-organisms are those required in largest amount, and it remains today an exacting task to demonstrate requirements for elements such as copper, molybdenum, zinc, and even manganese. To complicate the task, substantial differences exist from one organism to another in their quantitative requirements, and probably also in their qualitative requirements, for inorganic ions. We shall consider bases for some of these differences later.

In a medium that lacks a single essential ion but is otherwise adequate, growth of an appropriate test organism fails to occur. Upon addition of the essential ion in graded amounts, the growth obtained will increase regularly with concentration until a maximum cell yield determined by other factors is reached. The realization that such growth responses to essential inorganic ions could be used as a semiquantitative or even quantitative method of estimation is not of recent origin. Butkewitsch in 1909 suggested use of *Aspergillus niger* as a qualitative biological indicator of available potassium and phosphorus in soils,[20] and the method was rather widely studied and used in the ensuing period. References to several of these early procedures are given by Mehlich, Fred, and Truog,[21] who showed excellent correlation between available potassium determined in soils by assay with *A. niger* and that indicated by field tests and chemical procedures. Similar results were obtained in phosphate analyses of soils with *Cunninghamella blakesleeanus*.[22] These methods served as prototypes for more recent procedures such as that of Nicholas,[23] who uses *A. niger* or *Penicillium glaucum* in a similar fashion to determine available Fe, Cu, Zn, Mn, and Mo in soils, with results that show excellent

* A growth response of several bacteria to cobalt has been observed in media that contain chelating agents such as 8-hydroxyquinoline.[19]

correlation with availability of these trace elements as indicated by growth of higher plants.

Concurrent with these studies, the past 15 years have seen an enormous increase in our knowledge of the organic nutritive requirements of bacteria and other micro-organisms. Each of the B-vitamins and the common amino acids is required by one or another such organism, and accurate and reliable methods for the quantitative determination of these substances based upon growth of micro-organisms have been widely used and accepted.[24-26] The principles that govern use of these methods are no different from those involved in the determination of essential inorganic ions by similar methods, and the general techniques required are essentially identical. Aside from the technical problem of obtaining ion-free media, therefore, there appear few reasons why microbiological techniques for determining inorganic trace elements should not be much more widely used, and applied not only to the problem of determining available ions in soils, but to the quantitative determination of these elements from materials of all types. Few such methods have, in fact, appeared, and it will be more useful to discuss here several special problems that relate to use of micro-organisms for this type of assay than to tabulate the several inadequately tested methods that have appeared. A few of these methods will be discussed later. These problems relate to the *specificity* of the requirements for inorganic ions, to the phenomenon commonly termed *ion antagonism,* and to the *availability* of inorganic ions in the presence of organic materials, such as chelating agents. These factors, all of which contribute to the observed variability in the requirements of micro-organisms for inorganic elements, are discussed briefly below.

THE FUNCTION OF INORGANIC IONS IN NUTRITION

The high requirement for substances such as Ca^{+2} and PO_4^{-3} in higher animals results from their use as major structural materials in bone formation. Similarly, the microscopic diatoms require silicates for skeleton formation. For most micro-organisms, however, there are no major inorganic structural elements; the nutritionally essential metal ions, like the trace elements required by higher plants and animals, serve principally or exclusively as activators or active groups of a great variety of enzymes.

This fact has interesting and important consequences in nutrition. Some enzymes, such as the heme enzymes, may require a single specific

metal ion as activator; for others, a variety of different metal ions may serve in this capacity. Examples of the latter category are a soluble aldehyde dehydrogenase ($CH_3CHO + DPN^+ + H_2O \rightarrow CH_3COOH + DPNH + H^+$) of yeast that requires either K^+ or Rb^+ for activation and is inhibited by Na^+ or Cs^+,[27] a histidine deaminase (histidine → urocanic acid + NH_3) from a soil bacterium that is activated by Zn^{+2}, Cd^{+2}, or Hg^{+2}, but is inhibited by Mn^{+2} and Fe^{+3},[28] and the enzyme "isocitrase" (isocitrate ⇋ glyoxylate + succinate) from *Pseudomonas aeruginosa* that requires either Mg^{+2}, Mn^{+2}, Fe^{+2}, or Co^{+2} for activity, Mg^{+2} being the most effective.[29]

A great many enzymes belonging both to the metal-specific category and to the category activated by several different metal ions are known; a partial listing has been made by McElroy and Nason.[30] Enzymes of the metal-specific category we may view as those possessing such a specifically constructed active center that it can combine with only a single ionic species to produce a catalytically active metalloenzyme. Enzymes of the metal-unspecific category we may view as having a less exacting architecture that permits them to combine with a restricted number of related ions to form catalytically active metalloenzymes, as illustrated for an enzyme activated by K^+ and Rb^+ in Fig. 1. An

$$\text{Protein}_1 + \begin{cases} K^+ \rightarrow \text{K-enzyme}_1 & \text{(active)} \\ Rb^+ \rightarrow \text{Rb-enzyme}_1 & \text{(active)} \\ Na^+ \rightarrow \text{Na-protein}_1 & \text{(inactive)} \\ Cs^+ \rightarrow \text{Cs-protein}_1 & \text{(inactive)} \end{cases}$$

FIG. 1. Nonselective activation or inhibition of a hypothetical enzyme by related alkali-metal ions.

enzyme of this type may also combine with other related ions (e.g., Na^+ or Cs^+, Fig. 1) to form an enzymatically inactive metalloprotein. These latter ions would function as inhibitory metal ions, and, depending on the reversibility of the association reaction, the inhibition might be either noncompetitive (for irreversible association reactions) or competitive (for freely reversible association reactions). Where the enzyme is required for growth, the general relationship between metal activator and inhibitor described by Fig. 1 provides an explanation for the phenomenon of *ion antagonism*.[31, 32]

Since different organisms vary in their enzymatic constitution and protein specificity, we should expect variability in metal-ion requirements from one organism to another. Since the chief function of trace elements in the nutrition of plants and micro-organisms is as activators

for enzymatic processes, we must also expect from the above considerations to find that the requirement of a single organism for a given metallic ion will vary considerably, depending on the nature and amounts of other metal ions present.[7,31–33] Such variability arises from (1) partial or complete functional replacement of one ion by another; (2) enhancement of the requirement for an essential ion by a related inhibitory (antagonistic) ion; and (3) effective bypassing of a metalloenzyme-catalyzed process: e.g., by provision in the growth medium of the product of this reaction. Conversely, the necessity for metabolizing a given product might enhance the requirement for a given metalloenzyme, and hence for its metal component. Some examples of variability of these various types follow.

VARIABILITY IN INORGANIC-ION REQUIREMENTS

Variability between Different Organisms. Related organisms grown under similar conditions may vary appreciably in their quantitative requirements for inorganic ions, even though cell yields are similar. Such differences, a few of which are illustrated in Table II,

TABLE II

VARIATIONS IN METAL-ION REQUIREMENTS OF DIFFERENT ORGANISMS IN SIMILAR MEDIA

Metal Ion	Organism	Approximate Requirement for Maximum Growth, ppm	Reference
Mg^{+2}	*Pseudomonas aeruginosa*	2	
	Aerobacter aerogenes	3	
	Azotobacter chroococcum	32	(34)
Mn^{+2}	*Lactobacillus arabinosus*	0.1	
	Lactobacillus casei	0.03	
	Streptococcus faecalis	<0.03	(8)
K^{+}	*Lactobacillus casei*	30	
	Streptococcus faecalis	15	(35)

must reflect differences in enzymic constitution (i.e., in metabolic pattern) and in the specificity of species-specific proteins for metal ions.

Variability Due to Partial or Complete Functional Replacement of One Metal Ion by Another. An apparently complete functional replacement of K^{+} by Rb^{+} occurs for growth of *Streptococcus faecalis*

as shown in Fig. 2. The phrase "apparently complete" should be emphasized in all instances of this kind, since contaminating traces of K^+ may fill a vanishingly low specific requirement for this ion. An organism of this type obviously would not be valuable for the specific estimation of K^+. Results of its use as an indicator of "available potassium" in soils might not be misleading, however, since potassium is more common than rubidium in soils, and since rubidium, although

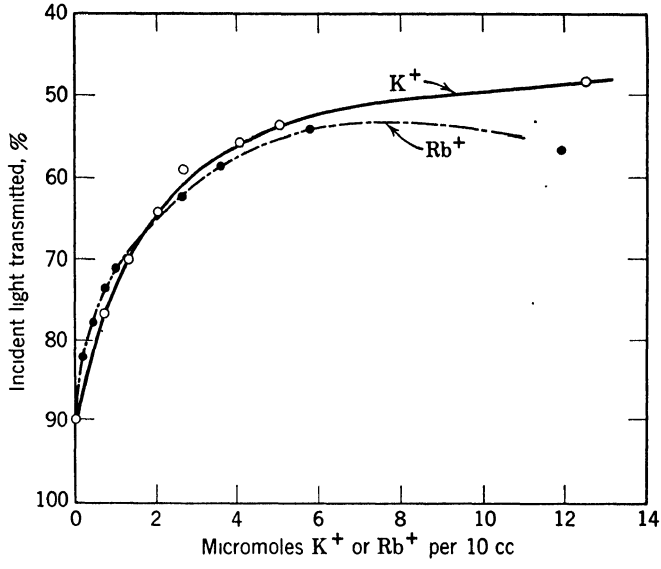

FIG. 2. Comparative effects of K^+ and Rb^+ on growth of *Streptococcus faecalis*.[35] (Redrawn by permission of the *Journal of Biological Chemistry*)

not a complete substitute for potassium for growth of higher plants, frequently does reduce ("spare") markedly the potassium requirement.[33,36] A similar complete replacement of K^+ by Rb^+ occurs in the nutrition of some varieties of *Chlorella*.[36] For this latter organism,[14] and also for the phycomycete, *Allomyces*,[38] a requirement for Ca^{+2} for growth can be met equally well by Sr^{+2}. As pointed out elsewhere,[31,32] it is meaningless to ask which is the essential ion in these cases. Whichever one is available can be used efficiently. Our prejudices favor considering K^+ rather than Rb^+, and Ca^{+2} rather than Sr^{+2} as essential simply because K^+ and Ca^{+2} are used by a wider array of organisms, or, in enzymatic terms, activate a wider array of enzymes. Such prejudices obscure the fact that living organisms apparently make more general use of metal ions in their environment than is indicated by their minimum nutritional requirements.

Organisms such as these cannot, however, be used for specific estimation of K^+ or Ca^{+2}.

Much more common than complete substitution of one metallic ion for another is the *partial* replacement (sparing effect) that one ion may show in relation to another. This is illustrated by Fig. 3. For *Streptococcus equinus* (Fig. 3*B*), Rb^+ does not fully replace K^+, as it

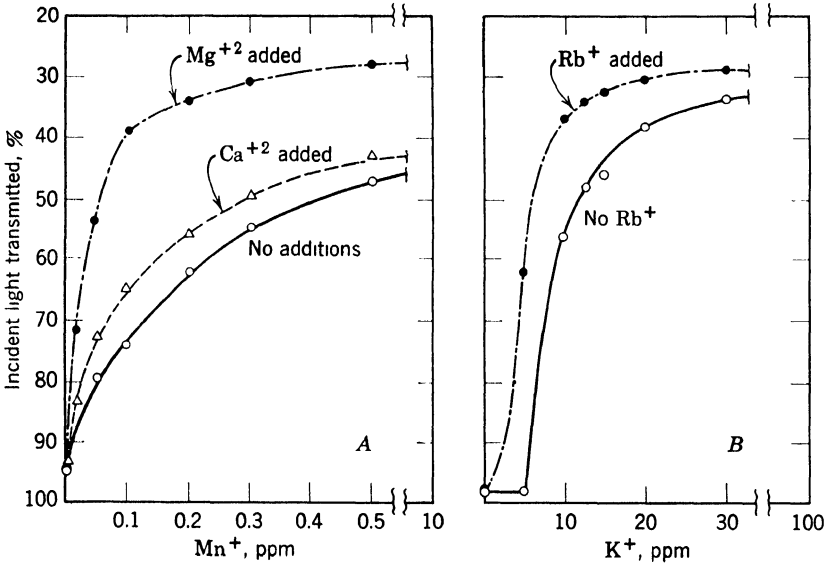

FIG. 3. (*A*) The effect of Ca^{+2} and Mg^{+2} on the Mn^{+2} requirement of *Lactobacillus arabinosus*. (*B*) The effect of Rb^{+2} on the K^+ requirement of *Streptococcus equinus* (*Leuconostoc mesenteroides* P-60).[32] (Redrawn by permission of the *Journal of Bacteriology*)

appears to for *S. faecalis* (Fig. 2); however, it does materially reduce the requirement for K^+, presumably because it fills some, but not all, of the enzymatic functions of K^+ in this organism.[32] Only in an appropriate medium containing optimum amounts of Rb^+ could this organism be used for the specific estimation of K^+. Similarly, the Mn^{+2} requirement of *Lactobacillus arabinosus* is greatly decreased by the presence of Mg^{+2} (Fig. 3*A*), while Ca^{+2} (and Sr^{+2}) have a definite but smaller sparing effect.[32] Thus, while neither Mg^{+2} nor Ca^{+2} has been shown to be required by *L. arabinosus* (both may prove to be required in small amounts on more highly purified media), an accurate assay for Mn^{+2} could not be conducted with this organism in a medium that lacked these metal ions. Many examples of this type involving additional metal ions are known.

Occurrence of this behavior as a general phenomenon emphasizes that knowledge of the nature and magnitude of sparing effects is essential to development of specific assay methods for trace metals. In the absence of such detailed knowledge, it would be safer for the analyst to err on the side of including an unnecessarily large variety of different metal ions related to known essential ions than to operate with minimal media that include only the known essential trace elements.

Variability Due to Functional Bypassing of the Requirement for a Metalloenzyme. Steinberg in 1937 observed that *A. niger* required much more molybdenum for growth with nitrate than when ammonium salts were the nitrogen source. He postulated that molybdenum was concerned with the reduction of NO_3^- to NH_4^+.[39] The observation has been repeatedly confirmed.[23,40] The validity of the postulated mechanism of the effect was established when it was shown that molybdenum was required specifically for the formation of nitrate reductase in *Neurospora crassa,*[40] *A. niger,*[40] and *Escherichia coli,*[41] that the purified fungal enzyme contained molybdenum,[42] and that, after inactivation by appropriate dialysis, the enzyme was reactivated specifically by molybdenum compounds.[42] Molybdenum is required for additional functions when NH_4^+ is supplied, but in smaller amounts, since one quantitatively important function of the substance is thereby eliminated.

Several other instances in which the requirement of an organism for a metal ion is reduced by addition of appropriate nonmetallic substances to the medium are known. Although the mechanism of these effects has not been investigated, one suspects that here, too, the function of certain metalloenzymes may be bypassed and the requirement for the metal ion thus decreased. Examples of this behavior include (1) the marked decrease in Mo requirement observed in nitrogen-fixing organisms when the necessity for fixing nitrogen is bypassed † by addition of ammonium salts,[43] (2) the significant decrease in the Mg^{+2} requirement of several bacteria when amino acids are supplied in the medium,[44] and (3) the increase in the requirement of *S. faecalis* for iron when serine is omitted from the medium.[45]

Effects of this nature indicate that direct bioassay without ashing of samples rich in organic materials (e.g., tissue extracts, etc.) might very probably give high results for trace metals if conducted in simple

† Nitrogen fixation and nitrate reduction appear to have some common features, and it may be that the demonstrated role of Mo in the latter explains also its apparent role in the former.

inorganic media. Assays of organic materials should be specifically tested for reliability by comparison of results on ashed and unashed samples.

Organic materials in the sample for assay may also change the response to a trace metal through chelate formation. This effect is discussed on page 557.

Variability Due to Ion Antagonism. Counteraction of the effects (stimulatory or inhibitory) of one ion by another is referred to as *ion antagonism.* The phenomenon has been widely studied; the antago-

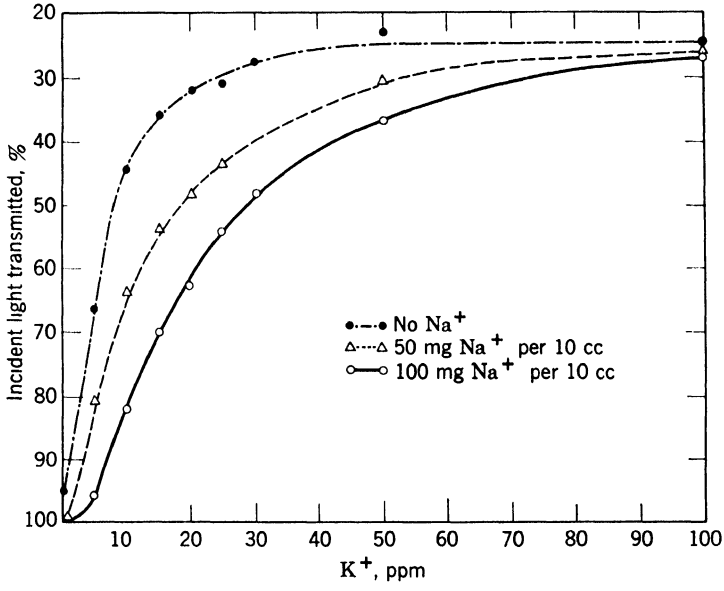

FIG. 4. The effect of Na^+ on the K^+ requirement of *Lactobacillus casei.*[35] (Redrawn by permission of the *Journal of Biological Chemistry*)

nistic ions vary from organism to organism.[46,47] MacLeod and Snell [31,32,35] showed that many instances of ion antagonism could be explained on nutritional grounds, i.e., ions that suppressed growth frequently did so by interfering with one or more essential metabolic (i.e., enzymatic) roles played by an ion required for growth. If, in Fig. 1, the functional metalloenzyme is required for growth, then K^+ (or Rb^+) is a nutritionally essential ion and Na^+ and Cs^+ would be antagonists. In Fig. 4 the effect of Na^+ on the K^+ requirement of *L. casei* illustrates the effect of an antagonistic ion (Na^+) in increasing the requirement for an essential ion (K^+). Ammonium ion has an effect similar to Na^+ in this and several other organisms.[35,18]

The effects are frequently complex, since a single "ion analog" may replace the related ion for some functions but antagonize it for others; the two effects may or may not be separately observable at different concentrations. For example, at low concentrations Rb^+ substantially decreases the requirement of *S. equinus* for K^+,[32] but at higher concentrations inhibits this organism by competing with K^+ for sites that specifically require this latter ion.[35] Similar phenomena undoubtedly occur between each of the essential cations and related, nonessential ions, and additional examples are available.[32,33]

Results of this nature indicate that, in quantitative bioassay with micro-organisms, attention should be paid to the possibility that the amount of an essential trace element is being underestimated, owing to presence in the sample of an excess of a related, antagonistic ion. In most cases, fortunately, the ratio of antagonistic ion to essential ion required for suppression of the response is higher than will be found in most samples of natural origin; this possible source of error should, however, be recognized.

Variability Due to Chelate Formation. The avidity with which cells of various organisms absorb inorganic ions varies considerably. For example, with some lactobacilli (e.g., *L. casei, L. arabinosus*), addition of sufficient citrate to an adequate medium inhibited growth because complex formation with Mn^{+2} and Mg^{+2} rendered the concentration of these ions insufficient for growth.[8] The toxicity could be alleviated by additional metal ions. However, although *S. faecalis* requires both Mn^{+2} and Mg^{+2} for growth,[48] it is not inhibited by the usual amounts of citrate.[8] We may consider with Hutner et al.[18] that absorption of metal ions involves chelate formation with constantly renewed chelating groups on the cell surface. Absorption of a metal ion involves a competition between ligands on the cell membrane and those in the medium. The chelates of Mn^{+2} and Mg^{+2} thus formed with *S. faecalis* must have considerably higher stability than those formed with *L. casei.*

This effect of chelating agents in increasing the quantitative requirements for certain trace metals has been used to demonstrate the requirement for the latter by some organisms.[8,18] The magnitude of the effects obtained varies, of course, with the nature and concentration of chelating agent as well as with the organism. Illustrative effects in two organisms where requirements in the presence and absence of chelating agent have been determined are shown in Fig. 5. Additional work is required to demonstrate that the metal ions required in the presence of chelating agents are the same in all cases

as those required in the absence of the chelating agent.[19,49,50] In some cases an appropriate complex or chelate of a metal ion may be *more* available than the free metal ion. Walker, for example, showed that, for *Chlorella,* Fe^{+2} added as its ethylenediamine tetraacetate complex or as potassium ferricyanide was more available than ferrous sulfate.[37] In contrast, the response of this organism to Mn^{+2} and Zn^{+2} was inhibited by ethylenediamine tetraacetate.

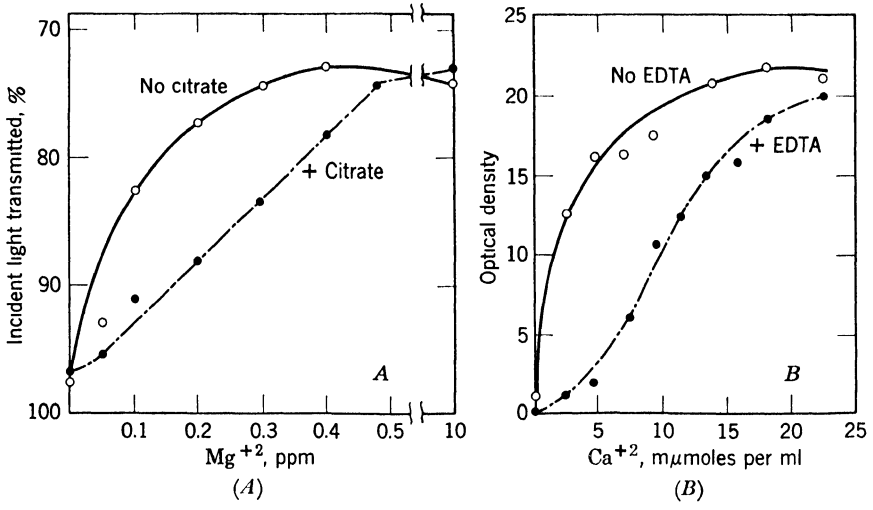

FIG. 5. (*A*) The comparative response of *Streptococcus faecalis* to Mg^{+2} in the presence and absence of citrate (20 mg citrate ion per ml).[48] (Redrawn by permission of the author and publisher) (*B*) The comparative requirement of *Chlorella* for Ca^{+2} in a glucose–urea–salts medium versus a glucose–urea–ethylenediamine tetraacetate(2.5 mg per 5 ml)–salts medium.[14] (Redrawn by permission of the author and publisher)

The significance of these considerations for the microbiological determination of the micronutrient ions lies in the fact that, in solutions containing organic materials of biological origin, the trace elements are always present as chelates of varying stability,[51] and the test organism may or may not be able to utilize these with the same efficiency as it does the standard preparation. Assays of organic extracts may not always yield, therefore, results that agree exactly with those obtained in ashed samples, even where these extracts do not supply growth-stimulating substances that affect growth independently of the trace element being determined. A general way of eliminating complications from this source would be to employ only ashed samples. Conversely, perhaps a chelating agent of appropriate complexing

ability could be added alike to standards and samples before their addition to the assay medium.

SUGGESTED ASSAY METHODS

Several of the factors that make for variability in the response of any test organism to a trace-metal ion and methods for circumventing these have now been considered. In practice, aberrant responses to samples in microbiological assays can be detected just as in any other quantitative assay method: i.e., through tests of the duplicability of the assay method, the deviations from known values in test samples, through quantitative recovery experiments, by comparison of the results of bioassays with those of other methods, and through application of the several tests for validity which have special applicability to microbiological assay methods. These latter include absence of drift in assay values determined at different concentration levels, agreement in assay values obtained with independent assay organisms, etc., and have been discussed fully elsewhere.[24,25] We may now discuss briefly the nature and applications of the few methods that have been suggested, and point out a few unexplored possibilities in this area. We shall not consider here the very early methods, referred to in the introduction, that have been used only for determination of availability of inorganic nutrients in soils.

Suggested Microbioassay Methods with Lactic Acid Bacteria. *Potassium.* Barton-Wright [52] showed that a regular dose-response curve to potassium could be obtained with *S. faecalis,* and recommended use of this organism for determination of K^+. The limitations of this organism, including its response to Rb^+, have been described above (see Fig. 2). However, quantitative methods for K^+ that employ bacteria as test organisms could readily be devised. For this purpose, organisms such as *S. equinus* (*Leuconostoc mesenteroides* P-60 (= strain number)), which have a specific requirement for K^+ should be used in media supplemented with sufficient Rb^+ (and perhaps with Na^+, NH_4^+, and Cs^+) to eliminate the sparing effect of this ion on the potassium requirement. The *sensitivity* of such a method would not be extraordinarily high—the assay range would be approximately 5 to 30 ppm. Any number of other micro-organisms could also be used as the basis for a method for this metal ion.

Manganese. A sensitive method for estimation of manganese with *L. arabinosus* was developed by Bentley, Snell, and Phillips.[53] The basal medium, shown in Table III, illustrates the complexity of media

TABLE III

COMPOSITION OF BASAL MEDIUM FOR ASSAY OF MANGANESE WITH *Lactobacillus arabinosus* [53]

Constituent	Amount per liter *	Constituent	Amount per liter *
Enzymatic casein digest	800 ml †	Guanine	20 mg
Glucose	40 g	Adenine sulfate	20 mg
Sodium acetate	20 g	Calcium pantothenate	1.0 mg
K_2HPO_4	0.5 g	Nicotinic acid	1.0 mg
KH_2PO_4	0.5 g	Pyridoxamine	1.0 mg
Salt mixture ‡	40 ml	Riboflavin	1.0 mg
Cystine	400 mg	Thiamine	1.0 mg
Uracil	20 mg	Biotin	250 γ
		Folic acid	250 γ

* This medium is twice its final concentration.

† A pancreatic digest supplying organic solids equivalent to 40 grams of casein together with some sodium acetate.[53]

‡ 10 grams $MgSO_4 \cdot 7H_2O$, 0.5 gram NaCl, 0.5 gram $FeSO_4 \cdot 7H_2O$ in 250 ml of water.

necessary for growth of lactic acid bacteria. The difficulty in freeing such media from the trace quantities of mineral elements required for growth explains the fact that so far, only K^+, Mn^{+2}, Mg^{+2}, and possibly Ca^{+2} have been shown to be required by representatives of this group. For manganese assay, this medium was freed of interfering

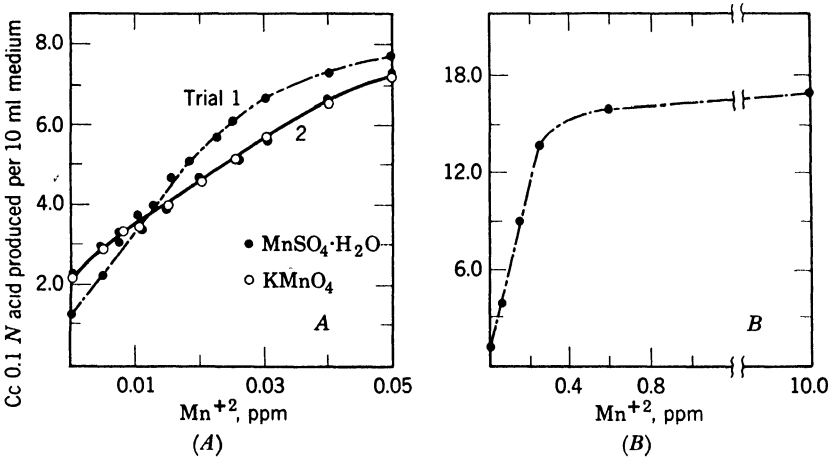

FIG. 6. The response of *Lactobacillus arabinosus* to low (*A*) and high (*B*) concentrations of Mn^{+2} in manganese-deficient medium.[55] (Reproduced by permission of the *Journal of Biological Chemistry*)

traces of Mn^{+2} by growing the test organisms in it. Such growth absorbs the contaminating traces of Mn^{+2}, thus reducing its concentration below interfering limits. The cells are then removed by centrifugation, and the medium is neutralized and supplemented with other nutrients that might also be removed by this procedure. Standard and samples are then added to a series of test tubes with 5 ml of water, and the volume is made to 10 ml by addition of the basal medium. The tubes are capped, sterilized, and inoculated with a dilute suspension of the test organism. The response of the test organism to Mn^{+2} can be determined either by quantitative measurements of culture turbidity or by titration of acid produced during growth. Results obtained in the latter fashion after 72 hours' incubation are shown in Fig. 6. The assay range lies between about 0.005 and 0.03 ppm of manganese.

Results of application of this procedure to ashed samples of low Mn^{+2} content gave results that agreed well with those obtained by a chemical periodate [54] method (Table IV). Quantitative recoveries of

TABLE IV

COMPARATIVE VALUES FOR Mn^{+2} OBTAINED BY MICROBIOLOGICAL ASSAY AND BY A PERIODATE METHOD IN ASHED AND ACID-EXTRACTED SAMPLES [53]

	Mn^{+2} Content, Ashed Samples			Acid-Extracted Samples	
Sample	Chemical, γ per g	Microbiological, γ per g	Recovery, %	Microbiological, γ per g	Recovery, %
Alfalfa	28 8	28.2, 27.4, 25.9, 30.4, 31.7, 25.4, 26 5, 27.1, 28.7	97.5, 111	31.3, 29.1	113
Dried liver, 3	11.3	10.2, 12 05			
Dried liver, 4	11.3			17.7, 18.0, 18.8	
Dried kidney	5.8	6.7			
Dried adrenal tissue		2.12	97		
Dried muscle tissue		2.4			
Blood		5–8 *			

* γ per ml.

added Mn^{+2} also were obtained. In contrast to the chemical method, the microbiological method could be applied to ashed materials high in iron but low in manganese, such as blood, with apparently accurate results.

The feasibility of direct assays for Mn^{+2} on neutralized acid extracts of organic materials was tested by comparing results on such samples with those obtained on the ashed samples. In some cases, good agreement was obtained; in others, higher results were obtained with the acid extracts (Table IV). Whether these are high because of

sparing effects of unidentified organic compounds on the Mn^{+2} requirement, as discussed earlier, or whether the results with ashed samples are low because of losses in the ashing process is not known.

To my knowledge, this represents the only instance in which a quantitative comparison of the results of microbiological assay with those of chemical assay has been made. It shows that sensitive microbiological methods of satisfactory accuracy and specificity can be obtained. At the same time, it indicates the need for further study before these methods are applied routinely to biological materials containing large amounts of organic matter. The lactic acid bacteria have an unusually high requirement for Mn^{+2}, so that other test organisms should provide methods of even higher sensitivity.

Suggested Microbioassay Methods with Fungi. These methods have been used almost exclusively for determination of available nutrients in soils. Good correlation of the results with those of field trials have been reported, but the absolute values obtained have not, in general, been compared with those obtained by other procedures. There is every reason to assume, however, that quantitatively accurate values using these general procedures and test organisms could be obtained, perhaps with few or no variations in the procedures now employed. One of the more recent and generally applicable of these methods is that of Nicholas,[23,55] which will be described briefly.

Nicholas employs *A. niger* as the principal test organism; *Penicillium glaucum* also is useful. The complete basal medium is given in Table V; the trace element to be determined is omitted from this

TABLE V

BASAL MEDIUM FOR DETERMINATION OF TRACE ELEMENTS WITH *Aspergillus niger* OR *Penicillium glaucum*

Component	Amount per liter	Component	Amount per liter
Dextrose	50 g	$CuSO_4 \cdot 5H_2O$	1 mg
KNO_3	5 g	$MnSO_4 \cdot 4H_2O$	3 mg
KH_2PO_4	2.5 g	$Na_2MoO_4 \cdot 7H_2O$	1 mg
$MgSO_4 \cdot 7H_2O$	1 g	$Ga(NO_3)_2 \cdot 8H_2O$	0.2 mg
$Ca(NO_3)_2$	0.5 g	$Co(NO_3)_2 \cdot 6H_2O$	0.1 mg
$FeCl_3 \cdot 6H_2O$	20 mg	$Ni(NO_3)_2 \cdot 2H_2O$	0.08 mg
$ZnSO_4 \cdot 7H_2O$	20 mg	$NaVO_3 \cdot 4H_2O$	0.08 mg

medium, and special purification procedures are used to eliminate traces of it from other components of the medium. Ga, Co, Ni, and V are included in the medium, even though no requirement for them

has been detected; as we have seen earlier, this is sound practice and could be extended to even additional elements. The macronutrients N, P, K, and Mg are essential and could be determined; the method has been applied primarily, however, to the micronutrients Fe, Cu, Zn, Mn, and Mo. To carry out the test, 50-ml portions of the sterile base medium in separate 500-ml Erlenmeyer flasks and supplemented with appropriate amounts of soil, soil extracts, or other samples are inoculated with a spore suspension in water and incubated at 25° for 6 days (*A. niger*) or 20° for 10 days (*P. glaucum*). The growth produced is then compared (visually or by determination of dry weight of the mycelium produced) with that from a standard series. With Mn^{+2}, growth may not be affected by the deficiency, but the quality of the growth changes and can be assessed visually. Copper is required for pigment formation in *A. niger,* and the amount present can be assessed either by quantitative comparisons of growth or by visual comparisons of color. Details of each assay, such as the initial *p*H of the medium desirable to obtain maximum differences between normal and deficient soils, vary slightly. The assay ranges for the various ions are shown in Table VI. The sensitivity in all cases is high,

TABLE VI

CONCENTRATIONS OF METAL IONS APPROPRIATE FOR ASSAY WITH *Aspergillus niger* AND *Penicillium glaucum* [23]

Micronutrient Element	Concentrations Suitable for Standard Curve, γ per 50 ml
Mo	0, 0.0005,* 0.001, 0.002, 0.003, 0.004, 0.005, 0.0075,* 0.01
Mn	0, 0.01,* 0.025, 0.05, 0.1, 0.5, 1.0,* 5.0
Cu	0, 0.05,* 0.1, 0.2, 0.4, 0.6, 0.8,* 1, 1.5, 2.0, 4.0
Fe	0, 0.1,* 0.5, 1, 2.5, 5,* 10
Zn	0, 0.5,* 1, 2, 3, 4, 5,* 10

* The asterisks enclose the best assay ranges.

and the bioassay procedure is stated to possess greater sensitivity and also higher accuracy at low concentrations than available chemical procedures.

With Cu, Zn, Mo, and Mn the method has shown marked differences between soils yielding crops showing deficiency symptoms and normal soils. The deficiency in mineral elements noted in deficient soils also appears in plants grown on those soils (Table VII).

It is unfortunate that the results obtained with this method have not been compared with those given by standard chemical procedures. In addition to its application to soils, the method has been used to

TABLE VII

MOLYBDENUM CONTENT OF MOLYBDENUM-DEFICIENT AND NORMAL CAULIFLOWER LEAVES AS INDICATED BY *Aspergillus niger* [23]

Mo in Ash, γ per g Dry Plant Leaves		Mo in Water Extract of Leaves, γ per g Fresh Weight	
Normal	Mo-deficient	Normal	Mo-deficient
0.5–1.0	0.03–0.1	0.1–0.2	0.001–0.01

determine the Mo content of the enzyme, nitrate reductase, at various stages of purity.[40]

Application of a similar procedure for Zn^{+2} has been described by Tucker, Kurtz, and Lynch.[56] Florida soils known to respond to zinc fertilization were found to contain 0.6 to 2.88 ppm of Zn^{+2}; Illinois soils ranged from 1.6 to 21.5 ppm.

THE OUTLOOK

From this summary of the factors that influence the inorganic nutrition of micro-organisms, and from the success that has attended the few methods already suggested for inorganic ions, the possibilities for development of a wide variety of sensitive, accurate bioassays for the physiologically important trace elements is apparent. For routine application to large numbers of samples, such methods are frequently more convenient and less time-consuming than more customary chemical procedures. Obviously, the less the protoplasmic mass involved, the less of a given ion will be required for growth, other things being equal. For this reason the fungi, now chiefly used for bioassays because of their ability to grow in simple inorganic media, do not provide such sensitive assay organisms as unicellular organisms: e.g., yeasts, some algae, and bacteria, with which much smaller masses of growth can be accurately estimated. Application of the latter organisms to such assay methods involves no new principles, but awaits simply the development of less tedious procedures for freeing growth media from metal-ion impurities.

The requirement of specific enzymes for metal ions has been referred to previously. Where such activation is specific for a single metal ion, another sensitive technique for estimation of that ion becomes available that may prove useful in special circumstances. No

such methods have so far been suggested, and the sensitivity of such methods would not be high.

The possibility that enzymes and micro-organisms may also prove useful in specific instances for estimation of trace quantities of toxic ions that normally play no role in their metabolism is suggested by recent findings of Shaw and Lawrance,[57] who achieved a rather accurate estimation of the solubility product of silver chloride by determining the toxic action for guppies of dilutions of a solution initially saturated with this compound.

SUMMARY

Micro-organisms, like living organisms in general, require a variety of trace metals for growth. Because of nonspecificity in the metal requirement for enzyme activation, however, the minimum requirements for growth do not show all of the metal ions that play a useful physiological role in the metabolism of a given organism. Where micro-organisms are to be used for specific quantitative bioassay of a single essential metal ion, therefore, the addition of an assortment of nonessential ions to the assay medium may be necessary. Organic materials, too, may influence greatly the requirement for a given metal ion. They may render nonessential a previously essential metal-ion-catalyzed process; alternatively, their presence may enhance the need for such a process. They may also increase or decrease the availability of a trace element through complex (chelate) formation. All of these undefined effects may be rendered inoperative through the use of ashed samples for assay, through the use of sufficiently complex organic media for the assay, or by addition of appropriate chelating agents to the medium. Antagonistic relationships between certain ions exist, and their possible interference in a quantitative bioassay should be borne in mind.

A brief review of the published methods of microbiological assay for inorganic metal ions reveals that no single method has been studied adequately from each of these points of view. Despite this fact, these methods have yielded useful results, in some cases of proved accuracy. Because the methods are among the most sensitive available and, once put on a routine basis, are conveniently carried out in large numbers, they offer excellent possibilities for the analyst. Metal ions proved to be required for growth of one or another micro-organism, and that might be determined in this fashion include K, Mg, Ca, Mn, Zn, Cu,

Fe, Mo, and V. In addition, Co and perhaps Ga (and other metal ions not so far studied) are required for growth. The primary factor retarding development and use of such methods is the difficulty in rendering growth media specifically deficient in a single metal ion.

DISCUSSION

Discussion Leader
Alvin Nason
McCollum-Pratt Institute
Johns Hopkins University

THE USE OF FUNGI IN THE MICROBIOLOGICAL ASSAY FOR METALS *

The food or nutritional requirements of an organism may be defined as those chemical substances that are necessary for growth, by virtue of their function in the generation or release of energy, the building and repair of protoplasm, or the regulation of metabolic processes. Food materials are usually classified into those groups that can be used as (1) an energy source, namely, carbon compounds such as carbohydrates, fats, or proteins, (2) a nitrogen source such as amino acids or some form of inorganic nitrogen, (3) growth factors which are organic compounds needed in small amounts, since they cannot be synthesized by the organism and must therefore be supplied to it intact, and (4) mineral salts or inorganic nutrients.

Pasteur was the first to show the importance of inorganic nutrients for micro-organism by observing improved growth of yeast by incorporating the ash of yeast in the nutrient media. Raulin [58] in 1869 reported that the fungus *Aspergillus niger* required iron and zinc for maximum growth. As a result of further work on pure cultures of this organism by others,[59-62] it has been possible to show that copper, manganese, molybdenum, and possibly gallium and vanadium are also essential trace elements for *A. niger.* It is also known that *A. niger* (Mulder strain) requires Mg, S, K, P, and N for maximal growth. When one of these elements is present in insufficient amounts in the nutrient media, the growth of the fungus is retarded as shown by reduction in dry weight and sporulation. An increased supply of the deficient element results in a specific and quantitative increase in

* Contribution no. 184 of the McCollum-Pratt Institute.

growth and therefore in weight yield. Thus a series of standard growth curves for each of the essential nutrients can be prepared,[62] as shown in Fig. D1. After appropriate treatment the material to be assayed is added at different concentration levels to the nutrient medium. The development of this principle to the point of an effec-

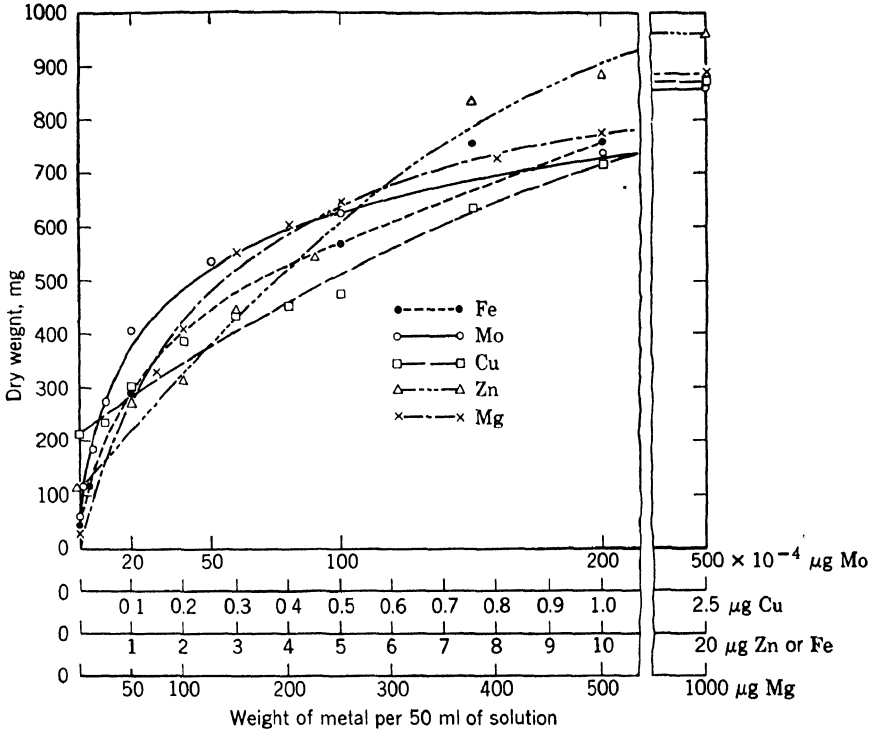

FIG. D1. Standard growth curves of *Aspergillus niger* with different levels of molybdenum (and other metals) in the nutrient medium. (From Perlman[5])

tive microbiological assay tool using the fungus *A. niger* or *Penicillium glaucum* (Keilling strain [63]) has been accomplished principally by the Long Ashton research group at the University of Bristol.[62] The microbiological assay for micronutrient elements has in its favor an extremely high sensitivity and specificity. In particular its application in these respects as a tool for molybdenum determination very likely exceeds all chemical and physical methods of analysis. Some of the disadvantages of the assay are the steps necessary for insuring the removal of contaminating metals from glassware, water, and chemicals; the long incubation period (6 to 10 days) needed for growth; the possibility of introducing inhibitory compounds accompanying the test

material; and the usual precautions in checking for possible contaminations and mutations of the test organism.

Materials and Methods. The details of the various procedures and precautions in preparing and conducting the microbiological assay using *A. niger* has been described by Nicholas.[62] Briefly they include the preparation of redistilled water by two- or threefold distillation through Pyrex glass; thorough cleaning of glassware with hot water and an organic-detergent, cleaning solution mixture, or chelating agents; and removal of micronutrient element contaminants from the macronutrient solutes including glucose by various coprecipitation procedures, chelating agents, and ion-exchange resins. The composition of a suitable culture solution for *A. niger* and *P. glaucum,* the preparation of spore inoculum, the preparation of standard growth curves for each of the inorganic nutrients, and the preparation of various samples for testing such as soils and tissues have also been provided.[62] The minimum amounts of metal in micrograms per 50 ml of culture solution detected by the assay method are: copper 0.05, zinc 0.25, iron 0.01, manganese 0.01, and molybdenum 0.0001. It seems feasible that this microbiological technique can be applied to the problem of assaying the elements in materials of all types.

Application of Assay in Determination of Biochemical Role of Molybdenum. Although it has been known for some time that molybdenum is important for the metabolism of nitrate in fungi and higher plants, its role as an electron carrier in the enzyme nitrate reductase has only recently been demonstrated. Nitrate reductase, purified from the fungus *Neurospora,* catalyzes the reduction of nitrate to nitrite by reduced triphosphopyridine nucleotide (TPNH). The enzyme is a sulfhydryl flavor protein with flavin adenine dinucleotide (FAD) as the prosthetic [64] group and molybdenum as the metal component.[65,66] In the early stages of these studies, the significant sensitivity of the enzyme to a number of metal binding agents such as cyanide, azide, potassium ethyl xanthate, *o*-phenanthroline, and 8-hydroxyquinoline suggested a metal constituent. Further indirect evidence for a metal component of the enzyme, suggestive of molybdenum, was the observation that, of all the individual metal deficiencies induced during growth, only a molybdenum deficiency resulted in a significant decrease in nitrate reductase in cell-free extracts of *Neurospora.* Table DI shows that there is a linear relationship between the molybdenum supplied to the nutrient medium and the nitrate reductase in vitro of *Neurospora.*[65] At low levels of molybdenum, e.g., 1×10^{-5} μg, the enzyme activity provides a more sensitive assay pro-

TABLE DI

EFFECT OF GRADED LEVELS OF MOLYBDENUM ON NITRATE REDUCTASE ACTIVITY CELL-FREE EXTRACTS OF *Neurospora crassa* WILD TYPES 146 AND 5297a [65]

Units of Enzyme Activity per mg Protein

Fungus	Molybdenum * added, μg	% Growth	Nitrate Reductase
146	0	21	24
	0.001	36	46
	0.01	49	118
	1	70	113
	10	100	84
5297a	0	25	16
	0.001	42	26
	0.01	62	71
	1	80	92
	10	100	71

* Per 125-ml basal solution.

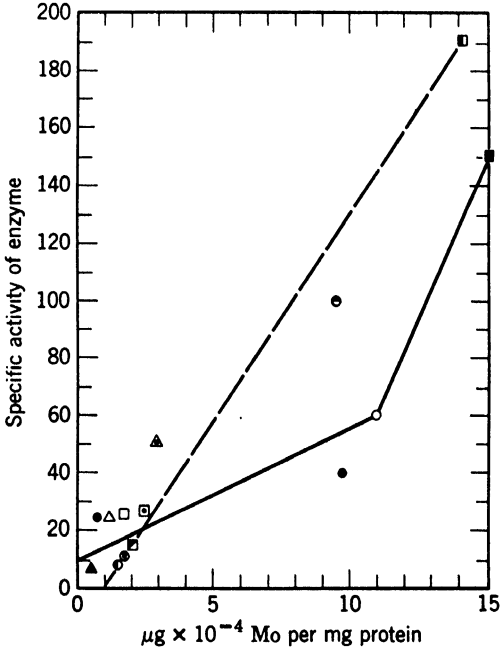

FIG. D2. Relation between molybdenum content and specific activity of nitrate reductase of various protein fractions from *Neurospora*. (From MacLeod and Snell [9])

cedure for the micronutrient than the dry-weight yields of the mycelia. This suggests a possible microbiological assay for molybdenum dependent on nitrate reductase activity instead of dry-weight yields.

Figure D2 shows the direct relation between the specific activity of nitrate reductase and the molybdenum content per milligram of protein in enzyme fractions at different stages of purification.[66] Molybdenum was determined by the microbiological assay employing *A. niger,* after the cell-free extracts and various enzyme fractions were first digested with redistilled nitric acid. Further evidence identifying molybdenum as the metal component of nitrate reductase was obtained by dialysis of the enzyme against cyanide, resulting in an inactivation of nitrate reductase and a corresponding decrease in the micronutrient element.[66] This is shown in Table DII. After a 3-hour

TABLE DII

ENZYME ACTIVITY AND MOLYBDENUM CONTENT OF DIALYZED NITRATE REDUCTASE [66]

Unit of Enzyme Activity per 0.05-ml Enzyme

Treatment	Dialyzing Solutions *		Dialysis Time, hr 0	3	6	9	12
1	0.2 *M* phosphate + 10^{-3} *M* glutathione	Enzyme	19	17	15	15	14
		Molybdenum †	13	13	14	13	13
2	As for (1) + 10^{-3} cyanide	Enzyme	4	3	2	1	1
		Molybdenum	15	0.5	0.5	0.2	0.5
3	As for (2) but after 3 hr dialyzed against (1)	Enzyme	4	3	5	9	11
		Molybdenum	15	0.5	1	5	8
4	As for (3) but using Mo-free solutions	Enzyme	4	3	1	1	0
		Molybdenum	15	0.5	0.2	0.2	0.2

* Membranes bathed for 12 hr in the solutions listed and 5 ml of enzyme subsequently dialyzed against 1 liter of the same solutions unless otherwise stated.

† Molybdenum as $\mu g \times 10^{-4}$.

dialysis against cyanide, the molybdenum content fell to a thirtieth of that of the enzyme dialyzed against a control solution of phosphate buffer and glutathione. Subsequent dialysis against glutathione and phosphate solution, free from molybdenum, was performed to remove the cyanide. Molybdenum was determined by the microbiological assay utilizing *A. niger.* There is a sufficient molybdenum impurity in the phosphate, also confirmed by microbiological assay, to necessitate the use of molybdenum-free salt in the second

dialysis step above. The addition of molybdenum trioxide or sodium molybdate reactivates the enzyme to 85% of the original value. Pre-incubation with other trace metals including iron, zinc, manganese, nickel, cobalt, mercury, tungsten, uranium, vanadium, or boron were ineffective in place of molybdenum. It has also been possible to show [67] that the flavin and molybdenum function as electron carriers in the nitrate reductase enzyme in the sequence: TPNH → FAD → Mo → NO_3^-. In support of this sequence was the observation that reduced molybdate, prepared with $Na_2S_2O_4$, enzymatically reduces nitrate to nitrite in the absence of added FAD. The molybdenum appears to be undergoing an oxidation–reduction reaction from an oxidation state of +6 to a more reduced level, probably +5.

Thus the microbiological assay for molybdenum using *A. niger* has proved to be an important tool in the identification of molybdenum as the metal component of nitrate reductase in *Neurospora.* Similar results have been obtained with nitrate reductase from the leaves of a representative higher plant such as soybean [68] and from the bacterium, *Escherichia coli.*[69]

QUESTIONS AND ANSWERS

JOHN H. YOE: Dr. Nason, you have mentioned fungi and bacteria as indicators for certain metallic ions or elements. What about the green algae?

ALVIN NASON: I think green algae as well can serve very nicely for this sort of thing. The cell size of certain green algae is quite small, almost down to cell size of bacteria. As long as one can get reproducible and rapid growth and show a requirement for the particular metal that one is concerned with, it should serve quite well. For example, the photosynthetic flagellate, *Euglena,* is used for the assay of Vitamin B_{12}, a cobalt-containing factor necessary for growth of this organism.

DISCUSSANT: Is iodine needed in plant growth?

ALVIN NASON: Iodine is required by higher animals. We all know it is an atom of the thyroxin or thyroglobulin molecule, but there is no sound evidence at this moment for its essentiality in plant growth. A number of claims have been made for it, but as yet the evidence is by no means convincing.

CYRUS FELDMAN: I was wondering, is it possible to get around having to wait so long for results by using turbidimetric methods?

ALVIN NASON: In the particular case that I used here with the fungus, *Aspergillus,* it seems quite unlikely. The minimum growth period for the assay is 5 or 6 days. However, with bacteria we can get a much faster rate of growth. In the matter of a few hours we can get some excellent growth which can be rapidly determined by turbidity measurements. Perhaps a microscopic count may further shorten the period.

HAROLD A. FREDIANI (*Bristol Laboratories*): Within the last few months I have seen applications of the remarkable versatility of micro-organisms wherein they are induced to carry out complex oxidations or reductions for organic syntheses. Many of these are almost impossible to carry out chemically in the test tube except with extremely low yields. It means that one can develop an organism to do almost anything from an analytical point of view if one knows how to go about it.

Once an organism sensitive to a specific constituent is available, it is possible, by process of selectivity, to increase both sensitivity and specificity; but how does one get this first organism that is, say, sensitive to the thing one wants to test? Is it trial and error?

ALVIN NASON: There is a standard procedure for getting this first organism, known as the "enrichment culture" technique. You might go down to some mud puddle, or take a sampling from a sewer of some of the mud you find there, and set it up on agar plates. Observe the cell count or number of colonies on these plates when incubation takes place, using minimal medium. You have a mixed group of bacteria here. Now, you aseptically pour on—you are interested in finding an organism that requires, let us say, riboflavin—a solution of riboflavin.

Having already marked the sites where colonies have appeared, allow the plate to incubate for a time, and then notice the appearance of the new colonies. The new colonies that appear will be the ones that require riboflavin. They did not appear earlier since you did not provide riboflavin. If they do appear, it is a simple matter of isolating these particular colonies and growing them in culture.

A simpler alternative would be to select a desirable organism from the "American Type Culture Collection" catalog.

HERMAN A. LIEBHAFSKY: Have modern statistical methods been used in planning the experiments in this complex field?

ALVIN NASON: This is essentially an empirical type of analytical procedure. One sets up reproducible standard curves and works from this basis under standard conditions. Generally, one uses 3 or 4 different aliquots in duplicate of the material to be tested. As for the problem of standard error in this particular method, I am not quite sure.

HERMAN A. LIEBHAFSKY: I was not thinking of the error, but of the experimental design.

ALVIN NASON: In answer to your original question, the method is for the most part experimental.

EDWARD C. DUNLOP (*E. I. du Pont de Nemours*): Are these results reported for the total element or for a particular oxidation state? You mentioned something about molybdenum being +5 or +6.

ALVIN NASON: The results are reported for the most highly oxidized state of molybdenum, namely, +6. Molybdenum is not too stable in a +5 oxidation state, and in due time it will be oxidized to +6. In most of these procedures, in any of the substance one tests, the organic material is not added as such. It is ashed, and the ash is dissolved in an acid or digested in nitric acid solution and then assayed. The inorganic elements for the most part are in the oxidized state.

The micro-organisms can be used on any of the reduction states. Iron can

be used in ferrous form. We know that the ferrous form will not stay around for very long since it will be oxidized. We can use molybdenum in the +5 or +6 oxidation state.

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Part II

INSTRUMENTATION

— 21 —

Instrumentation

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ABSTRACT Instrumentation is a science that is developing at a prodigious rate, and its techniques find immediate application in every field of scientific investigation. Modern analysis leans heavily on instrumental methods and may be expected to become influenced to an increasing extent by new instrumental methods. A contemporary picture is given of instrumentation with respect to primary elements or transducers, electronics, servomechanisms, data assimilation, and computers.

Instrumental techniques for the measurement and recording of the back-scattering of beta particles by matter are briefly described. These studies have led to a general relationship in which the back-scattering can be predicted for any element or any compound. Some interesting analytical applications are beginning to emerge from these studies.

Analytical chemistry has depended on instruments from its very beginning. A precise analytical balance is one of the finest examples of an elegant instrument, and it has always been an indispensable adjunct to precise quantitative work. Dozens of other instruments of an electrical or optical nature were developed to supplement the

classical procedures of quantitative and volumetric analysis. Because a large fraction of all analyses are now conducted by essentially instrumental methods, it has become customary to speak of these techniques as instrumental analysis. While the use of this term requires no defense at this time, it does have some unfortunate consequences. The classical analyst is mildly infuriated by the inference that it is something new. The physicist and instrumentation specialist regard present-day instrumental analysis as an interim solution which is still hampered by old-fashioned thinking and practices.

As long as classical methods were merely refined or improved on by better electrical, electronic, or optical means, we could, in all truth, say that this was nothing radically new. But what are we to say about microwave absorption, nuclear magnetic resonance, activation analysis or a half-dozen other essentially physical developments which are becoming increasingly useful to the analyst? These appeared as fully developed physical principles, the analytical uses of which were merely useful by-products.

Instrumentation has had precisely the same impact upon analysis. Until a few years ago, almost every analytical technique was improved here and there by a special electrical, electronic, or optical method. This approach has been changed almost completely, and therein lies the possibility of entirely new analytical methods.

There now exists a completely generalized theory and practice of instrumentation, and its methods are equally applicable to every branch of science. This has had some remarkable effects. There was a time when each science had its special techniques and tools. As far as measurement is concerned, the mode of attack is becoming unified.

In any phenomenon with which we have to deal, we require some element, detector, or transducer to convert the phenomenon into a signal, preferably electrical. So far, the problem is still specific, distinctive, and restricted to the practices of the particular science. The chemist will need an electrode responsive to a particular ion, the physiologist a tambour to detect delicate pressure fluctuations, and the astronomer a refrigerated phototube to detect starlight.

Beyond this, the modern instrumental treatment of the primary signal is almost completely standardized. In general, the signal will be amplified electronically, and with stability, to a level at which it can be presented on rugged instruments—on oscillographs, recorders, or meters. The augmented signal can initiate power devices to control a process, if it is subject to control, and the information can be printed, fed to computers, or stored in memory circuits for future reference.

Thus, the chemist's electrode potential can control a process, the encephalograph output can control and maintain a desired level of anesthesia, or the astronomer's phototube can move a gigantic telescope and "track" a star automatically.

The steps that are involved in an over-all instrumental approach are in general (1) a primary element or transducer, (2) electronic amplification, (3) a servomechanism. Beyond this, the particular requirements may call for recording, computing, and data assimilation.

TRANSDUCERS

To list all known transducers would require one to record the entire history of physical measurement. We have, in phototubes, thermopiles, bolometers, and related devices, means for detecting and measuring radiation in the entire electromagnetic spectrum. There are transducers for the measurement of temperature, pressure, flow, humidity, and almost every conceivable variable. The advent of electronics permitted the revival or radical improvement of many obscure devices. The search for new and better transducers is unending. Especially with electronic adjuncts, indirect methods find widespread use. One might assume that in order to weigh a truck it would have to be trundled onto a large platform scale. In a recent development, strain gages are fastened to the supporting pillars of a floating section of highway. Trucks moving at 48 miles per hour are weighed by this device. It records front-axle loading, median weight, and tandem rear-wheel loading. It can transmit these data to a remote inspector, enabling him to flag down violators of permissible loadings.

An interesting example of the use of the same transducer arose in the development of mechanical manipulators. The latter are widely used in the remote handling of hazardous materials such as highly radioactive substances. In this instance, the manipulator provided adequate dexterity but the sense of feeling was lost to the operator. Although he was able to pick up a fragile glass vessel with the remote mechanical hand, it was difficult to tell whether it was held tightly enough to avoid slipping or whether the applied force was enough to crush it. Either extreme was easy enough to detect, but either could be equally disastrous. The solution in this case was to provide the operator with a glove containing strain-gage elements sewn in the fingers. Electrically operated tension elements were also incorporated in the glove. When pressure was exerted, the strain-gage elements varied in electrical resistance, which caused current to be fed back

into the tension elements. The glove thus exhibited resistance to compression and in proportion to the effort exerted. This restored the sense of feeling to the operator's hand.

ELECTRONICS

The earliest applications of electronics to physical measurement were an unmitigated nuisance to all except their proponents. The latter had sufficient faith and patience to realize that electronics would eventually revolutionize physical measurement. The advent of Black's principle of inverse feedback was a milestone in electronic amplification. This broad principle, which finds application in other ways, simply resorts to a high degree of amplification with the feeding back of a portion of output voltage or current into the input in a degenerative sense. The net gain, which is still useful, becomes relatively independent of tube characteristics or power-supply voltages.

Electronic circuitry has almost unlimited possibilities. Relaxation oscillators or trigger circuits provide rapid means for counting, scaling, frequency division, timing, or introducing delay functions.

At a time when the uses of the vacuum tube and associated circuitry seemed unlimited, the by-products of modern solid-state physics provided the transistor, which threatened to make the vacuum tube obsolete. This has by no means happened, but transistors can duplicate many of the functions of the vacuum tube with power requirements 1000 times less. The field of transistor circuitry is now highly developed, and many useful devices are now available in extremely compact form, requiring only tiny pen-light batteries for their operation. However, a radically new form of vacuum tube, practically as small as a transistor, is built of ceramic insulation and tantalum electrodes with microscopic spacing of electrodes. It has one advantage over transistors in that it can be operated at temperatures as high as 500° C.

Effective amplification is not limited to circuits employing tubes or transistors. The magnetic amplifier is particularly useful in power applications at line frequency. It depends on the saturable-reactor principle, in which a small direct current passing through a winding of a special transformer can saturate the core and produce large changes in the effective reactance of the a-c windings. In this way small direct currents can produce large changes in the a-c output. Several units can be cascaded to produce high amplification. To do

this it is merely necessary to rectify the a-c output with crystal rectifiers and use the direct current so obtained to control the next saturable reactor. Feedback principles can be used to stabilize the magnetic amplifier as a whole.

Among purely electronic devices, none is as versatile or more useful than the cathode-ray tube. The latter is indispensable in most electronic work as a means of delineating electrical phenomena at almost unlimited speed. It has found direct application in many scientific problems. Polarograms and spectrophotometric curves can be presented instantly and continuously on the screen. The cathode-ray tube is the basis of all present-day television receivers, and, although television has instructional and entertainment values which are a matter of personal taste or preference, direct televising has its scientific uses, such as remote display of hazardous regions or the multiple display of information from restricted areas (surgical operations). These are more or less obvious applications. The use of the cathode-ray tube as a flying-spot optical scanner is much more significant. It has been used to scan specimens under a microscope and thereby count blood cells or cancerous cells with precision and at high speed. A variation of the flying-spot technique has produced the photoformer. In this device, the luminous spot can be made to traverse the contour of an opaque mask in contact with the tube face. This is achieved by detecting the luminous spot with a photomultiplier, the output of which constantly tends to pull the spot below the opaque mask. As a consequence, the spot will ride along the contour during its uniform horizontal trace. If the ordinates of the mask are proportional to the square of the abscissas (horizontal sweep), then the feedback photocurrent will vary as the square of the horizontal displacement. Any mathematical function cut into the mask will be developed electrically by the photoformer. This is an electronic version of mechanical computing elements such as cams or linkages and can, of course, operate at much higher speed. An improved version of the photoformer uses a high-speed sampling scheme and can follow a line instead of the edge of an opaque screen.

These few isolated topics represent a minute fraction of the resources of electronics. In any instrumental problem one can usually find the necessary electronic techniques in the vast literature of that subject. Despite this, current developments are constantly offering new approaches.

SERVOMECHANISMS

The general principle of inverse feedback can be applied to electromechanical systems, and these are designated as servomechanisms. A servomechanism can be defined as a power-amplifying device, the output of which is at all times proportional to the difference between input and output. An analogy with the classical principle of the null measurement is useful. In the classical Poggendorf method of measuring electromotive force, the unknown voltage is opposed by an equal and opposite voltage, set in by an operator, until a detector (galvanometer) indicates no difference between them. A servomechanism accomplishes the same task but eliminates the operator. A simple, but very useful, example is shown in Fig. 1, the General Electric Company's Autopot or self-balancing potentiometer. The potential to be measured is applied to a mirror galvanometer. A beam of light is reflected from the mirror to a twin phototube. The unequal illumination of the two photocathodes produces a signal between grid and cathode of one half of a twin triode. This unbalances the bridge circuit, of which the other reference triode forms the adjacent arm. Current therefore flows between the two plates, through the output meter A and a standard resistor R_3. The potential drop across the latter is applied to the galvanometer and input signal in series opposition. This action restores the galvanometer to zero position. The feedback current through A is directly proportional to the input voltage and is sufficiently high to operate a rugged (0–5-ma) meter. This electronic optical feedback scheme has many other applications. The entire instrument is not much larger than an ordinary enclosed lamp-and-scale galvanometer. Six ranges of voltage between 100 μv and 1 volt full scale can be selected by plugging standardized resistors R_3 into the front of the case. In the complete circuit of this instrument, damping characteristics are introduced by capacitors.

Electronic recording potentiometers, which are used by the thousands, employ a different principle but one that illustrates the servomechanism principle in a more general fashion.

In this class of instrument, the unknown potential is connected to the conventional slide-wire-type potentiometer. In place of a galvanometer, the unbalance voltage or "error signal" is received by a synchronous switch or chopper which directs the small d-c signal in alternate directions through the primary winding of a transformer. The output of this transformer is an approximation to alternating

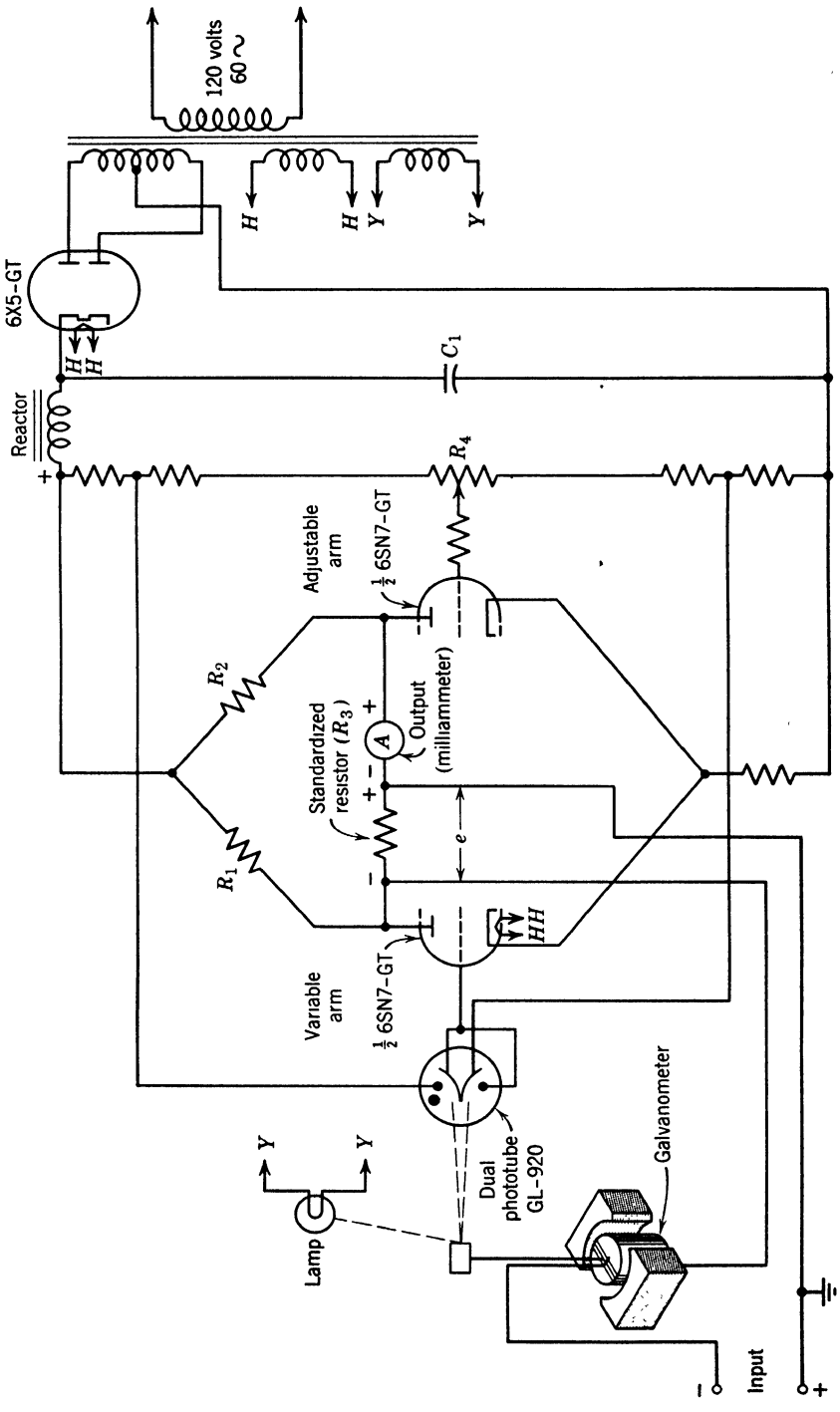


FIG. 1. Self-balancing potentiometer.

current—more nearly a square wave—and can be accepted by a high-gain voltage amplifier. The output of the latter feeds a power stage which drives one winding of a two-phase motor. Since the other winding of the motor is excited in quadrature from the line, the rotor will turn in a direction determined by the polarity of the original unbalance d-c potential. The shaft of this motor rotates the potentiometer slide wire in the correct direction and reduces the error signal. This action continues until the error signal is canceled. The same mechanism drags a pen across the recorder chart. This balancing action is continuous and within the inertia limits of the system, which can be varied widely, affords a continuous and rapid record of the varying input potential.

The same principles of transducer, amplifier, rebalancing system apply whether one is measuring a nerve potential or positioning a heavy gun turret, but it cannot be assumed that they are equally simple in execution. Whenever very large masses are to be positioned or where very abrupt changes occur, there are other factors that must be considered. Thus, in swinging a gun turret or positioning a huge astronomical telescope, much more information is required by the servomechanism in order that it shall avoid overshoot, oscillation ("hunting"), or even tearing itself to pieces. The theory as well as practice in these cases is highly developed. In general, one must consider a constant force (frictional forces), a velocity, and an acceleration. These correspond to a constant signal and first and second derivatives. The computation of these factors and their embodiment in proper circuits can exactly duplicate, and indeed exceed, the "judgment" which a human operator would exercise in avoiding over control. Stability criteria, first expounded by Nyquist, enable one to predict the conditions under which a servosystem will be stable and not be thrown into a mechanical tizzy by transients or abrupt changes.

All serious servomechanism developments are based on careful theoretical considerations, but no one with a sense of responsibility would fail to check performance with a model or prototype. Research in servomechanism design has been greatly simplified by the advent of elegant mechanical components which can be set up in great variety and with no tools other than a few socket wrenches. Particularly since machining operations are costly and time-consuming and the fact that these components have manifold uses in the research laboratory, it may be useful to describe and illustrate some of them. Several firms manufacture these components; among them we illustrate those supplied by Servomechanisms, Inc., of Mineola, N. Y.

Consider the typical configuration shown in Fig. 2. It is a fairly complex assembly of motor, gearing, couplings, potentiometers, rotatable transformers, and a transition from rotary to linear motion with indicating scale. A terminal plate for electric connections is included. This would be costly and time-consuming to build in a shop, and in most well-organized laboratories careful shop drawings would be required before construction would be started. This assembly can be put together from standard components in less than one hour, and, after it has served its purpose, it can be torn down and the components returned to stock in one-half to one-third the time.

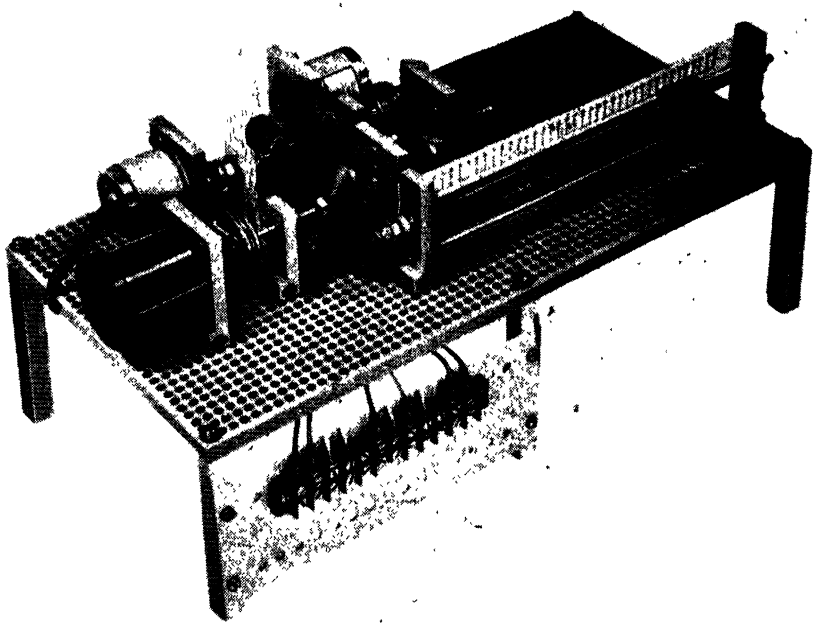
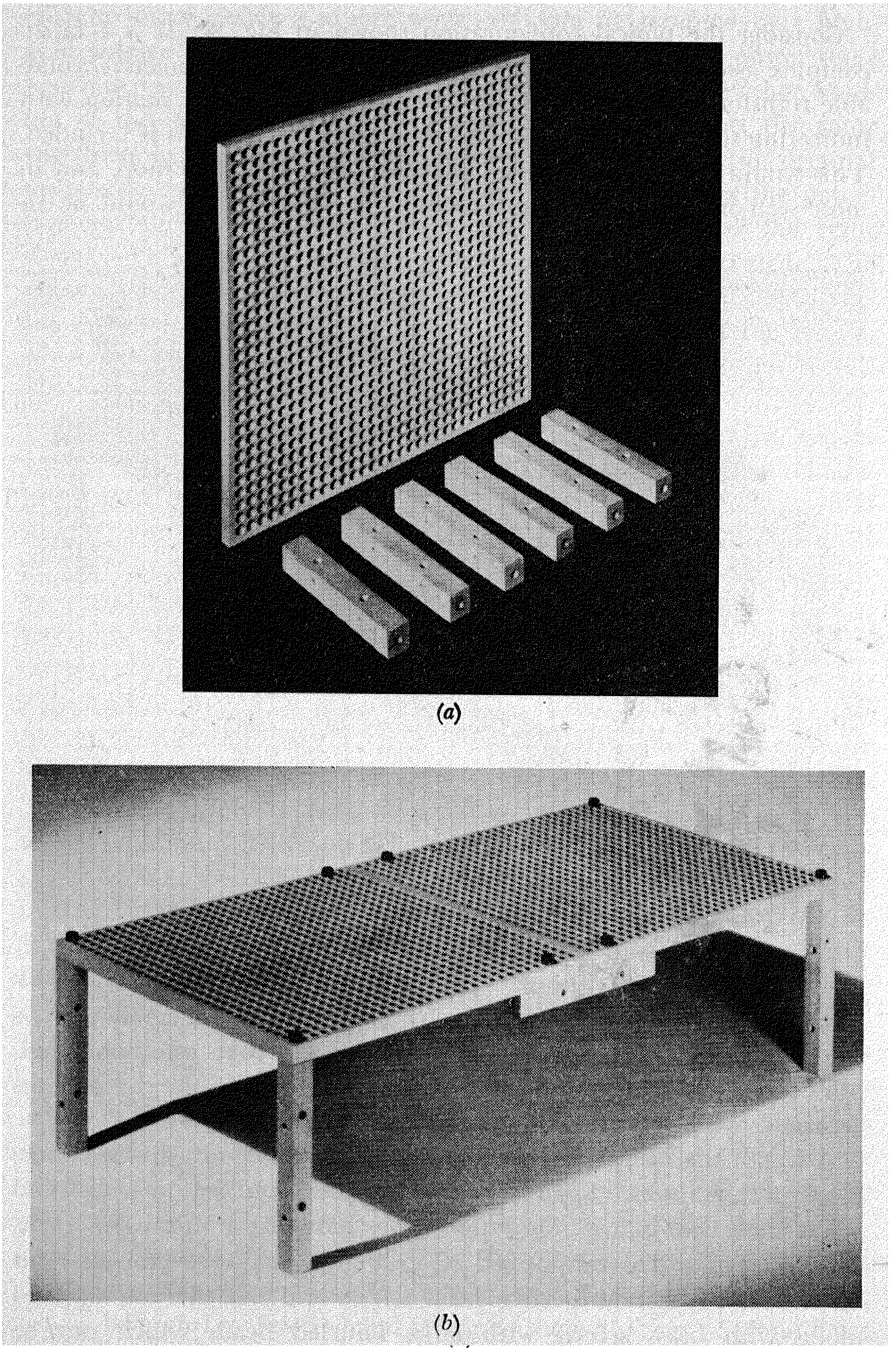


FIG. 2. Typical configuration of a servomechanism.

A few of the many components that are available are shown in successive illustrations. Figure 3 shows a typical foundation board and legs. The boards are aluminum alloy, bored at uniform intervals. The legs are drilled and tapped at both ends, and at several intervals on the sides. Standard Allen head screws and washers are supplied along with brass screws with large knurled heads which can be tightened sufficiently with one's fingers.

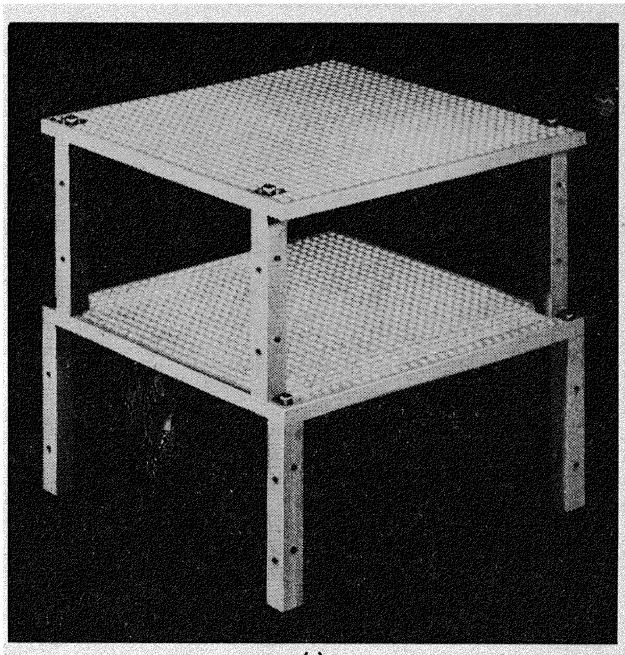
Bearing blocks and mounting blocks are available in wide variety.



(*a*)

(*b*)

FIG. 3. Typical foundation board and legs of a servomechanism.



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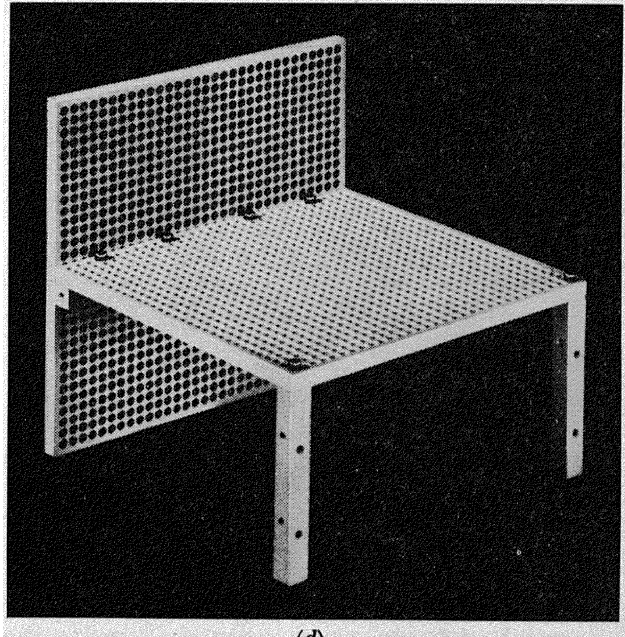

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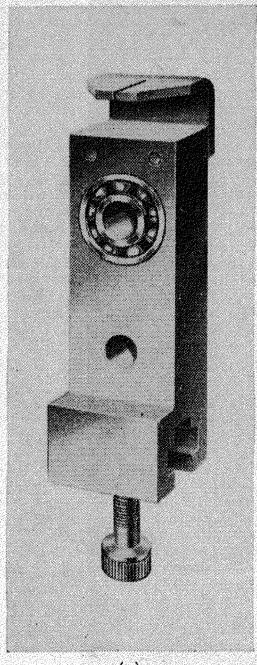
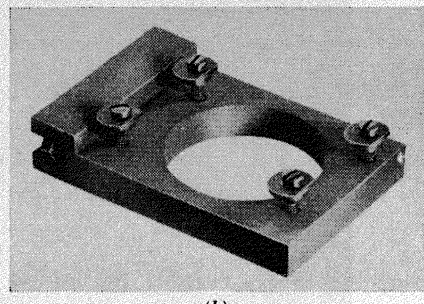
FIG. 3 (*continued*).



(*a*)



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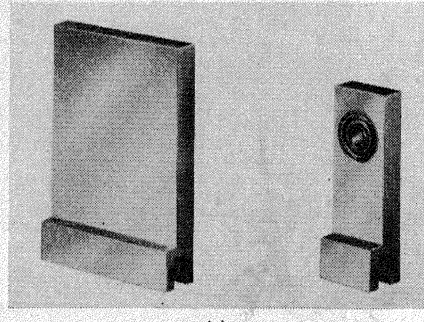

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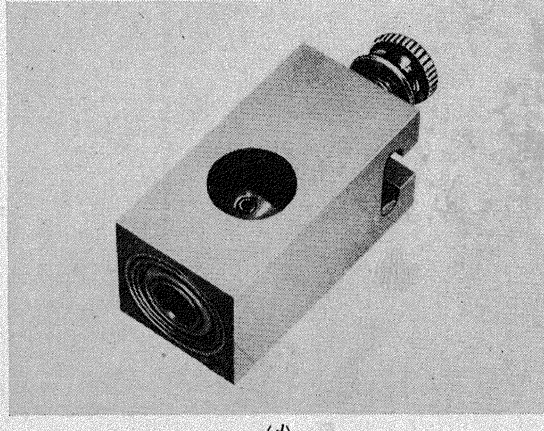


(*d*)

FIG. 4. Bearing blocks and mounting blocks.

The latter can be obtained counterbored to fit standard accessories from electrical manufacturers: i.e., for Beckman Helipots, Fairchild potentiometers, Diehl and Kearfot motors, and for synchros, rotatable transformers, and resolvers. The base of every block contains a precision T slot holding two or more captive nuts. The bearing blocks are press-fitted with precision ball bearings to accommodate standard shafts. All blocks have a standard module between center and base

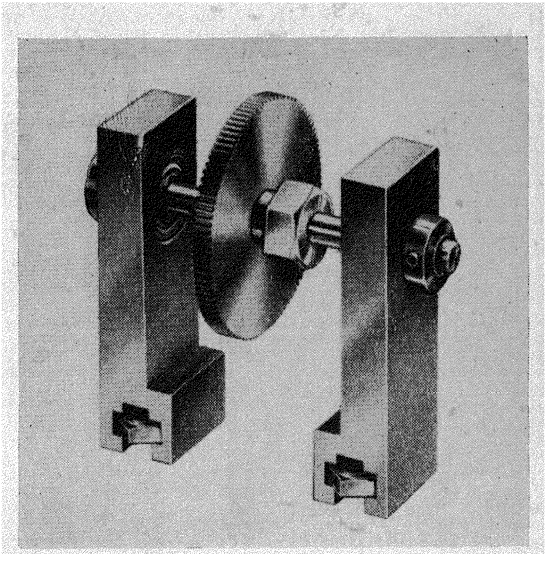


FIG. 5. Typical gear shaft and bearing block assembly.

within ±0.001 in., so that there is no difficulty in shaft alignments (Fig. 4).

Shafts, adapters and standard-stainless steel gears are available in wide assortment. The shafts are centerless ground to high finish and tolerance. Gears are not mounted by means of setscrews but use tapered split collets which are squeezed onto the shafting by a cap screw. This practically eliminates gear eccentricity. Spring-loaded pairs of gears are also obtainable for the elimination of backlash.

A typical gear shaft and bearing block assembly is illustrated in Fig. 5.

Beyond this, there is a wide choice of universal joints, Oldham couplings, indicating dials with index, microswitch mountings, bevel gears for right-angle drive and rotary stops. Inasmuch as every possible requirement cannot be foreseen, many components are offered

in blank form, wherein a special hole can be drilled or other fittings can be added.

It is not to be assumed that these components are toys, nor that they are cheap. They are fashioned of the best materials and to very close tolerances. The great economy arises from the saving of time and machining costs and the fact that the parts can be used over and over

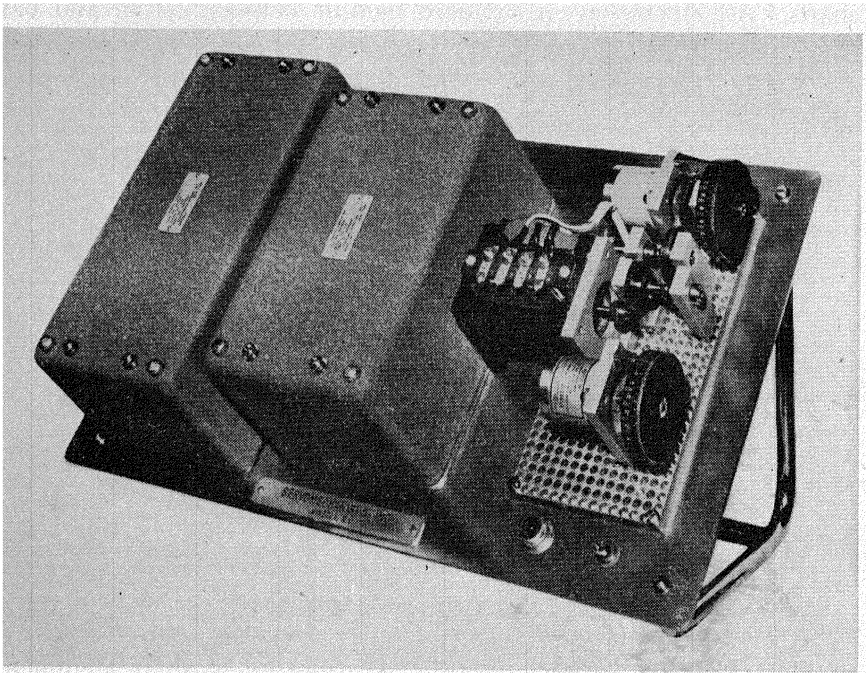


FIG. 6. Special mounting racks for combining the mechanical unit and associated electronic components.

again. Despite these advantages, many investigators have found that a particular device, so erected, was well worth keeping intact and consequently ordered more parts in replacement.

As shown in Fig. 6, special mounting racks can be obtained for combining the mechanical unit and associated electronic components in convenient form.

When the proper theoretical treatment and experimental design has furnished the servo engineer with complete information, it may be desirable to "freeze" a practical design for production. Then such matters as compactness, serviceability, shock mounting, and ready replacement of components become of importance.

I have used these components for many years in assembling polarographic scanners, chromatographic scanners for ordinary or C^{14}-labeled

chromatograms, for simple analog-computing devices, as timing or switching devices, and as follow-up mechanisms.

One might well assume that servomechanism design is the sole prerogative of the servo engineer. Although it is true that some of the more advanced and complicated systems are best left to his skill and judgment, a knowledge of what can be done is becoming essential for the research man. One of the most startling, significant, and valuable developments has been the use of electronic systems, servomechanisms, or a combination of these, to simulate a complex set of phenomena. It is now possible to set up such systems to simulate in every conceivable manner the flight characteristics of a multimillion dollar aircraft and at a small fraction of the cost. This hypothetical aircraft can be "flown," stalled, looped, or put into a terminal dive, and in other respects flown to destruction. Even the cheap simulator survives destruction, but in the interim affords voluminous data on performance, idiosyncrasies, impending failures, etc. It can afford extensive data on an aircraft that has never been built, never left the ground, or never killed any of the crew.

A complex chemical process is difficult to control, and, if an attempt is made to render the entire production line automatic, much information is required. We have long since passed the day when instruments were "hung" onto the line. These things are now introduced at the drafting-room stage. In order to evaluate the performance of the entire projected system, analogs are set up that reproduce or simulate temperatures, pressures, flow, delays, lags, and upsets. These are complicated because most chemical systems exhibit nonlinear response to environmental factors.

This electronic or servo analog of the plant can now be subjected to the most outrageous treatment. Sharp step functions can be introduced and one can see what will happen. Notable advances in this technique have been made by Philbrick and others. This approach to the study of real systems is no substitute for careful calculation; it supplements the latter and in many cases it provides an immediate answer to the behavior of a system for which straightforward computation would be hopelessly laborious or even impossible.

The older scientist may feel that we have "fallen on evil days" when he is faced with the prospect that his skill and ingenuity in manipulation are challenged by a mess of vacuum tubes, motors, gearing, and computers. He may yield grudgingly if these things do tiresome and essentially menial tasks without attention, but he resents the intrusion nevertheless. We are all too prone to consider these things that work

by pushing a button as a means of saving time, money, or human effort. Their real value to the scientist is to provide more information and more precision in a given time.

Perhaps the situation in which we find ourselves is a consequence of the history of science itself. When science, or natural philosophy, was concerned with the study and observation of nature, it was sufficient to observe, delineate, and record observations, and apply dignified Latin terms to what was seen. When man chose to experiment, he proceeded to modify circumstances and to duplicate natural phenomena, to create new phenomena and nonexistent substances. In short, he sought, and has been seeking ever since, to control his environment. That control, by man, should exceed the capabilities of his own senses was inevitable. His instruments and machines are far better—only his mind is still supreme. Professor Wiener, in his book *Cybernetics,* was concerned about the economic and social aspects of automatic control. When he says, "The second industrial revolution is rapidly developing a state of affairs in which the person of mediocre ability has little to offer which is worth anyone's money to buy," this may well apply to anyone who is unwilling to accept the implications of all scientific and technical accomplishments.

That the mind of man is supreme and still vastly superior to his best instruments and machines brings us to the question of computing and data assimilation and their proper function in modern instrumentation.

COMPUTING AND DATA ASSIMILATION

Although modern computers can perform some fantastic feats, it is unfortunate that such terms as "giant brain" have been applied to them. As far as thinking is concerned, they are in the amoeba class compared with even a mediocre human brain. Computers can perform a host of repetitive functions at prodigious speed and can follow elaborate instructions which are "coded" into them by a mathematician. If the coding or instructions are incorrect or foolish, the answer is also nonsense and turned out with the same speed.

Two important classes of computers are recognized: analog and digital. An analog computer is any mechanical or electrical device by means of which mathematical information can be represented by a physical quantity or electric current, voltage, or resistance. A slide rule is one of the oldest analog computers. Simple operations of addition, subtraction, multiplication, etc., can be performed by cams and

various mechanical linkages. If a potentiometer is short-circuited, the resistance with respect to the slider is proportional to the squares of the displacement, measured from the center. If a rectangular card is uniformly wound with resistance wire and a slider moves in one quadrant of a circle across the card, a sine function can be generated. Integration and differentiation can be duplicated mechanically; and electronically, one can differentiate or integrate signals at high speed. A Wheatstone bridge, driven automatically by a servosystem, can multiply or divide. One does not build this elaborate system to prove that $2 \times 2 = 4$. Its value lies in the fact that it can turn out products (or quotients) continuously and at high speed. Complete computing servos have been built which solve complicated ballistic or navigational data continuously. Simple analog computers have unlimited possibilities in instrumentation. Their function and justification lie in the direction of feeding information out of the instrument in the most useful form: i.e., to eliminate calculations that might require more time than the measurement itself.

Digital computers, as the term implies, simply count. Although this seems like an elementary approach and needlessly laborious, the counting can be done at enormous speed. The binary system is widely used because it places so little burden on the reliable functioning of electronic circuits. The use of radix 2 instead of 10, as we are more accustomed to in decimal notation, is an old story to mathematicians who have long used arithmetical systems based on this or any other radix. In the binary system all numbers are represented by arrangements of either 1 or 0. Electronically this simply calls upon a tube to pass full current or none at all. In effect the tube merely has to say definitely "yes" or "no." One does not have to use the final answer in binary form; there are many binary-to-decimal converters.

An important and essential requirement of digital computers is the element of memory. If the computation involves many steps, it is obvious that several pieces of computed information must be stored until they can be combined in the next operation. There are dozens of acceptable memory devices. The information can be stored temporarily on magnetic drums, tape, as charges on the face of a cathode-ray tube, or in tiny magnetic cores. When the coding mechanism calls for this information it is "read out" of the memory device and further acted upon. In some systems routine operations corresponding to a standard operation, which is required very frequently, are kept on standard tapes or punched cards. These can be called upon by the coding device.

In this brief discussion, one example is selected in order to illustrate a high order of computing and storage. The device employs tiny magnetic cores which are toroids of a special magnetic material. This material, of which the alloy Deltamax was one of the earliest examples, depends on an almost rectangular hysteresis loop pattern. This means that, if one plots the magnetizing force against the induction, a narrow, almost rectangular loop is obtained instead of the more usual open sigmoid loop. If, by means of a wire passing through the core a current pulse is sent through the wire, the core will saturate. Fur-

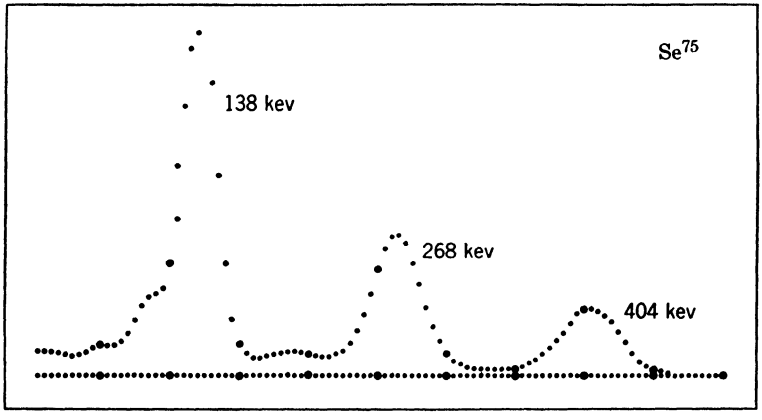
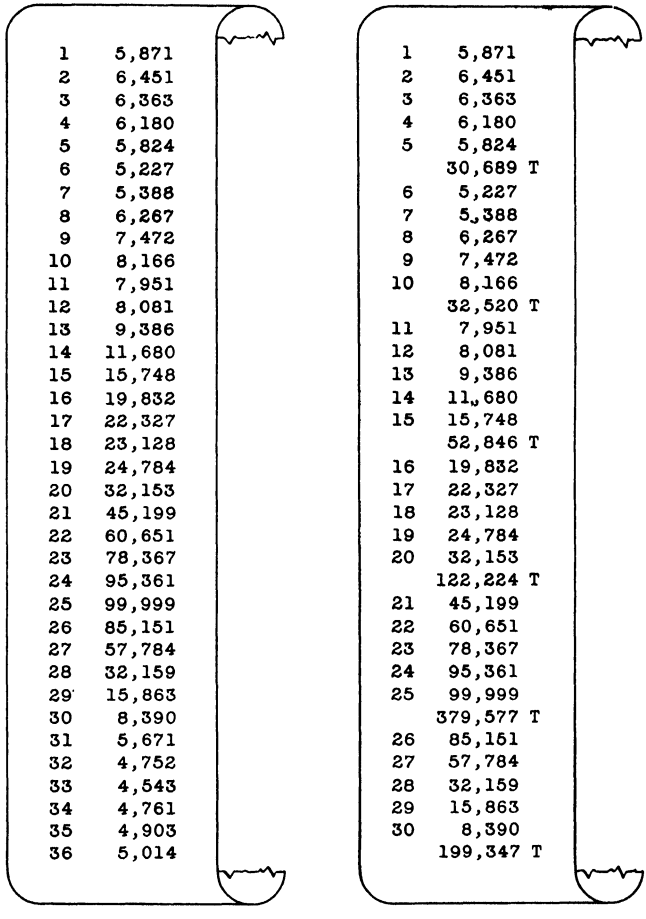


FIG. 7. Energy distribution for the decay of the Se^{75} isotope.

ther pulses in the same direction will have no further effect, and one can say that a "bit" of information is thus stored in the core, where it will stay indefinitely. This "bit" can be "read out" by sending a pulse in the opposite direction. Since the core now saturates in the opposite direction, no further information can be "read out"—it cannot be counted twice. Hundreds of these cores can be mounted on a frame of small dimensions.

The commercially available 100-channel pulse-height analyzer uses matrices of such memory units. This instrument gives a completely automatic presentation of the disintegration of a radioactive substance as a function of energy, and presents the count rate as a function of 100 discrete and equal energy levels. The first and most useful type of presentation for orienting purposes is by means of a cathode-ray tube. As each energy channel is interrogated, the counts are stored in the memory chassis. These are then read out automatically and presented on the scope as luminous dots with each tenth dot intensified for identification. Depressing a pushbutton causes

the dots to form a true base line. A typical example (Fig. 7) shows the energy distribution for the decay of the Se^{75} isotope. In common with general oscillographic practice, this pattern can be expanded in the vertical or horizontal direction for more detailed examination.



1	5,871
2	6,451
3	6,363
4	6,180
5	5,824
6	5,227
7	5,388
8	6,267
9	7,472
10	8,166
11	7,951
12	8,081
13	9,386
14	11,680
15	15,748
16	19,832
17	22,327
18	23,128
19	24,784
20	32,153
21	45,199
22	60,651
23	78,367
24	95,361
25	99,999
26	85,151
27	57,784
28	32,159
29	15,863
30	8,390
31	5,671
32	4,752
33	4,543
34	4,761
35	4,903
36	5,014

1	5,871
2	6,451
3	6,363
4	6,180
5	5,824
	30,689 T
6	5,227
7	5,388
8	6,267
9	7,472
10	8,166
	32,520 T
11	7,951
12	8,081
13	9,386
14	11,680
15	15,748
	52,846 T
16	19,832
17	22,327
18	23,128
19	24,784
20	32,153
	122,224 T
21	45,199
22	60,651
23	78,367
24	95,361
25	99,999
	379,577 T
26	85,151
27	57,784
28	32,159
29	15,863
30	8,390
	199,347 T

FIG. 8. Tabulated data.

Once the optimum conditions have been established, the pattern can be photographed or switched over to mechanical printing of the data. The data printer can accept the data and tabulate them in one of two forms shown in Fig. 8. The printed slip on the left shows the count in each channel. Thirty-six of the hundred channels are recorded on this section of the tape. An alternative scheme shown on the right permits a totalized count to be recorded at the end of each

fifth channel. This assists in integrating under peaks. In either case the data so printed are useful in making large-scale plots and, of course, serve as a permanent record of the measurements.

This is a costly device, as one might imagine from its versatility and speed. It is important to recognize that it is not merely the speed or the ease with which data can be taken here; it can delineate the behavior of a very short-lived species which might well be impossible to do by slower, manual methods.

Data assimilation is a serious problem for a number of reasons. It is an embarrassing problem with many existing instruments that data come out faster than they can be assimilated. Then, too, there is a vast amount of information that must be stored for further reference. In infrared spectroscopy, some 40,000 to 50,000 substances have been examined. It would be hopelessly laborious to take the recorded spectrogram of a substance and then hunt through previous records for a possible counterpart. For many years such information has been punched on standard IBM cards along with all related chemical information. These can be sorted or collated automatically in standard IBM machines, and in very short time a half-dozen closely related patterns will drop out which can then be subjected to closer examination for a final conclusion.

It is entirely possible at the present time to set up any form of handbook or reference data on machines and intercompare them at high speed with a new measurement. It is barely possible that some analytical processes of the future will depend on the automatic measurement of several nonspecific properties of the substance. If each of these is known to 3 or 4 significant figures, and these are interrogated automatically with data-assimilation records, a unique and positive identification will result. It is easy to compute the possibility of confusion. The precision and reliability probably exceed the known number of substances.

CONCLUSION

This symposium on trace analysis has given eloquent testimony to the originality and resourcefulness of the analytical chemist. He performs some elegant and convincing experiments with pieces of filter paper or with cooperative bacteria. In such cases he uses few or no instruments. Other methods employ elaborate and very costly instruments. Each has its proper role; each attempts to answer an essentially simple set of questions: What is it, how much is there—and occa-

sionally—where is it? It is difficult to say whether further progress will come through simplification or elaboration.

As far as the science of instrumentation is concerned, it has resources that can be used to increase speed and precision and to minimize cost. Best of all, it affords more detailed information about what is really going on, and this above all should be of interest to the analyst, whatever other obligations and commitments he may have.

Discussion of this chapter is given in Chapter 22.

— 22 —

The Interaction of Beta Particles with Matter *

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ABSTRACT The back-scattering of beta particles by matter has been examined for 34 elements and several hundred compounds. The back-scattering for the elements is a discontinuous function of Z, but linear in Z for each period of the periodic system. The scattering by pure compounds can be predicted with high precision from the intrinsic scattering of their constituent atoms and their known weight fraction in the compounds. That the rare gases mark the inflection points in the back-scattering versus Z relationship has been established by three independent methods. Synthetic crystals of NaF, KCl, RbBr, and CsI can serve as cardinal reference points since their scattering is practically identical with the rare gases (solid) Ne, A, Kr, and Xe. Isomers give identical back-scattering, but differ characteristically in transmission. The technique is applicable to substances in the solid or liquid state and to solutions. These regularities are astonishingly simple, and it now seems possible to find a relatively simple explanation for them despite the apparent complexity of the multiple-scattering problem.

* This work was performed under the auspices of the Atomic Energy Commission.

Whenever the peacetime uses of atomic energy are discussed, it is often assumed that these imply the production of power or further uses and applications of isotopic tracers. Although these are of enormous importance in the sense that the former offers hope for our eventual independence of fossil fuels, and the latter will continue to provide powerful tools for research, they hardly represent the exclusive promises of nuclear research. For many years the techniques of nuclear chemistry and physics have provided fundamental information on the nature of atomic and molecular structure. The nuclear atom, as first proposed by Rutherford, was a logical and inescapable consequence of his experiments on the manner in which alpha particles were scattered by thin films of matter.

The advent of a large number of artificial isotopes, in relatively large amount, offers the modern investigator powerful sources of alpha and beta particles, gamma rays, and neutrons. These are far more convenient than the radium sources and other naturally occurring radioactive elements with which some of the early discoveries were made. It is likely that these sources of radiation will give us improved methods for establishing new and important information concerning atomic and molecular structure.

This chapter concerns the general conclusions and laws that have been established by me in some three and a half years' study of the interaction of beta particles with matter. This study deals primarily with the manner in which beta particles are "reflected" by matter. The phenomenon is more commonly referred to as back-scattering.

If beta particles strike matter, a certain fraction of all particles are thrown back in the general direction of their origin. That this is not ordinary reflection is easily demonstrated by increasing the thickness of the target. The back-scattering increases with increasing thickness up to a definite limit, beyond which there is no further increase in back-scattering. This limiting thickness, for a given source of beta particles, can be designated as infinite thickness for back-scattering in the same sense that the term is used in transmission measurements, although the two values are not the same.

At the outset it should be stated that the phenomenon must be extremely complicated. For a substance like calcite, containing atoms of relatively low atomic numbers, simple calculations show that some of the back-scattered beta particles must have traversed several million interplanar distances before they returned and emerged from the target. To explain this behavior from fundamental principles, in quantitative fashion, is almost hopeless. Indeed, most of the the-

oretical treatments emphasize the necessity of confining oneself to a consideration of single scattering: that is, the motion of a beta particle and its deflection as it passes near the field of a single or, at most, a few atoms. It is therefore little short of astonishing that the relationship between back-scattering and atomic number is so direct and simple.

Almost 50 years ago it was observed that the back-scattering of beta particles increases rapidly at first and then more slowly as the atomic weight of the scatterer increases. Also, a definite indication of falling into distinct periods was noted, and the essentially atomic nature of the phenomenon was indicated by the fact that the scattering by compounds could be predicted on the basis of the intrinsic scattering, and the known amount, of the constituent atoms. These early studies were made before the concept of the nuclear atom had been established. With the necessary and exclusive emphasis on atomic weight, some of the conclusions were doubtful.

On the basis of measurements on 34 elements ranging between Be ($Z = 4$) to Bi ($Z = 83$) and several hundred compounds, we can now state that *the relative back-scattering of beta particles is a discontinuous function of Z, but strictly linear in Z within each period of the periodic system.*

Several other broad generalizations are possible at this time, and it is the object of this chapter to describe several of the definite conclusions that can be considered to be well understood at this time. Each of these is based on hundreds of measurements, and the intimate details thereof are being prepared in several papers.

Although the nature of this phenomenon has been obscure until these studies, it is curious to note that several eminently practical applications have been made of the interaction of beta particles with matter. It may not be out of place therefore to mention two of these applications.

An elegant study of the absorption of beta particles by liquid hydrocarbons has been made by Smyth and Otvos. By precise, semi-empirical measurements and calculations they were able to compute relative cross sections for the lighter elements. As a result, they developed a technique for the precise determination of hydrogen in liquid hydrocarbons. A precision of 0.1% is easily attained, if the density of the liquid is measured with comparable accuracy. An instrument for this purpose was developed by these authors with provision for automatic recording.

At least two forms of this instrument are commercially available.

One of these, for the precise determination of hydrogen or the hydrogen–carbon ratio is shown in Fig. 1. It is manufactured by the Central Scientific Company of Chicago.

Another application, considerably more empirical, is in the form of a most useful and rugged industrial machine. It employs a power-

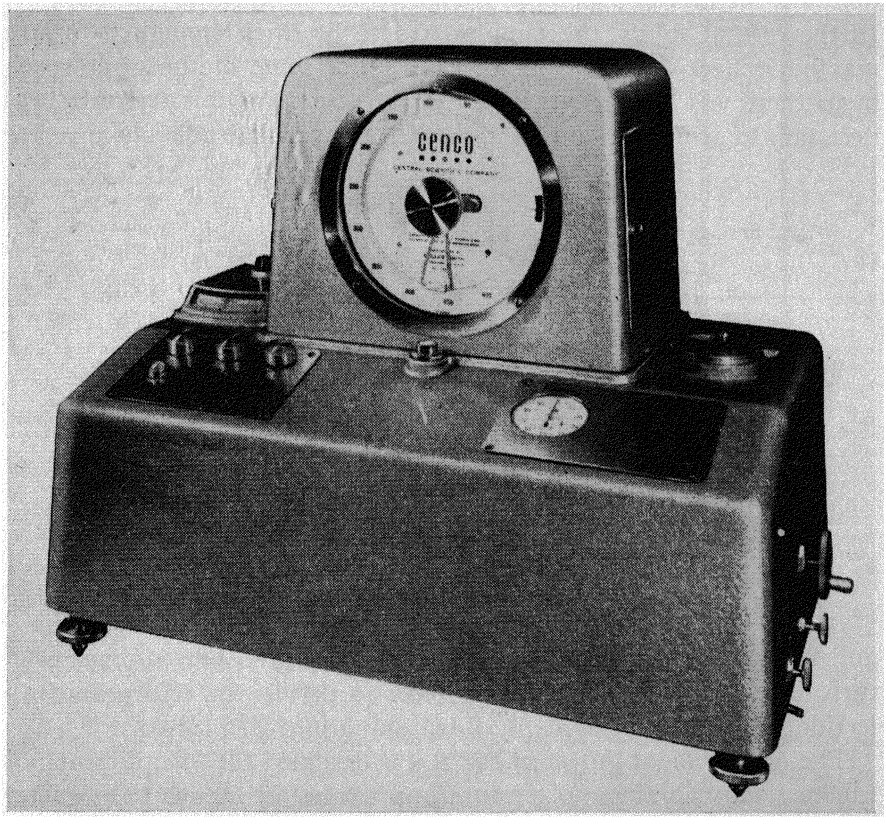


FIG. 1. Instrument for the precise determination of hydrogen by beta absorption.

ful beta source and suitable counting and recording equipment to provide continuous gaging of paper, cardboard, and thin metal foils during fabrication. The transmission of beta particles through the sheet affords a continuous indication of thickness. Beta-particle gaging can also utilize the back-scattering phenomenon in which it is useful for measuring the thickness of coatings.

From these and other direct applications of β-particle techniques it may be apparent that a more complete understanding of the manner in which β particles interact with matter is of very great importance.

MEASUREMENT OF THE BACK-SCATTERING OF BETA PARTICLES

In order to make a systematic study of the back-scattering, it was necessary to make several compromises. In the first place, it was desirable to measure as many elements and compounds as possible. In the case of solids, the samples had to be thick enough to insure maximum back-scattering in all cases. For some of the rarer specimens there was an obvious limitation in size. For this reason it was necessary to provide a sample stage with the smallest possible aperture

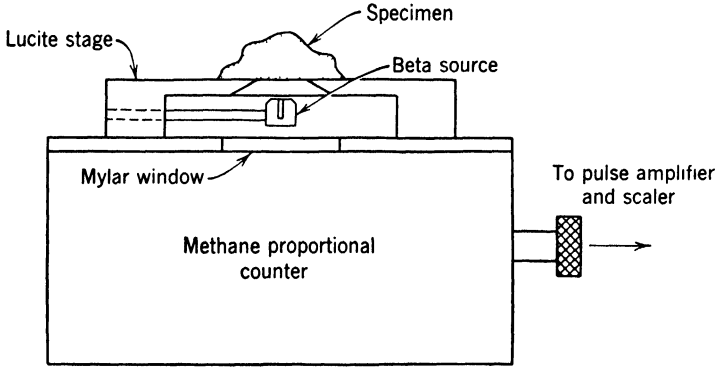


FIG. 2. Apparatus for measurement of beta-particle back-scattering.

and mount the collimated beta source below it. The latter was in turn to be as small as possible in order to permit the return and collection of as many betas as possible.

The arrangement shown in Fig. 2 was designed for this purpose. A Lucite stage contains an aperture approximately $\frac{3}{8}$ in. in diameter over which the specimen is placed. Immediately below it is the collimated source of beta particles. The source holder is a truncated cylinder of aluminum with a tiny cylindrical well, at the bottom of which is placed a few millicuries of Sr^{90}. The top of the cylindrical source is covered with a thin stainless-steel window of sufficient thickness to stop the 0.53-mev betas from Sr^{90} but to transmit most of the 2.18-mev betas from the daughter element Y^{90}. It is these betas that bombard the specimen, and within the permissible geometry of the arrangement, those that are back-scattered pass through the thin Mylar window into the proportional-counting chamber. The geometry of this system leaves much to be desired, the principal compro-

mise being the desire to use relatively small samples. It has the advantage of measuring only those betas that are back-scattered; the count with no sample over the aperture is relatively small and arises from small contamination of the source cylinder and a few betas that are scattered by the truncated underside of the stage. Some of the older methods mount the beta source directly on the window of the counter and then observe the increase in count rate when the source is backed with a scatterer. In this case, a more favorable geometry can be

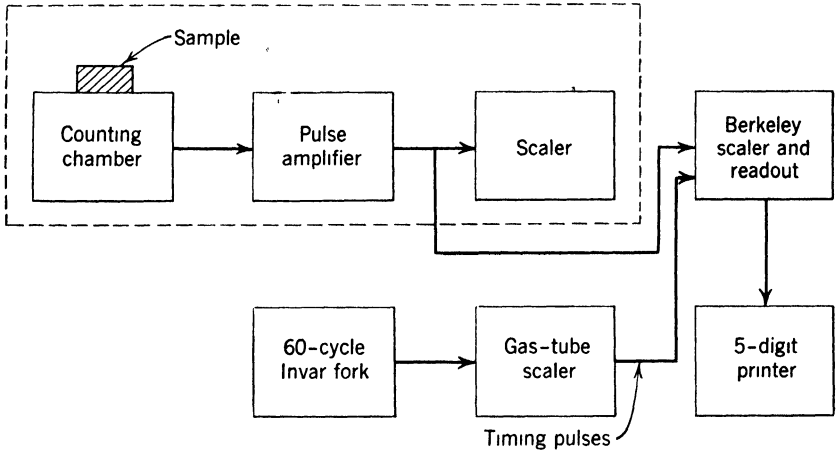


FIG. 3. Counting methods for back-scattering of beta particles: (*A*) manual operation (within dotted lines); (*B*) completely automatic timing and printing out.

achieved, but the desired answer is the difference between two large quantities, which is always an unfavorable state of affairs.

A good plateau of count rate versus high voltage was obtained in the region of 3700 volts, and this plateau does not shift with the nature of the specimen over a wide range of atomic member.

Figure 3 illustrates two schemes of counting which were employed in these studies. The system within the dotted lines represents a conventional pulse amplifier followed by a binary scaler. The latter was used on a scale of 1024 indicated by mechanical register, and interpolation lamps permitted reading to a single count. The scaler is provided with a high-speed synchronous clock which recorded elapsed time for the observed count.

After considerable data had been collected and the observations and regularities seemed to be so promising, means for automatic counting and recording became desirable. The remainder of Fig. 3 illustrates the completely automatic system. For timing and opera-

tional signals a 60-cycle Invar tuning fork was fed to a gas tube scaler. The latter permitted the choice of any desired counting interval from 1 to 59 minutes in 1-minute intervals and any "dead" time between 1 and 10 minutes. The pulse-amplifier output was fed to a Berkeley 5-decade decimal scaler which has a set of relays and stepping switch which, upon receipt of a command signal from the timing system, will stop the counting operation, read out the total count, and present the answer to a 5-digit printer. The automatic system was particularly useful for those substances showing low scattering and for which a large number of counts were necessary. Overnight runs were therefore convenient and time-saving. The automatic system does require more attention, because after continuous operation over many months relay adjustment, cleaning, and servicing are required.

PRECISION

As will be shown later, very high precision can be attained in back-scattering measurements; indeed for crystalline substances of high purity, predicted and observed values will differ by no more than 0.05 to 0.07%. From the random nature of radioactive decay it is well known that the precision of counting varies as $1/\sqrt{n}$, where n is the number of counts. Although this is quite fundamental, it applies only to the statistics of the disintegration which give rise to the beta particles. Instrumental and environmental variations contribute errors beyond this. For this reason all measurements were broken up into blocks of data—say, 50,000 counts—and many sets of these were taken. Any set of 50,000 counts should have a definite statistical error associated with it. When consecutive sets are intercompared, it is at once evident whether they conform to the expected statistical deviation. This criterion enabled us to detect an occasional malfunctioning of the equipment. It is an especial advantage of the automatic printing system that the data appear for successive groups of counts. These can be ruled off on the tape in blocks of ten and intercompared. It is all too easy for a lazy operator to wait for an accumulation of one million counts and then assume that the precision is 0.1%. The error may be many times greater, but it can be established with certainty if the component data are intercompared in blocks.

Beyond these precautions, the grand average of statistically acceptable groups was taken as a value for the intrinsic back-scattering of a substance. In a few special cases, where small or negligible dif-

ferences were expected, a least-squares method of evaluating data was used for finding the best straight line relating successive accumulated counts as a function of total elapsed time.

Alternative instrumental approaches to the measurement of back-scattering are discussed briefly at the end of this report. Although very elaborate techniques can be used, I wish to emphasize at this point that the essential laws and principles described in this chapter can be verified with extremely simple and cheap equipment. The problem does not require gigantic "atom smashers" or costly installations so frequently associated in one's mind with nuclear techniques.

GENERAL RELATIONSHIP BETWEEN BACK-SCATTERING AND ATOMIC NUMBER

By the middle of 1953 we had measured the relative back-scattering for 32 elements lying between Be = 4 and Bi = 83 and for some 22

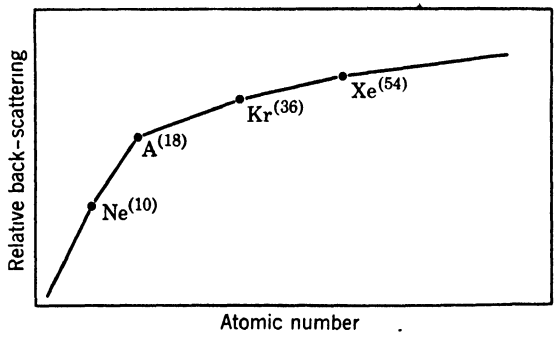


FIG. 4. Back-scattering of beta particles a discontinuous function of Z, but linear with Z in each period.

compounds. Although the data found in the literature for some half-dozen elements covering this range of Z could be fitted fairly well to a straight line when plotted against the cube root of Z, our more extensive data showed that this relationship was no longer satisfactory. The data required a broken straight-line plot of back-scattering against the first power of Z as shown schematically in Fig. 4 with abrupt discontinuities at atomic numbers 10, 18, 36, and 54, which correspond to the rare gases terminating each period of the periodic system. Additional evidence to support these points of inflection has been obtained in three different ways, as will be shown later.

This and several other generalizations were described in a letter to the *Physical Review*.[1] At that time it was also suggested that the scat-

tering for any compound could be predicted by computing an effective atomic number $\bar{Z}$ which for a compound BnCm has the form:

$$\bar{Z} = \frac{n(A_B Z_B) + m(A_C Z_C)}{\text{molec. wt. of BnCm}}$$

where A_B and A_C are the atomic weights of B and C. So defined, $\bar{Z}$ is simply the sum of each atomic number multiplied by the weight per cent of the atom which is present in the compound. On this basis the value of $\bar{Z}$ for calcite ($CaCO_3$) is 12.565. This indicates that back-scattering from calcite should be intermediate between Mg ($Z = 12$) and Al ($Z = 13$). Linear interpolation between the back-scattering for calcite and for these two metals yields a value of $\bar{Z}$ practically identical with the predicted value. More precise evidence of this will be shown later.

It was also shown at that time that the scattering from organic compounds is completely predictable with a definite anomaly due to hydrogen. Fortunately, the diminished scattering caused by hydrogen is exactly proportional to hydrogen content, and precise corrections can be made. Much additional information on organic compounds has been accumulated since that time, and an extensive paper on this subject by D. C. Müller of this laboratory is in preparation.

The $\bar{Z}$ concept, as described above, is subject to a slight refinement, as we have found from further inquiry. Strictly speaking, it is perfectly accurate when all elements in the compound are in the same period or closely adjoining periods. Over the entire range of atomic numbers, however, it is more precise to state that the scattering of a compound is equal to the sum of the intrinsic scattering of the individual atoms, each multiplied by the weight fraction of that element in the compound. The degree to which the $\bar{Z}$ value, as defined above, will hold, when confined to elements in the same period or adjacent periods, is illustrated in Fig. 5. The three substances, all very pure crystals, are calcite ($CaCO_3$), halite (NaCl), and a synthetic KCl crystal. If the values for NaCl and KCl are used to define the linear equation for period III, then the observed $\bar{Z}$ value for calcite is 12.556, whereas the computed value is 12.565. The agreement, within 0.072%, is almost within the limit of precision of the technique; nevertheless, it is the result of partial compensation of very small errors. Of the constituent atoms in calcite, two of them, carbon and oxygen, are in period II, and the third, calcium, is in period IV. Calcium constitutes

40.04% of calcite and carbon and oxygen 59.96%. Inasmuch as the slopes for periods II and III do not differ very greatly, the source of total error is greatly diminished.

Where still less disparity in location in the various periods occurs, the agreement is even better. Thus, when NaCl and KCl were used to define period III, a measurement of the back-scattering of native quartz (SiO_2) agreed almost perfectly with prediction. In this case the measurements were used to compute the atomic weight of silicon, assuming the value of oxygen as 16.0000. The result was 28.04,

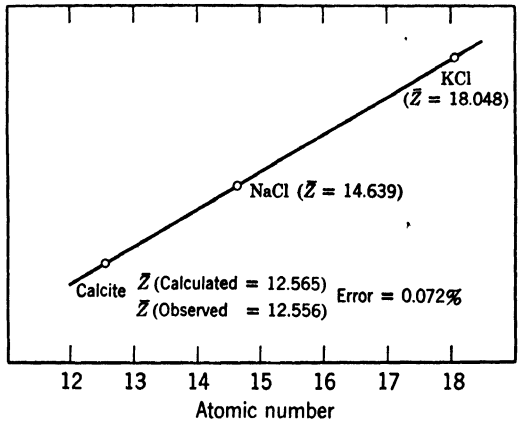


FIG. 5. Relative back-scattering.

whereas the accepted chemical atomic weight is 28.06. In general, and especially for elements of higher atomic number, it can be said that the back-scattering from crystalline substances of high purity can be predicted with a precision only slightly less than that with which the chemically determined atomic weights are known.

The validity of the $\bar{Z}$ concept as originally defined is thus clearly established. A more generally useful relationship is to have the intrinsic scattering for all elements. Another way of stating this is to say that we require accurate constants for each of the linear equations which relate back-scattering to atomic number in each period.

Inasmuch as every new back-scattering instrument would have slightly different geometry, it would seem to be extremely laborious to calibrate the system for the entire periodic system. This is not the case. It turns out that a few properly chosen crystals of high purity can establish the appropriate straight lines. We shall discuss this under the heading of cardinal calibration points.

CARDINAL CALIBRATION POINTS

The first set of extensive measurements which we made indicated clearly enough that the rare gases marked the inflection points in the linear-back-scattering–atomic-number relationship. This would suggest that one should measure samples of each of the rare gases (as solids). This would be the hard way to do it. Simple calculation showed that crystals of NaF, KCl, RbBr, and CsI should scatter almost identically with the rare gases (solid) Ne, A, Kr, Xe. The extent to which the $\bar{Z}$ values of these crystals agree with Z of the respective rare gases is shown in Table I. It will be noted that each alkali metal

TABLE I

SYNTHETIC CRYSTALS AS REFERENCE POINTS FOR BACK-SCATTERING (ALMOST IDENTICAL WITH RARE GASES WHICH TERMINATE PERIODS)

Crystal	$\bar{Z}$	Z of Nearest Rare Gas
NaF	10.092	10 (Ne)
KCl	18.048	18 (A)
RbBr	36.034	36 (Kr)
CsI	54.032	54 (Xe)

in these crystals is one atomic number above the corresponding rare gas, and each halogen is one atomic number below it. A $\bar{Z}$ value computed for each crystal will therefore be subject to very small errors. This has been confirmed with high precision. Earl Fullman of this laboratory has grown large perfect crystals of these alkali halides by growth from a melt. The purity of these crystals, even though made from pure starting materials, is enhanced by the crystal-growth method because trace impurities tend to be rejected by the slowly growing lattice, much in the manner of the zone-purification process. Fullman has also grown many other crystals for me, and these are invaluable as further check points. These investigations will be published elsewhere.

The use of these crystals, as convenient substitutes for the rare gases, at once sets the appropriate equations for periods III, IV, and V. This still leaves helium, at the bottom of period II and radon at the top of period VI, which would be necessary to define these periods. Period II has been settled by using a synthetic crystal of LiF and also by scattering from teflon which contains only carbon and fluorine and is thus immune from the anomaly of hydrogen. The upper end of period VI is not established to our complete satisfaction although

bismuth ($Z = 83$) of spectroscopic grade seems quite satisfactory. One might infer at this point that periods II and VI are difficult to define. At the risk of being repetitious, it can be said that we have hundreds of reliable measurements in these periods. What is lacking, especially in period VI, is a single convenient reference sample of unquestioned reliability which can be connected with the xenon point as given by CsI.

BACK-SCATTERING BY SOLUTIONS OF ALKALI HALIDES

The back-scattering of solutions is of great interest, not only from the point of view of solution anomalies which they might reveal, but because many substances can be examined which otherwise would be

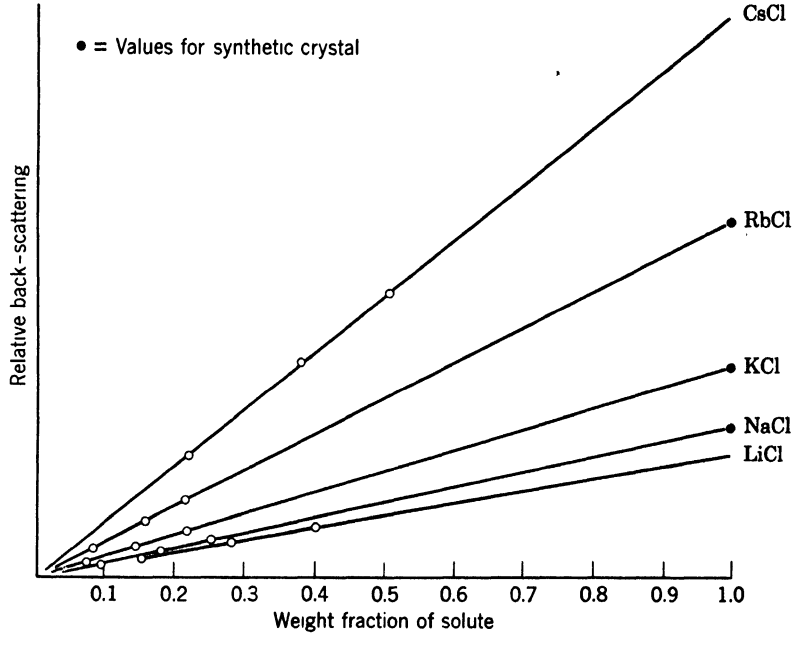


FIG. 6. Back-scattering by alkali halide solutions.

very difficult to prepare in well-defined crystals. During the past summer, Lonadier and I studied solutions of LiCl, NaCl, KCl, RbCl, and CsCl. In addition, solutions of NaF, NaBr, and NaI were examined. In the former series, which is illustrated in Fig. 6, the relative back-scattering is strictly a linear function of weight fraction of solute. The data are limited by solubility considerations. When the straight

lines are extrapolated to unit weight fraction, one gets the value that would be exhibited by the pure solid. These might be considered to be heroic extrapolations, but the three cases illustrated in Fig. 6 by solid dots indicate points for which pure crystals were available as a direct check on the extrapolation. Indeed for these systems, the entire concentration range for back-scattering can be established with two measurements: one with pure water and a second with a pure crystal, if the latter is available. Of course, there is absolutely no proof here that all solutes in various solvents would exhibit strict linearity. As a matter of fact, some current investigations here on binary mixtures of liquids, which in other properties exhibit marked deviations from ideality, reflect some of these anomalies in their back-scattering.

Lonadier's results permit the calculation of intrinsic scattering for the alkali metals which begin each period and for the halogens which are but one atomic number from the end of the periods. These and other aspects of solution behavior will be the subject of another chapter.

The choice of weight fraction as the concentration index seems most direct, especially if the back-scattering of the pure solid phase is of primary interest. It is curious to note that, among the very early measurements made around the turn of the century, Allen measured several solutions, but this good physicist was misled by his well-intentioned chemist friends who must have stated that all good chemists use molarity, molality, or the normal solution as a concentration basis. Estimates for the solid-phase scattering in such cases must be made at infinite concentration. Some of Allen's results, when recalculated to a weight-fraction basis, are nearly linear, but the precision was not very high, and no very definite conclusions can be drawn from them.

STRUCTURAL EFFECTS OF BACK-SCATTERERS

It is naturally of interest to see what this powerful tool of back-scattering can reveal about molecular structure. One of the first tests made in this direction was to study the back-scattering from isomers. The first case examined was diethyl ether and normal butanol, both $C_4H_{10}O$. Their back-scattering is identical. This would please any physicist and disappoint every chemist because one can tell the difference just by smelling them. However, they do differ in their ability to transmit beta particles and very closely in proportion to their densities (inversely, of course). Lonadier has proved this also for

anthracene, phenanthrene, and tolan. His initial attempts were made with pellets compressed in a die with a hydraulic press. More precise values were obtained from solutions with values extrapolated to unit weight fraction of solute. In transmission, only phenanthrene and tolan could be compared; anthracene was not satisfactory owing to solubility limitations.

These conclusions are summarized in Table II. Another interesting case deals with nonisomeric substances. It happens, accidentally,

TABLE II

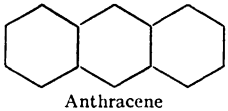
BETA-PARTICLE BACK-SCATTERING AND TRANSMISSION

Isomers

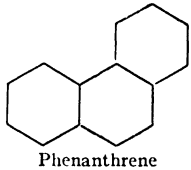
$(C_2H_5)_2O$ Back-scattering identical

Both $C_4H_{10}O$

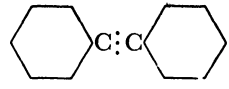
$CH_3(CH_2)_2CH_2OH$ Transmission inversely as densities



Anthracene



Phenanthrene



Tolan (acetylene diphenyl)

All $C_{14}H_{10}$

Back-scattering identical, transmission, phenanthrene/tolan, inversely as densities

Nonisomeric, but almost identical $\bar{Z}$ values

Halite (NaCl) Fluorite (CaF_2)

$\bar{Z} = 14.639$ $\bar{Z} = 14.646$, $\Delta\bar{Z} = 0.048\%$

Back-scattering identical ($<0.1\%$), halite slightly lower

that NaCl and CaF_2 have practically identical $\bar{Z}$ values; consequently they should scatter identically. Exhaustive counts on these two diverse substances show indeed that they differ by less than 0.05%, but, as expected, halite is very slightly lower. We are having thin plates of halite and fluorite ground to identical thickness. They will undoubtedly show marked differences in transmission.

Combined measurements of back-scattering and transmission will answer many fundamental questions, and we now have sample holders and a measuring system to do this.

This general survey of the phenomenon illustrates many startling regularities. We have not indulged in any extensive speculation regarding the explanation of these regularities, although we are now in possession of enough information to do so. It might be claimed that too much empiricism is attached to these studies. Against this we submit the evidence that the best theoretical treatments of this complicated phenomenon can offer little more than "order of magnitude" results. What has been observed is, in most cases, susceptible to check and measurement with high precision. It would seem strange indeed that if Nature exhibits such simple regularities that a relatively simple explanation would fail to explain them. This matter is now occupying our earnest attention. It is almost unnecessary to point out that these studies have raised more questions than they have answered. Hundreds of new approaches and systems present themselves as interesting and intriguing possibilities. Not the least of these are new experimental techniques. Although I have spent a good part of my life in instrumental developments, in this instance the earliest results were so interesting that it soon became advisable to "let well enough alone" and get more information. This does not alter the fact that great experimental and instrumental improvements are possible and highly advisable. Many of these can be in the direction of low cost and simplicity. For example, fairly strong sources of pure beta emitters are quite inexpensive, and with one of these it is possible to observe these phenomena with a gold-leaf electroscope and a stopwatch, thus reducing the technique practically to a high school experiment.

Serious objections might be raised to the entire basis of these studies, for the reason that the beam of beta particles is not monoenergetic, and one might argue that a beta spectrometer or a monoenergetic and steady source of electrons from a high-voltage accelerator would yield more convincing information. There are several good reasons why this is not necessarily so. There is some collateral evidence that the back-scattered betas are not greatly changed with respect to energy distribution. On the basis of cost, these more elegant devices are more expensive by factors of 10^3 to 10^4.

Newer and better methods of measuring beta particles are an unquestionable means of improving these techniques. With strong sources, direct counting of the collected electrons is quite feasible.

The so-called "atomic battery" using strong Sr^{90} sources is a typical example. The bombardment of nonlinear devices such as transistor junctions is another promising approach.

DISCUSSION

Discussion Leader
W. Wayne Meinke
University of Michigan

I am sure that you all enjoyed Dr. Müller's talk on instrumentation as much as I did. This last method of analysis by back-scattering of beta radiation is particularly intriguing. The idea that this can be carried out with such precision, I think, is very interesting.

I am not an instrumentation expert. For a few minutes I would like, however, to make a few general statements on instrumentation and then try to make some comparisons of several of the methods we have heard about here.

Dr. Müller allowed me to read part of his manuscript for this symposium, and I was intrigued by one statement which I would like to read to you. The statement is this: "To weigh a sample, cremate it and weigh it again has proved its worth to the analyst, but as a scientific story it eliminates most of the interesting plot."

I think this is important, especially in line with Dr. Müller's comments that the trend of analysis is toward nondestructive methods. In many of our present-day standard methods we burn our samples in flames of thousands of degrees, we shock them with high-powered arcs, we leach or dissolve them with powerful acids or fuse them with powerful bases. All these techniques, of course, destroy the material. Some of the more recent methods such as X-ray diffraction and nuclear magnetic resonance are more subtle and depend on nuclear properties of the atoms of the sample. Even activation analysis is basically "nondestructive" when it is possible to make the analysis without dissolution of the sample. I am sure that in the future we will see the development of more of these nondestructive methods.

In my concluding remarks I would like to try to compare several of these trace methods that we have heard about.

There seem to be two tendencies on the part of analysts. One is to stick completely with a method with which they are familiar, no matter what advances come up in other fields. Then there is another

group of people who, once they find some new method or technique, go over to it completely. They want to do everything by that method and insist that it is the only method that is any good, and it really is the answer to all analytical problems.

Of course we want to try to find some median position between these two extremes. It seems to me the best way to find this happy medium is to try to make some comparisons between old and new methods, no matter how imperfect these comparisons might be.

A year and a half ago I attempted to make sensitivity comparisons of this sort for the Gordon Research Conference in Analytical Chemistry. These order-of-magnitude comparisons were later published in *Science* and can be used as a starting point in comparing a number of analytical methods. The entire article from *Science* is reprinted in Chapter 23.

QUESTIONS AND ANSWERS

J. T. HORECZY (*Humble Oil and Refining Co.*): Are commercial back-scattering instruments for hydrogen determination available? I am familiar with the absorption instrument on the market, where a density determination must be made.

RALPH H. MÜLLER: In my brief discussion I omitted two important commercial instruments. One, to which you no doubt refer, is based on the elegant work of Smyth and Otros in which beta-particle absorption provides a precise means for determining the hydrogen content of liquids. There are two commercial instruments utilizing their principle. The other is the use of beta particles in measuring and recording the thickness of paper or thin metal foils during fabrication. They utilize both transmission and back-scattering. Such machines are built by Tracerlab, General Electric, and Pratt and Whitney.

J. T. HORECZY: I want to get around measuring the density, to omit the measuring.

RALPH H. MÜLLER: To eliminate the burden of measuring the density?

J. T. HORECZY: Yes.

RALPH H. MÜLLER: At present I do not know what hopes there are in that direction.

ERIK ODEBLAD (*illustrating on blackboard*): I did not realize how you obtained the point for back-scattering from gases. Did you compare the same substance in the liquid and the gas state?

RALPH H. MÜLLER: I wonder if everyone heard Dr. Odeblad's question? He is worried about the question of the density of the sample: i.e., Would it be vastly different between vapor and liquid in all cases? The answer to this is "yes." Our back-scattering measurements were confined to solids, liquids, and solutions, all of sufficient thickness to insure maximum back-scattering. Did I give the impression that we had actually measured the rare gases?

ERIK ODEBLAD: No.

RALPH H. MÜLLER: All our results indicate that the linear relationships between back-scattering and atomic number should change abruptly at the rare gas which terminates each period in the periodic system. Rather than attempt to prepare samples of each solid rare gas, we found that crystals of NaF, KCl, RbBr, and CsI should yield back-scattering values almost identical with solid Ne, A, Kr, and Xe, respectively. The evidence is thus indirect but very convincing. Further evidence for the fact that a rare gas marks an inflection point was afforded by measurements of a large number of elements and compounds in two adjoining regions. When the linear equations for the two periods are solved simultaneously, their common point or intersection corresponds unmistakably to the atomic number associated with a rare gas. The same conclusion follows from extensive measurements on solutions of alkali chlorides and sodium halides.

ERIK ODEBLAD *(illustrating on blackboard)*: Earlier data indicate that the relation between back-scattering and Z should be a slightly curved line, but you say these data are fitted better by a broken straight line. Do you have data that are accurate enough to draw this conclusion?

RALPH H. MÜLLER: Yes. The early data dealing with a few elements can be fitted fairly well to a straight line when back-scattering is plotted against the cube root of Z. It is only when a large number of elements and compounds are measured that deviations from the smooth curve become apparent. These can be fitted only by a linear function of Z changing slope abruptly at atomic numbers 10, 18, 36, and 54.

REFERENCE

1. *Phys. Rev., 93,* no. 4, 891 (1954).

Part III

SENSITIVITY, SEPARATION, CONCENTRATION, AND CONTAMINATION

— 23 —

Trace-Element Sensitivity

Comparison of activation analysis with other methods *

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ABSTRACT Rough comparisons of the sensitivity of activation analysis with other standard methods give the investigator a feel for the elements for which activation can be used as a powerful tool. Although improvements in technique for an entire method or for an individual element will invalidate the figures given in Table II, the over-all picture will be changed only slightly. Furthermore, other considerations such as speed of analysis, interferences of other elements, and availability of analyzing instruments and facilities may indicate the use of one method in preference to another. The increasing availability of nuclear-reactor facilities, however, means that analysis by activation is an important new tool for general use in trace analysis.

This work and similar studies on activation analysis have been supported in part by the University of Michigan Memorial Phoenix Project. The assistance of Kenneth K. Kendall Jr. of Beckman Instruments, A. A. Shurkus of Applied Research Laboratories, and C. E. Shuter of Jarrell-Ash Co., in locating authoritative summaries of sensitivities for standard methods of analysis is gratefully acknowledged. The material given in this chapter was presented before the Gordon Research Conference in Analytical Chemistry at New Hampton, N. H., in Aug. 1954.

* Reprinted from *Science, 121,* 177–184 (1955) with the permission of the publishers.

Activation analysis is a method for determining the constituents of a sample by utilizing certain nuclear properties of the isotopes of the elements in the sample. Nuclear particles are used to produce radioactive isotopes by activation of the nuclei of the sample elements. These radioisotopes can be detected and measured by their nuclear radiations. Thus with an exact knowledge of nuclear characteristics, a determination of the amount of element present can be made. This method is applicable to determination of trace elements in many types of materials used by the biologist, chemist, metallurgist, physicist, and others. Solid, as well as liquid, samples can be used for the analysis; a few milligrams or less of material is sufficient for precise work. When chemical separations are required in activation analysis to remove interfering ions, no errors are introduced by trace contaminants in the reagents.

Analysis by radioactivation was first suggested by Hevesy and Levi [1] in 1936 when they used neutrons from a 300-mc radium-emanation beryllium source to activate certain rare-earth elements. It was not until the advent of the nuclear reactor, however, that sources of sufficiently high neutron flux became available to give high sensitivity to this method.

In the past decade activation analysis has been viewed by many as representing the ultimate in sensitivity. The completion of new reactors with increasingly high fluxes brought a steady improvement in this sensitivity. Most analysts, however, had to forego the reportedly high sensitivities and had to use the more conventional methods of analysis because there were few reactor facilities available and access to them was limited.

In 1952 Oak Ridge National Laboratory made available to the public an activation analysis service [2,3] that has since enjoyed a favorable response. At the present time several non-AEC laboratories are building research-type reactors,[4] and the prospects for many more reactor facilities being built in the near future are good.

This increase in access and availability of sources of high neutron flux will proportionately increase the ease of obtaining analysis by activation. Not only will it be possible to send samples out to any of several laboratories for this type of analysis, but also in areas near a reactor an investigator should be able to make his own analysis at the reactor facility.

Thus some critical evaluation of activation analysis as a method for determining trace quantities of elements is necessary to ascertain its advantages and limitations relative to other existing methods of

trace analysis. Limits of sensitivity for various methods can be found in the literature. Although these limits vary with investigators, types of equipment, experimental procedure, and so forth, rough comparisons of these limits for each element can provide invaluable insight into the value of activation analysis relative to other methods of trace analysis.

ACTIVATION ANALYSIS

A number of general papers [5-15] on activation analysis should be consulted for background information on the method. The general equation governing analysis by activation is

$$\text{Grams of } X = \frac{\text{act.} \times \text{at. wt.}}{6.02 \times 10^{23} f(\sigma_{at})[1 - \exp(-0.693t/t_{1/2})]}$$

where

act. = activity, disintegrations per minute
at. wt. = atomic weight of isotope activated
f = flux of neutrons per square centimeter second
σ_{at} = atomic cross section of element (isotopic cross section × percentage abundance of isotope) †
$1 - \exp(-0.693t/t_{1/2})$ = saturation factor which depends on the ratio between length of bombardment and length of half-life of isotope formed
t = time of bombardment
$t_{1/2}$ = half-life of isotope produced

From this equation it can be seen that the activity for a given number of grams of material is directly proportional to the neutron flux. Furthermore, the number of grams of a material that gives a specified activity is inversely proportional to the flux. Thus the higher the neutron flux, the higher the sensitivity for detection of a given isotope.

Table I is a list of the average thermal neutron flux available from different sources of neutrons. A corollary to the fact that higher neutron fluxes give higher sensitivities is the fact that lower-level neutron sources can be used whenever lower sensitivities can be tolerated for an analysis. Hence even the portable low-level radium–beryllium and antimony–beryllium sources can be used for analysis on occasion.[10,16]

† The cross section is a measure of the probability of activation for a particular isotope; a compilation of isotopic cross sections can be found in the literature.

TABLE I

NEUTRON SOURCES AND USABLE FLUX

		Neutrons per square centimeter second
Ra–Be, 25 mg		10^2–10^3
Sb–Be, 1 c		10^3–10^4
Van de Graaf		10^7–5×10^9
Cyclotron		10^8–10^9
Reactor		
Oak Ridge	X-10	5×10^{11}
Oak Ridge	LITR	1×10^{13}
Brookhaven	BNL	2×10^{12}
Chalk River	NRX	2×10^{13}
Idaho	MTR	2×10^{14}

Order-of-magnitude sensitivities of various elements for activation analysis in the 5×10^{11} neutrons per square centimeter per second

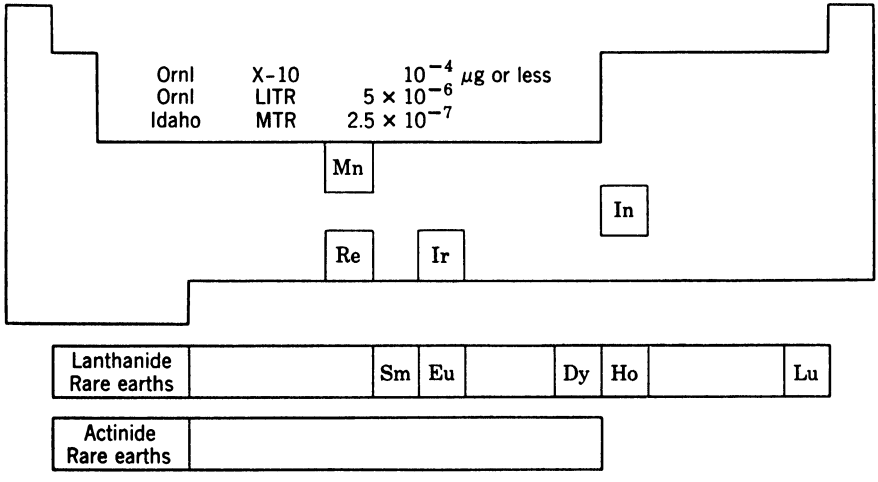

FIG. 1. Elements with thermal neutron-activation sensitivity of 10-4 μg or less in the Oak Ridge X-10 reactor.

flux of the Oak Ridge X-10 reactor have been published.[2] The sensitivities are shown in Figs. 1 through 4.‡ One additional chart could be made showing only europium and dysprosium with a sensitivity of

‡ Thanks are due to G. W. Leddicotte and S. A. Reynolds for permission to extend their sensitivity chart (ref. 9) in Figs. 1–4. Two changes could be made in the charts to bring them up to date. The detection limit for nickel should be 1 μg, and thorium should be added to the figures with a limit of 10-3 μg.

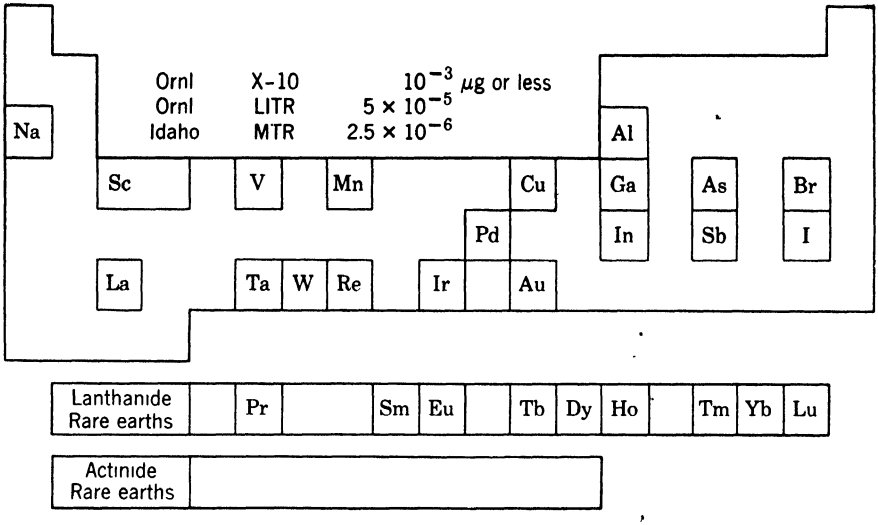

FIG. 2. Elements with thermal neutron-activation sensitivity of 10^{-3} μg or less in the Oak Ridge X-10 reactor.

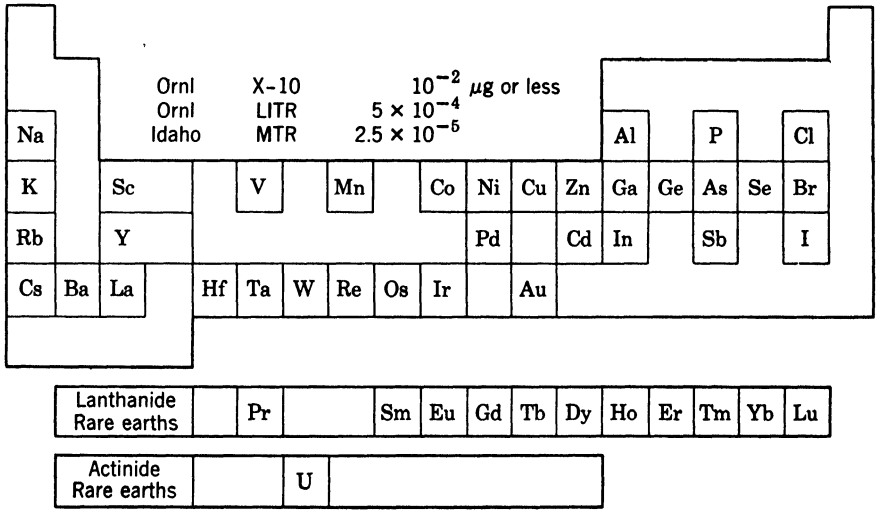

FIG. 3. Elements with thermal neutron-activation sensitivity of 10^{-2} μg or less in the Oak Ridge X-10 reactor.

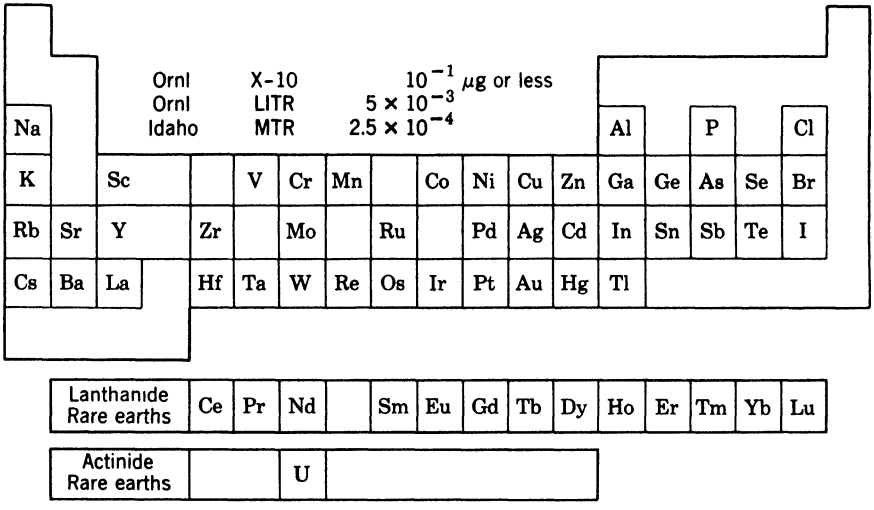

FIG. 4. Elements with thermal neutron-activation sensitivity of 10^{-1} μg or less in the Oak Ridge X-10 reactor.

10^{-5} μg or less for the X-10 reactor. Values for higher flux reactors are also listed on the charts.

COMPARISONS

Table II is a comparison of the sensitivities for the elements reported for activation analysis by Leddicotte and Reynolds [9] with those for five other typical trace-analysis methods for solutions and soluble salts. These methods do not necessarily represent the five best methods for trace analysis but were chosen as typical methods in general use.

It was necessary to make a number of assumptions in order to make the comparison. Lists of sensitivity limits as published by an authority in the field were taken without any further individual evaluation. Higher sensitivities for one element or group of elements may now be possible because of recent perfection of new procedures. In addition, sensitivities for an entire method might be increased by suitable improvements in technique. Neither of these possibilities has been taken into consideration in making these comparisons. Gross normalizing factors were introduced for each method to adjust the values to the same units as the activation-analysis values.

The problem of interfering ions and the necessity, in some cases, for chemical separations before the indicated sensitivity can be ob-

tained are completely neglected, even though they play a very important practical role in the problem. The speed with which the analyses are made is not considered. Finally the problem of multiple-element analysis in a single sample is not considered.

Regardless of these limitations, the comparison is worth while in that it readily points out the few elements for which activation analysis is particularly well suited and also the few for which it is not. It also shows elements for which activation gives only relatively fair sensitivity but for which it is very useful because other methods are at present much less sensitive.

In Table II sensitivities that can be obtained by activation analysis with two different nuclear reactors are compared with sensitivities for the other methods. Sensitivities for each element and method are those reported in the compilations of data listed here. Certain gross normalizing factors, however, were used for each method to adjust the values to the common sensitivity basis of "micrograms per milliliter" and an error of about 10%. It was assumed that as much as 25 or 50 ml of the unknown solution was available.

The values listed in the table represent an *order of magnitude only,* since peculiarities of experimental procedure, investigators, and so forth, introduce considerable variation. For activation analysis they represent conservative values, although values for other methods may be somewhat optimistic. Several of the methods are capable of an increase in sensitivity of a factor of 10 or more by a change of equipment or the use of larger samples, and so forth, if they were to be pushed to the limit.

The table, although not complete, is presented as a basis for general comparisons of the methods or as a type of work sheet. Pertinent information on each method follows.

Oak Ridge X-10 Reactor. Values are listed by Leddicotte and Reynolds [9] for activation analysis in the Oak Ridge X-10 reactor at a flux of 5×10^{11} neutrons per square centimeter second to give 40 disintegrations per second. Samples are activated to saturation or 30 days, whichever is shorter. It is assumed that a 1-ml sample is evaporated per analysis. The sensitivity of the method could be substantially increased by increasing the volume of sample used. The primary limitation is the effect of self-absorption of the bulky sample on the radiations emitted by the radioisotope. If it were desired to increase the sensitivity further, samples with activity less than 40 disintegrations per second might be accepted. With special techniques, an activated isotope having about one tenth of this activity could be determined.

TABLE II

Sensitivity Comparisons

Micrograms per milliliter

		Methods						
Z	Element	Oak Ridge X-10 Reactor	Oak Ridge LITR Reactor	Copper Spark	Graphite D-C Arc	Flame Spectro-photometer	Sensitive Color Reaction	Ampero-metric Titration
1	H							
2	He							
3	Li			0.002		0.02		
4	Be			0.002		250	0.04	
5	B			0.1		10		
6	C							
7	N							
8	O							
9	F			0.1				0.25
10	Ne							
11	Na	0.007	0.00035	0.1	20	0.002		
12	Mg	0.6	0.03	0.01	0.1	1	0.06	
13	Al	0.001	0.00005	0.1	0.2	20	0.002	300
14	Si	1	0.05	0.1	2		0.1	
15	P	0.02	0.001	20	50		0.01	15
16	S	4	0.2					5
17	Cl	0.03	0.0015				0.04	10
18	A							
19	K	0.08	0.004	0.1		0.01		100
20	Ca	3.8	0.19	0.1		0.03		100
21	Sc	0.002	0.0001	0.005				
22	Ti			0.1		2	0.03	~10
23	V	0.001	0.00005	0.05		2	0.2	3
24	Cr	0.2	0.01	0.05	2	1	0.02	1
25	Mn	0.0006	0.00003	0.02	0.2	0.1	0.001	0.0003
26	Fe	9	0.45	0.5	0.2	2	0.05	2
27	Co	0.02	0.001	0.5		10	0.025	100
28	Ni	0.03	0.0015	0.1	4	10	0.04	0.5
29	Cu	0.007	0.00035		0.2	0.1	0.03	10
30	Zn	0.04	0.002	2	20	2000	0.016	10
31	Ga	0.007	0.00035	1		1		
32	Ge	0.04	0.002				0.08	
33	As	0.002	0.0001	5	10		0.1	0.4
34	Se	0.05	0.0025					
35	Br	0.003	0.00015					200
36	Kr							
37	Rb	0.03	0.0015	0.2		0.1		
38	Sr	0.6	0.03	0.5		0.1		
39	Y	0.01	0.0005	0.01		50		
40	Zr	0.3	0.015	0.1			0.13	
41	Nb	10	0.5	0.2		20	50	
42	Mo	0.1	0.005	0.05		30	0.1	5
43	Tc							
44	Ru	0.1	0.005			10	0.2	
45	Rh					1	0.2	

		Methods						
Z	Element	Oak Ridge X-10 Reactor	Oak Ridge LITR Reactor	Copper Spark	Graphite DC Arc	Flame Spectro-photometer	Sensitive Color Reaction	Ampero-metric Titration
46	Pd	0.005	0.00025	0.5		1	0.1	
47	Ag	0.11	0.0055		0 1	0.5	0.1	1
48	Cd	0.05	0.0025	2	4	20	0.01	5
49	In	0.0001	0.000005	1		1	0.2	100
50	Sn	0.2	0.01		0.2	10		2
51	Sb	0.004	0.0002	5	4		0.03	10
52	Te	0.1	0.005	0.5		100	0.5	
53	I	0.002	0.0001					1
54	Xe							
55	Cs	0.03	0.0015	0.5		1		
56	Ba	0.05	0.0025	0.1		3		25
57	La	0.002	0.0001	0 05		5		
58	Ce	0.1	0.005	0.5		20	0.25	~500
59	Pr	0.002	0.0001	0.2		100		
60	Nd	0.1	0.005	0.2		50		
61	Pm							
62	Sm	0.0006	0 00003	0.2		100		
63	Eu	0.00003	0.0000015	0.02				
64	Gd	0.02	0.001	0.1		10		
65	Tb	0.004	0.0002					
66	Dy	0.00003	0.0000015	0.5		10		
67	Ho	0.0004	0.00002	0.2				
68	Er	0.02	0.001	0.5				
69	Tm	0.002	0.0001	0.05				
70	Yb	0.002	0.0001	0.1				
71	Lu	0.0003	0.000015	2				
72	Hf	0.02	0.001	0.5				
73	Ta	0.007	0.00035	1				
74	W	0.003	0.00015	0.5			0.4	
75	Re	0.0006	0.00003	2			0.05	
76	Os	0.02	0.001				1	
77	Ir	0.0003	0.000015	5			2	
78	Pt	0.1	0.005	0.02			0.2	
79	Au	0.003	0.00015	0.2		200	0.1	
80	Hg	0.13	0.0065	5	2	100	0.08	
81	Tl	0.6	0.03		0.2	1		
82	Pb	2	0.1	0.05	0.2	20	0.03	3
83	Bi	~0.4	~0.02	0.2	0.2	300	1	300
84	Po							
85	At							
86	Em							
87	Fr							
88	Ra			0.1				
89	Ac							
90	Th			0.2				
91	Pa			2				
92	U	0.01	0 0005	1		10	0.7	

Oak Ridge LITR Reactor. Values are given for activation analysis in the Oak Ridge LITR reactor (or proposed university research reactors at full power) at a flux of 10^{13} neutrons per square centimeter second. All other conditions and comments are identical with those given for the X-10 reactor.

Copper Spark. The original sensitivity values from which these were derived are listed by Nachtrieb [17] for spectrographic analysis using the copper-spark method. It is assumed that for best results only $\frac{1}{20}$ ml of sample can be evaporated on the electrode. If more sample could be evaporated without interfering seriously with the analysis, the sensitivity relative to activation would increase. Nachtrieb gives his data in millimicrograms using a 50-lambda sample and estimating an error of ±3 to 8%. His values were divided by 1000 to convert to micrograms, multiplied by 20 to adjust to the basis of a 1-ml sample, and divided by 2 to allow for an error of ±10%. The net result was to divide the literature values by 100 for the comparison.

Graphite D-C Arc. The original values are also listed by Nachtrieb [17] for spectrographic analysis, using graphite d-c arc methods. It is assumed that $\frac{1}{20}$ ml of sample was evaporated on the electrode. All comments on normalization given for the copper-spark method apply to this method. The literature values were divided by 100 to obtain the comparison value.

Flame Spectrophotometer. The original sensitivity values for analysis using the flame spectrophotometer with an oxyhydrogen flame are given in Beckman reprint R-56.[18] Values were given directly in micrograms per milliliter. The errors given for the reported sensitivities were ±100%. The values were therefore multiplied by a normalizing factor of 10 to convert them to the same ±10% error as the other methods.

Sensitive Color Reaction. The original sensitivity values for spectrophotometric analysis using sensitive color reactions were taken from Sandell.[19] The values are given in micrograms per square centimeter. It was assumed that a standard 1-cm cell was used in the spectrophotometer, although the 10-cm cells available could increase the sensitivity by a factor of 10. As with flame photometry, the original values were given with a ±100% error and were multiplied by a normalizing factor of 10 to convert to the prescribed basis.

Amperometric Titration. The original values for amperometric titration are listed in a compilation made by Sargent and Company.[20] These values were not all converted to the same units in the compila-

tion; some were given as micrograms, others as molarity, and so forth, with different limits of error. For this comparison, the sensitivity values were converted as nearly as possible to the common basis of the other methods considered. The normalized values, however, are much less certain than values presented for the other methods. Recent work on coulometric analysis may have superseded some of the lower sensitivity values for a number of the elements.

COMPARISON GRAPHS

Comparisons of these methods can be seen much more readily when they are presented in graphic form. Therefore methods for certain elements of importance have been compared graphically.

Certain of the rare-earth elements show the highest sensitivity of any elements for activation analysis. These are shown in Fig. 5 com-

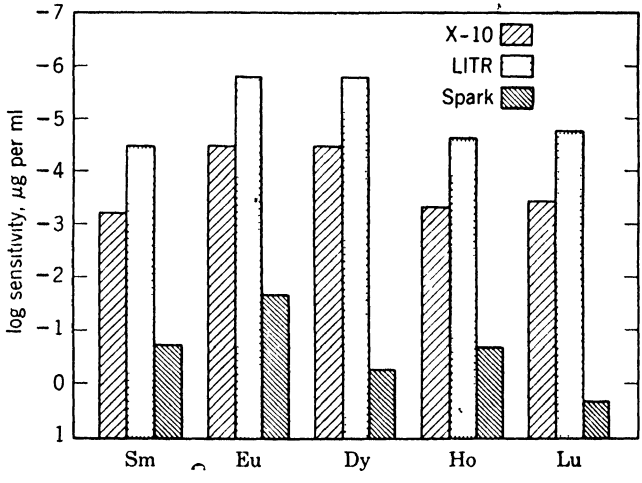

FIG. 5. Comparison graphs of some rare-earth elements having high sensitivity for activation analysis.

pared with the values for spark spectroscopy. For europium and dysprosium truly fantastic sensitivities can be obtained. For each of the elements shown in this figure, analysis by activation so far overshadows other methods that it is possible to obtain sensitive analysis with neutron fluxes much lower than those of a reactor.

Manganese, indium, rhenium, and iridium are four other elements that show high sensitivity on the chart of Fig. 1. The sensitivities for these elements are plotted in Figs. 6 and 7. The stars on the graph

under the symbol of the element indicate the zero point on the log-sensitivity scale. Although activation analysis is very sensitive for

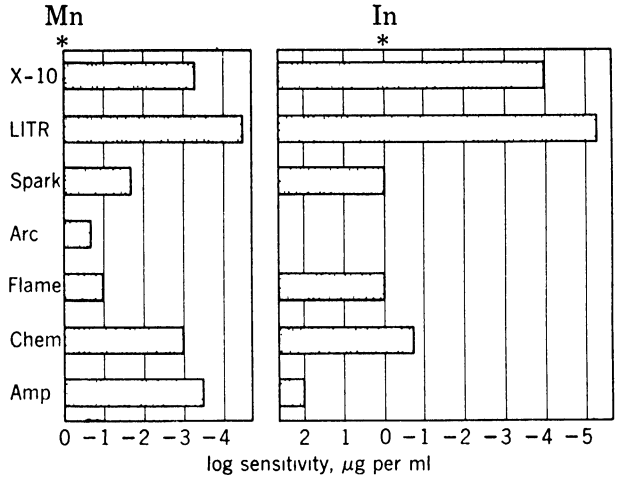

FIG. 6. Comparison graphs for manganese and indium.

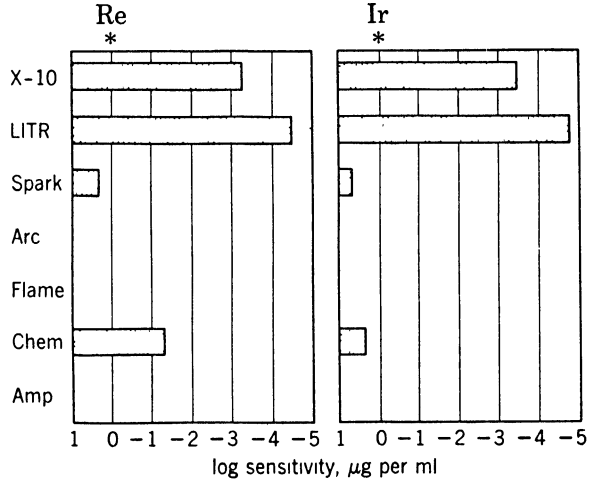

FIG. 7. Comparison graphs for rhenium and iridium.

manganese, the coulometric method of Furman and the spectrophotometric method using 4,4′-tetramethyldiaminotriphenylmethane give almost equivalent sensitivities. For indium, on the other hand, the other methods are not so highly developed, and activation would prove very useful. Activation would also prove invaluable for both rhenium

and iridium, since other methods available at present prove unsatisfactory for highest sensitivity.

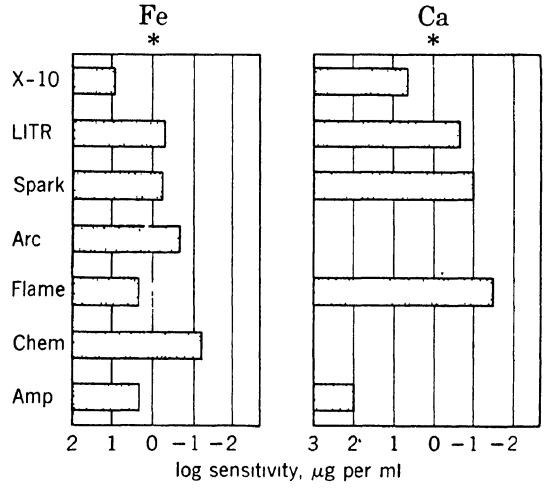

FIG. 8. Comparison graphs for iron and calcium.

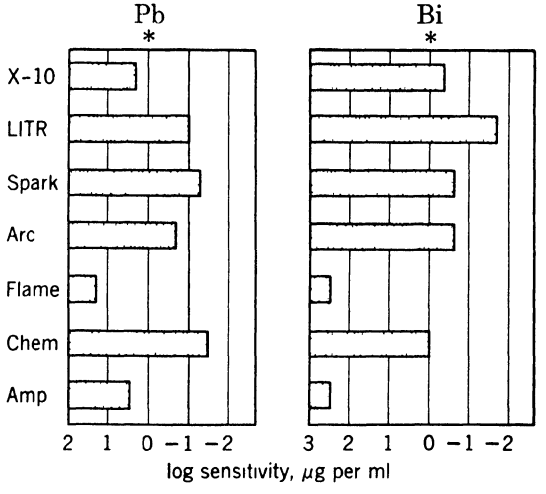

FIG. 9. Comparison graphs for lead and bismuth.

Figures 8 and 9 show comparison graphs for iron, calcium, lead, and bismuth, all of which show poor sensitivity for activation analysis. For analysis of these elements, it would be inadvisable to use activation analysis unless some special condition required it.

Figures 10, 11, and 12 show graphs for aluminum, vanadium, copper, gold, arsenic, and antimony, all of which show good sensitivity

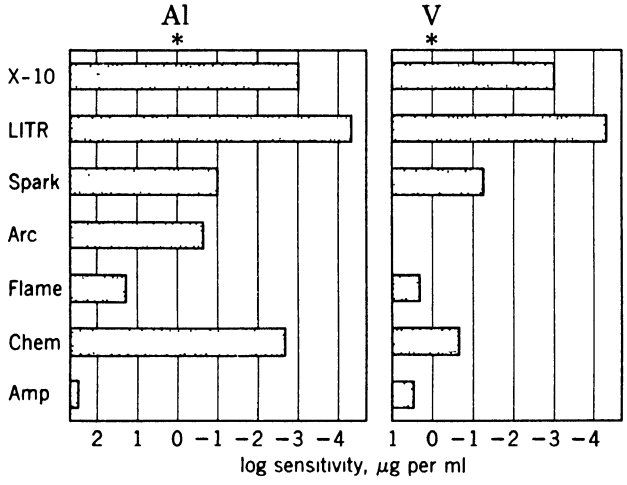

FIG. 10. Comparison graphs for aluminum and vanadium.

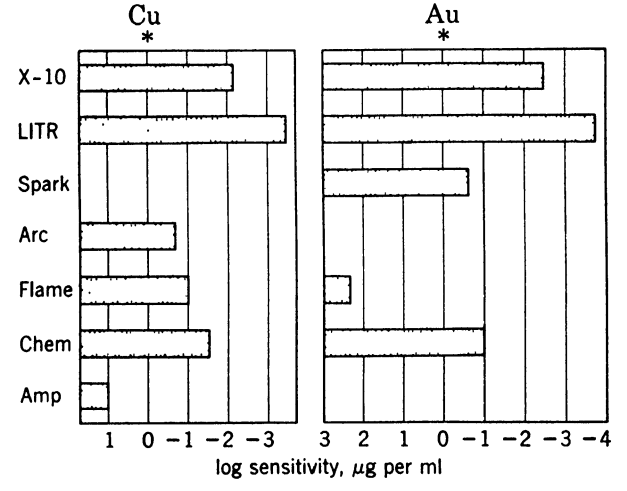

FIG. 11. Comparison graphs for copper and gold.

for activation analysis. Activation does not present enough advantage over conventional methods to warrant its use for copper and aluminum, but for the others it may provide some advantage.

Figures 13, 14, and 15 show graphs of several other elements that

are either neighbors in the periodic table or are in the same family. For each the activation sensitivity is only fair, but in some cases the method would be advantageous.

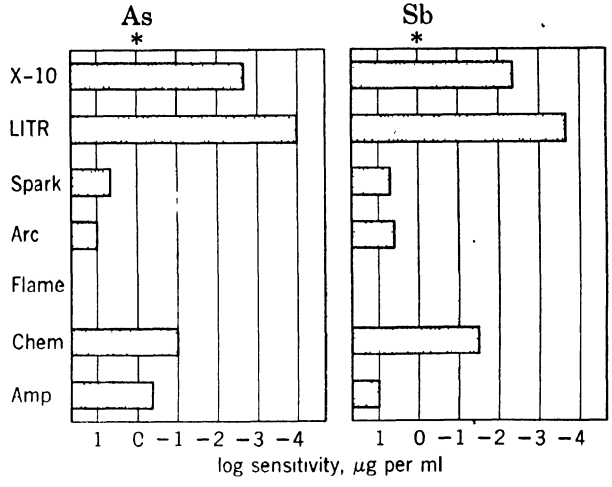

FIG. 12. Comparison graphs for arsenic and antimony.

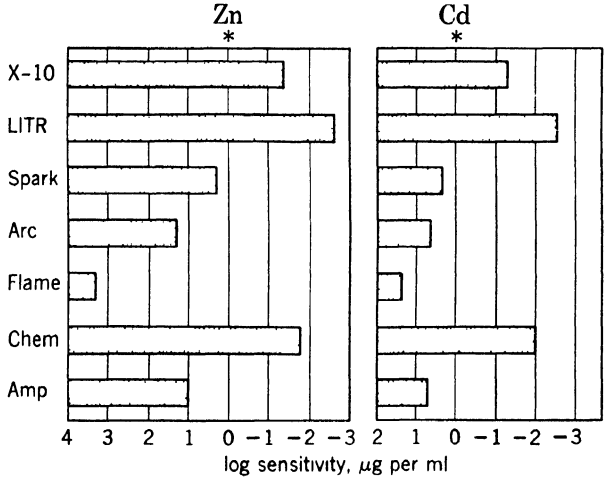

FIG. 13. Comparison graphs for zinc and cadmium.

Table III lists the chemicals that are reported to give the sensitivities indicated for the sensitive-color-reaction method on the graphs of Figs. 6 through 15.

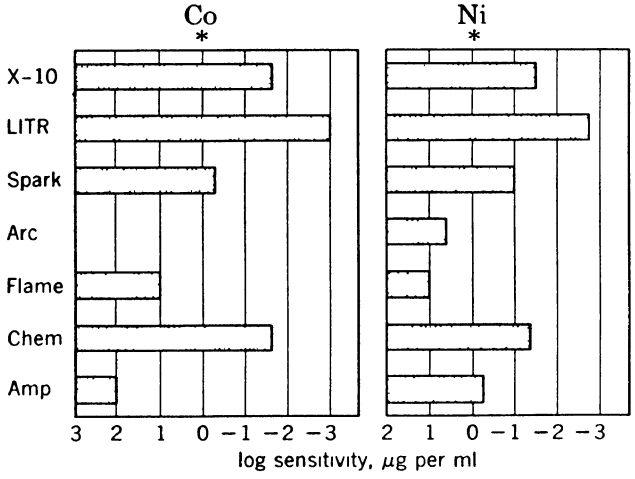

FIG. 14. Comparison graphs for cobalt and nickel.

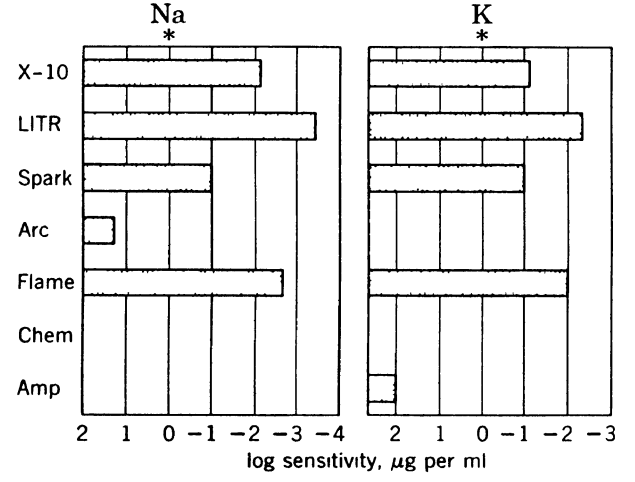

FIG. 15. Comparison graphs for sodium and potassium.

TABLE III

REAGENTS FOR HIGH-SENSITIVITY COLOR REACTIONS [19]

Element	Reagent
Mn	4,4′-Tetramethyldiaminotriphenylmethane
In	8-Hydroxyquinoline
Re	Thiocyanate–stannous chloride (ether solution)
Ir	$IrCl_6^{-2}$
Fe	*o*-Phenanthroline
Ca	
Pb	Dithizone (CCl_4)
Bi	Phenyldithiobiazolonethiol
V	Benzoylphenylhydroxylamine
Cu	Dithizone (CCl_4)
Au	*o*-Tolidine
As	Molybdate-hydrazine sulfate
Sb	Rhodamine B
Zn	Dithizone (CCl_4)
Cd	Dithizone (CCl_4)
Co	Nitroso R-salt
Ni	Bromine–dimethylglyoxime
Na	
K	
Al	Oxine

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— 24 —

Separation, Concentration, and Contamination *

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ABSTRACT When the determination of millimicrogram quantities is required, separation and concentration of the desired constituent is often necessary, either to provide enough material for test or to remove other materials that may interfere, if only from sheer bulk. Methods of separation, concentration, and determination for the millimicrogram range are reviewed. The problems associated with such methods are discussed, solvent extraction and ion exchange being used as examples. Prevention of contamination is of obvious and prime importance to this work. Some of the most common sources of contamination are considered, specific examples being used wherever possible.

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In many areas of analytical chemistry there has been a trend toward smaller and smaller samples and concentrations until now microgram amounts of metals are handled routinely and even millimicrogram separations and determinations are no longer rare.[50] Extremely sensitive methods are required in the separation and determination of fission products and carrier-free isotopes,[48,152,153] in the determination of trace impurities in metals or salts,[50,68,89] in the analysis of minerals,[5,6,129] in water analysis,[1,55,88] in petroleum analysis,[15,16,17] in trace analysis of human tissues,[25,116,165,166] of plants,[41,45,102,142] in the general fields of biochemistry,[41,153] histochemistry,[51,91] cytochemistry,[26] and physiology and pathology.[31,108,121]

Separation is a part of many of these high-sensitivity procedures,[171] and contamination is a great hazard to all of them.[155] To review the subjects exhaustively is virtually impossible. Therefore, this discussion will be confined to some of the more prevalent sources of contamination, the more general methods of separation and determination, and their applicability to the millimicrogram range. References will be given which review, exemplify, or bear on the discussion.

CONTAMINATION HAZARDS

One of the most unsatisfactory procedures from the point of view of contamination and losses is the destruction of organic matter. Although workers with some specific samples and elements have been able to avoid it,[10,32,94,112,140] wet or dry ashing methods have generally been used. This subject has been reviewed by Middleton and Stuckey,[99] and Piper.[113] The first work mentioned gives a comprehensive coverage of the literature and concludes that no satisfactory method exists, a conclusion in agreement with the experience of many.[90] However, until a better general method is found, wet or dry ashing must suffice.

Dry Ashing. The usual technique is to dry the organic matter in a platinum or silica dish, then place it in a cold muffle furnace and raise the temperature slowly over a period of hours, to about 500° C. The sample is then left at that temperature until it has completely ashed to a white powder. The advantages of dry ashing are that it is simple, requires little operator attention, and involves no reagents with the contamination they introduce.

It has several disadvantages:

1. Mercury and cadmium are almost invariably lost by volatilization.[29]

2. Unless the temperature is kept below 450° C, Zn is also lost. Keeping the muffle temperature below 450° C is not sufficient. The ashing must be performed so that the local temperature in the sample never rises above 450° C, which it tends to do with large samples because of the heat from the oxidation of the sample.[13]

3. Some organic materials ash extremely slowly at 450° C and leave a carbon residue even after 72 hours.[155]

4. Under some conditions, Ni and V are lost on dry ashing, and, unless sulfate is added, Pb volatilizes.[46]

5. After dry ashing, the alkali and alkaline-earth metals are extremely hard to dissolve. Mitchell[102] fused this residue with Na_2CO_3 to insure complete solution. Smit and Smit[148] found low results for cobalt and molybdenum when fusion was used.

6. When silica or porcelain dishes are used, the process of dry ashing slowly etches them and provides a source of serious contamination and loss.[177] A spectrogram of a piece of "clean" silica crucible by Vallee[161] showed lines of all the elements from blood samples which had previously been ashed in it.

7. Some materials, such as whole blood, tend to foam right out of their container when the temperature reaches about 300° C, in spite of the fact that they have been previously dried or lyophilized. Pre-ashing apparatus to prevent this have been described,[33,34] but they are gas-fired and not applicable to trace analysis.

In spite of these disadvantages, dry ashing has proved to be the more useful method of the two in our hands, largely on the basis of convenience. It has been made quite routine by means of a simple apparatus which permits contamination-free drying and pre-ashing without boiling or foaming of the sample. The apparatus, as shown diagrammatically in Fig. 1, is a Pyrex crystallizing dish with a side arm attached, which is placed upside down in a Pyrex Petri dish of slightly larger diameter. This forms a glass-enclosed chamber into which clean air can be blown through the side arm, to escape under slight pressure around the bottom of the upper dish. Evaporations (see Fig. 6) or pre-ashing steps carried out inside this chamber are in a constantly changing dust-free atmosphere. The gas is made to flow slowly in a circular path around the chamber, and the top of the chamber is arched. These two precautions respectively decrease condensation on the chamber wall and prevent anything that does condense from dripping back into the sample.

The ashing method employed is as follows. The sample of biological fluid or tissue is weighed into a clean platinum dish and placed

under the evaporation cover. A 250-watt infrared lamp placed above the covered dish is used to dry the sample in the current of filtered air, slowly at first, but with increasing intensity after the water has evaporated and the sample appears dry and brittle. The heat of the infrared lamp is continued and is augmented by the heat from a hot plate under the apparatus until the sample assumes a charred appearance. This preliminary charring prevents samples from foaming and

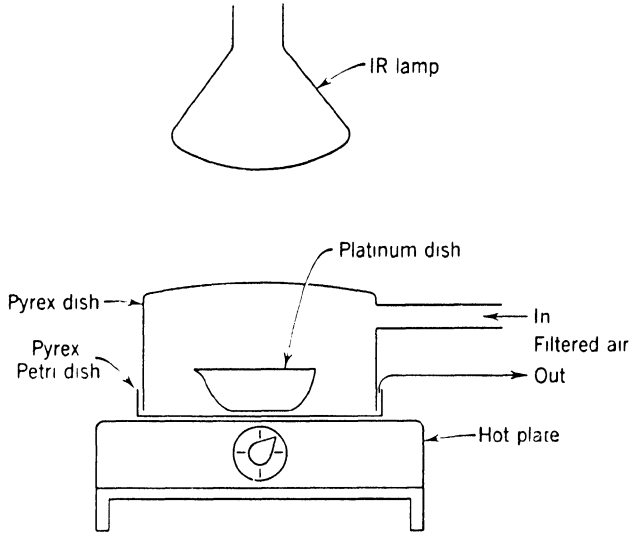


FIG. 1. Diagram of evaporation and pre-ashing cover.

spewing out of the dish when heated in the muffle furnace and is particularly helpful when large volumes of sample are ashed.

The dish containing the charred sample is placed in a silica-lined muffle furnace at 250° C or less. The temperature is brought up to 450° C over the course of an hour and is maintained there until the sample has ashed completely.

The sample ash is treated in the same dish with a minimum of purified 6 *M* hydrochloric acid. The dish is heated to aid solution of the ash, which is generally complete if enough acid and time are allowed.

Wet Ashing. Wet ashing has been thoroughly described by Smith,[149] Middleton and Stuckey,[99] Milton, Hoskins, and Jackman,[101] and others. Losses, like those associated with dry ashing, do not usually occur with wet ashing. This advantage is more than offset by the almost certain contamination that comes from the ashing reagents

and from their leaching action on the glass vessels employed. The oxidizing acids used, HNO_3, H_2SO_4 and $HClO_4$, almost invariably contain metals and require purification. It is often necessary to use ashing reagents in volumes ten times as great as the sample weight, which means that, if a blank of 0.5 mμg can be tolerated when a 10-gram sample is analyzed the acids must contain less than 0.05 mμg in 10 ml or only 0.005 part per billion (ppb). Purification methods for these reagent acids must be improved before such values are reached, although for water and hydrochloric acid such levels are now possible. Wet ashing also requires more operator time and attention than dry ashing, which is a disadvantage in routine work. Comparisons of wet ashing and dry ashing for oil samples have been made by Barney and Haight,[15] Bethge,[17] and Gamble and Jones,[46] and for plant samples by Hiscox.[70] Middleton and Stuckey [100] recommended ashing by keeping the sample moist with nitric acid at the temperature of a hot plate. This method has not been successful in our hands.

PURIFICATION OF REAGENTS

Water. Repeated distillation is the classical way of purifying water.[12, 62, 69] If the final condenser is made of fused quartz, very high purity may be achieved, but ordinarily distilled water contains magnesium, calcium, copper, aluminum, silicon, and iron, and changes the color of the chloroform layer when shaken with dithizone solution. Ion-exchange resins, particularly in mixed-bed operation, are replacing distillation in many laboratories. They are convenient, cheap, and effective. In distilled water, Liebig, Vaneslow, and Chapman [86] found 200 ppb (parts per billion) of copper. After the water had passed once through a column of cation-exchange resin, the copper concentration dropped to 3.5 ppb, and, after four more passages through the column, to less than 0.1 ppb. Healy, Morgan, and Parker [62] found in their twice-distilled water 1.4 ppb of zinc, 0.5 ppb of copper, 0.9 ppb of iron and 1.0 ppb of aluminum (no others were determined), but they found only 0.5 ppb of zinc, lead, copper, and iron, and 1.4 ppb of aluminum in water purified by ion exchange.

High-sensitivity spectrochemical analysis of Boston tap water, which had been purified by passage through a 1.5-meter column of mixed IR 120 and IRA 410 resins (Rohm & Haas Co.) showed the presence of 13 elements.[155] Table I gives the concentration of those elements for which standards were available in the required range. This water was far superior to distilled water, particularly with respect to the four

TABLE I

METAL CONTENT OF ION-EXCHANGE WATER

In parts per billion

Element	Concentration
Mg	2
Ca	0.2
Sr	<0.06
Ba	0.006
Pb	0.02
Zn	0.06
Cr	0.02
Mn	<0.02
Fe	0.02
Co	<0.002
Ni	0.002
Mo	<0.02

Identified but not determined: B, Si, Sn, Cu, Ag, V.

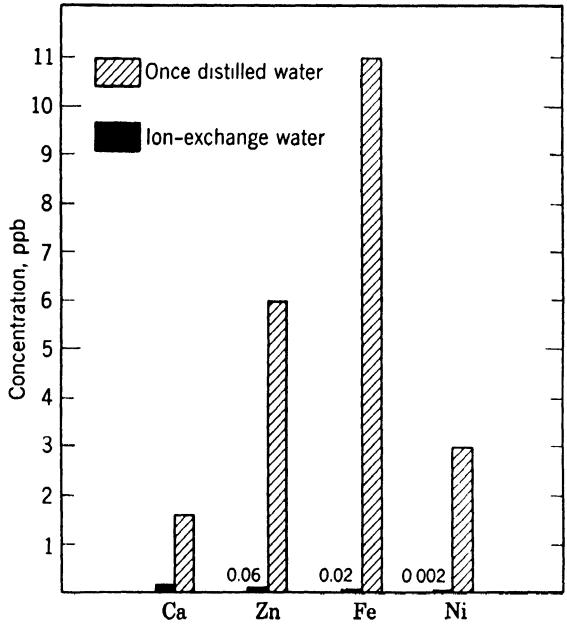


FIG. 2. Concentration of calcium, zinc, iron, and nickel in distilled and ion-exchange water.

elements shown in Fig. 2. It had a specific conductance of 3×10^{-7} reciprocal ohm (compared with 1.7×10^{-6} for triply distilled water),[69] and gave no color change at all to 20 ml of carbon tetrachloride solution of dithizone shaken with one liter.

Since non-ionic materials are not held by ion-exchange resins, and since traces of nitrogenous compounds are leached off the resins by water, probably the best treatment for obtaining purest water in *all* ways is ion exchange followed by distillation from fused quartz.

Hydrochloric Acid. Hydrochloric acid has been purified by redistillation of the constant-boiling mixture;[99] however, a much purer product can be obtained by dissolving the anhydrous gas in pure water. Anhydrous hydrogen chloride gas (Matheson Co.) is scrubbed in a bubble tower with concentrated sulfuric acid, filtered through approximately 1 meter of packed Pyrex glass wool, scrubbed again in a bubble tower with distilled water which has been saturated with the gas, and then dissolved in triply distilled water or its equivalent at 0° C to give an acid concentration up to 12 *M*. Sensitive spectrographic analysis of acid made by this method, using the ion-exchange water described above, showed that traces of only Na, K, Ba, Cr, Pb, and B had been added, but all at the fractional parts-per-billion range.[155] Table II shows this and includes a comparison with reagent-grade acid.

Nitric Acid. Repeated distillations of the azeotrope (65% nitric acid, 35% water) are generally used. However, significant quantities of sodium, magnesium, calcium, iron, aluminum, copper, silicon, chromium, and lead are found in the distillate when a Pyrex apparatus is used, and a fused silica condenser improves the product only with respect to magnesium, chromium, and aluminum.[156] Thus nitric acid, which is the reagent used in largest quantities for wet ashing, is one of the hardest purification problems there is.

Sulfuric Acid. Redistillation from a quartz still gives a very pure product.

Perchloric Acid. Very high-purity commercial grades which have been distilled during manufacture are available (G. F. Smith Chemical Co.).

Ammonia. This reagent can be distilled or produced from tank anhydrous NH_3 by a process similar to that for hydrochloric acid. A very high-purity product results.

Any reagent that can be vaporized at reasonable temperatures can be purified by using this property:[2] for example, hydrobromic acid,

TABLE II

METAL CONTENT OF HYDROCHLORIC ACID

In parts per billion

Element	Acid Made from Ion-Exchange Water	Reagent-Grade Acid
Mg	2 *	>2 †
Ca	0.2 *	>2 †
Sr	<0.06 *	>2 †
Ba	0.02	>2 †
Pb	0.06	0.6
Zn	0.06 *	>2 †
Cr	<0.06	2
Mn	0.02 *	>2
Fe	0.02 *	>2
Co	<0.002 *	0.2
Ni	0.002 *	0.2
Mo	<0.02 *	0.02
Elements Detected but Not Determined		
Na	+?	+3
K	+?	+3
Li	−	+
B	+	+3
Si	+ *	+
Sn	+ *	+
Cu	+2 *	+4
Ag	+2 *	+3
V	+ *	+2

* Same concentration as in the ion-exchange water.

† 2 ppb was the highest comparison standard used, the actual value may be orders of magnitude higher.

acetic acid, sulfurous acid, formaldehyde. Reagents that can be put into neutral aqueous solution and extracted with dithizone in carbon tetrachloride or 8-hydroxyquinoline in chloroform can be substantially improved with respect to the wide range of elements those reagents cover.[49] Organic compounds and solvents are usually much purer commercially and much easier to free of metal than are inorganic compounds.[110] Purification by electrodeposition[96,160] is possible for polarography in the microgram range, or before coulometry[123] of millimicrogram amounts. Mitchell[102] discusses the purification of several inorganic compounds. The new technique of zone melting may produce metals and inorganic compounds which are substantially purer than those obtained by any conventional method.[141]

STORAGE OF REAGENT SOLUTIONS

When analyses in the millimicrogram range are being made, one of the greatest hazards of contamination is storage. It is usually assumed that the concentration of the element in question will remain unchanged when the sample solution is stored, but there is ample evidence that serious changes can occur. These concentration changes are due to three processes: (1) contamination with the container material, (2) loss by adsorption on the container walls, and (3) contamination by desorption of previously adsorbed ions.

Horowitz and Paneth [72] noticed that radioactive tracers were adsorbed from dilute solutions on to the container walls, and subsequent workers have noted losses due to adsorption on container walls, phase interfaces, precipitates, and filter paper.[58-60,65,87,136] Erbacher and Nikitin [39] pointed out that adsorption had caused an earlier determination of the solubility of radium sulfate to be in error by almost two orders of magnitude. Hershenson and Rogers [67] found that adsorption of silver on pipets could cause errors even in the microgram range. Such difficulties are not restricted to the adsorption of metal ions on glassware.[27,42,128,130,133,135] Laug [83] and Richards [122] found that adsorbed chromium from chromic acid cleaning solution could not be easily washed off by repeated rinsing with pure water and that it is likely to contaminate samples stored in the vessels. Butler and Johnston [24] found that on Pyrex the retention of chromium amounted to 10 mμg per cm^2. Thus even a small beaker may retain over 1 μg of chromium. A one-to-one mixture of concentrated sulfuric and nitric acids makes an acceptable substitute for chromic acid cleaning solution.

Experiments by Leutwein [85] showed that the concentration of a 10-ppm solution of Ni, Mn, Mo, V, Au, Pt, Ru, and Ti decreased by a factor of 10 in 75 days when stored in 6% mineral acids in glass (Fig. 3). When fused quartz was used, only Ni and the precious metals decreased in concentration.

Losses like those described by Leutwein were specifically sought in the case of cobalt [172] by storing a very dilute solution of radioactive cobalt chloride at *p*H 3.0 in a Pyrex bottle which had been carefully cleaned with chromate cleaning solution, steamed out for one hour, and rinsed about ten times with triply distilled water. At the start of the experiment the solution used contained 13.3 mμg per ml of radioactive cobalt and less than 1 mμg per ml from other sources. The gamma activity of a 1.0-ml sample of this solution under reproducible

conditions was 1.02 times that of a chosen National Bureau of Standards Co^{60} standard, when the experiment was started. Repeated analyses of 1-ml samples of the solution over a period of 4 months gave counts that did not vary more than 2% from that of the standard and that showed no decreasing trend.

For about one year, frequent spectrograms have been made, using the same standard solutions of trace-elements which have been stored in mineral acid under conditions similar to those of Leutwein's experiment, except that they are in polyethylene bottles.[164] By plotting the

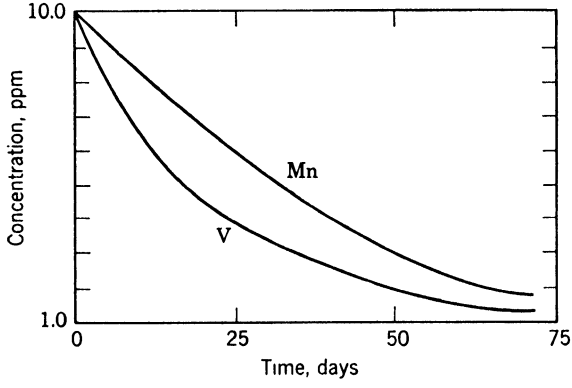


FIG. 3. Stability of standard solutions stored in glass. (After Leutwein [85])

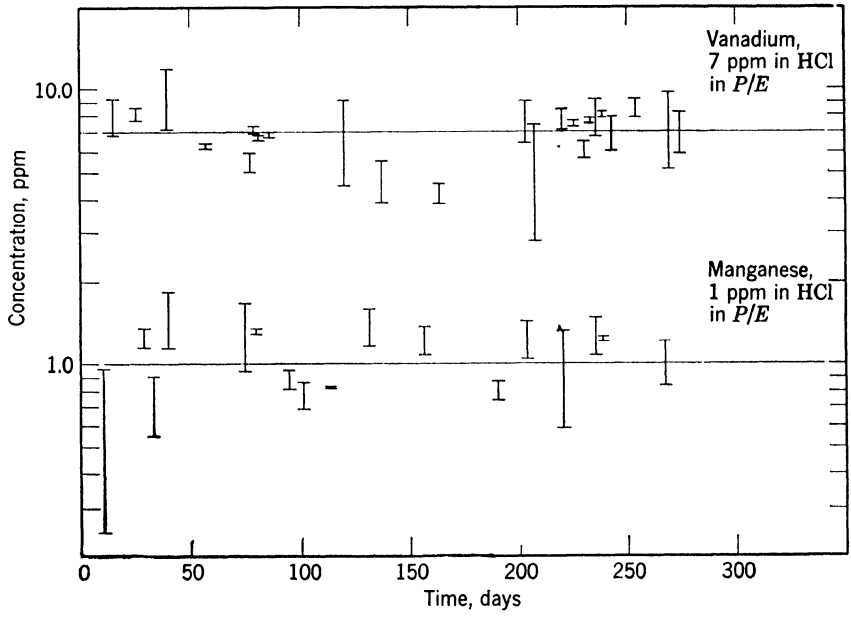
intensities obtained for manganese and vanadium with one particular spectrograph and densitometer, the data of Fig. 4 were obtained. They show that the solutions that contained 1 ppm of Mn and 7 ppm of V, respectively, have not changed their concentrations within the limits of error. The spread of the data is large, since an internal standard correction has not been used. Nevertheless, there is clearly no downward trend such as that noticed by Leutwein for storage of Mn and V in glass.

Gordon and Cook [55] faced this problem in analyzing routine samples of high-purity water. They adopted the recommendation of Van Tuyl and made their samples 0.1 *N* in HCl and 0.01 *N* in HF and stored them in polyethylene.

It has been shown that heavy metal contamination of distilled water which results from storage in glass [30] or wax is almost absent when storage is in polyethylene,[37, 62] although for sea water, Black and Mitchell [19] found either satisfactory. (See also Mullin and Riley.[106])

Metal-free water, aqueous solutions of acids and bases, and 50%

solutions of common-reagent acids have been routinely stored for weeks or months before use in spectrochemical analysis and no increase in blank levels or other signs of contamination have ever been noted.[156] Several experiments were performed to quantify such data. First, samples were cut from new polyethylene bottles and ashed, and the ash was analyzed spectrochemically. Table III shows the re-



Stability of standard solutions

FIG. 4. Stability of standard solutions stored in polyethylene. (Vallee and Thiers [164])

sults. The data are compared with the figures for another laboratory plastic, Tygon, analyzed simultaneously, and with the published figures for Pyrex 774 glass. Polystyrene and methyl methacrylate were analyzed also and found to contain more metal than Tygon. Polyethylene proved to be purer by orders of magnitude than other readily available bottle material. This is illustrated in Fig. 5. Therefore, dissolution of the container wall causes relatively little metal contamination if the container is polyethylene. Second, to determine whether the water permeability of the polyethylene was large enough to cause solution losses, a small bottle half-filled with water was tightly capped and stored, along with the others. Periodic reweighing showed no change beyond the normal balance error (0.0005 gram) over the 16-month period. Third, aliquots of a 0.1 *N* solution of NaOH were

TABLE III

METAL CONTENT OF APPARATUS MATERIAL

In parts per million

Element	Polyethylene *	Tygon	Pyrex 774
Al	0.3	55	10,000
B	0.09	3	50,000
Ca	0.2	5	1,000
Cr	0.3	6	—— †
Cu	0.004	10	——
Fe	0.6	50	3,000
Mg	0.08	120	600
Pb	0.2	200	——
Si	2	100	400,000
Ag	0.02	0.6	——
Mn	ND †	2	1,000
Ni	ND	200	——
Zn	ND	5	——
Na	ND	ND	30,000
K	ND	ND	3,000

* Median of six samples from various shipments of finished bottles, 1950 to 1953. Thirty-five elements were sought.

† ND indicates "not detected"; a dash indicates no data given.

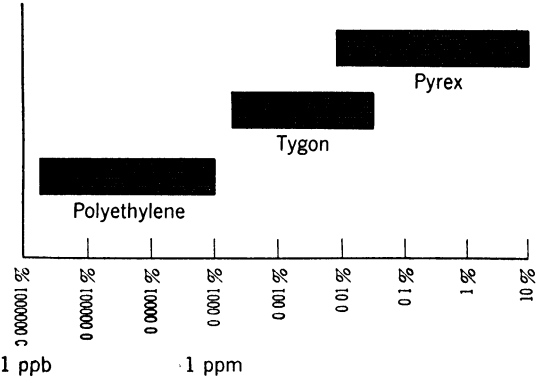


FIG. 5. Range of metal concentrations in container materials. Thirty-five elements were sought. Ag, Al, B, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Si, and Zn were found.

stored in soft glass, Pyrex 774 glass, and polyethylene bottles with caps of the same material for 16 months at 75° F. The solutions were analyzed before and after storage. The data, given in Table IV, show that only polyethylene did not contaminate the product. Fourth, an

TABLE IV

CONTAMINATION OF 0.1 *N* NaOH SOLUTION BY CONTAINER MATERIALS

In parts per billion

Element	Original Solution	After 16 months' storage in Polyethylene (Plax)	Soft Glass (Kimble)	Pyrex (774)
Al	<60	<60	4,000	1,500
B	<20	<20	400	1,500
Ba	<40	<40	500	<40
Ca	60	<60	1,200	<60
Cr	3	<3	<3	<3
Cu	2	<2	<20	<2
Fe	30	<30	<30	<30
K	<400	<400	<400	<400
Mg	2	<2	10	<2
Sr	30	<30	150	<30
Zn	<600	<600	2,000	<600

acid solution (*p*H 2.0 $\bar{c}$ HNO_3) of 19 elements at 1 ppm concentration was similarly stored and analyzed. Table V shows the elements with

TABLE V

CONTAMINATION OF ACID STANDARD SOLUTION BY CONTAINER MATERIALS DURING 12 MONTHS' STORAGE

Contaminating Elements From		
Pyrex (774)	Soft Glass (Kimble)	Polyethylene (Plax)
Al, B, Ca, Fe, Pb, Li, Mg, Mn, Na, Si, Sr	Al, B, Ca, Pb, Li, Mg, Na, Si, Sr	None

which the solution became contaminated during the storage period. In glass, contamination occurred with nine or ten elements; in polyethylene, with none.

It is apparent that polyethylene is of tremendous usefulness to the trace analyst, and is an almost indispensable tool in work with millimicrograms of metals. Polyethylene made by the Ziegler process using catalysis at low pressure has not yet been tested and may be quite different from the pure, unplasticized, high-pressure product discussed here.

Airborne Contamination. The ambient air can be a serious source of contamination, particularly where it is moving such as in a fume hood or near a window. Covering beakers or other apparatus with a

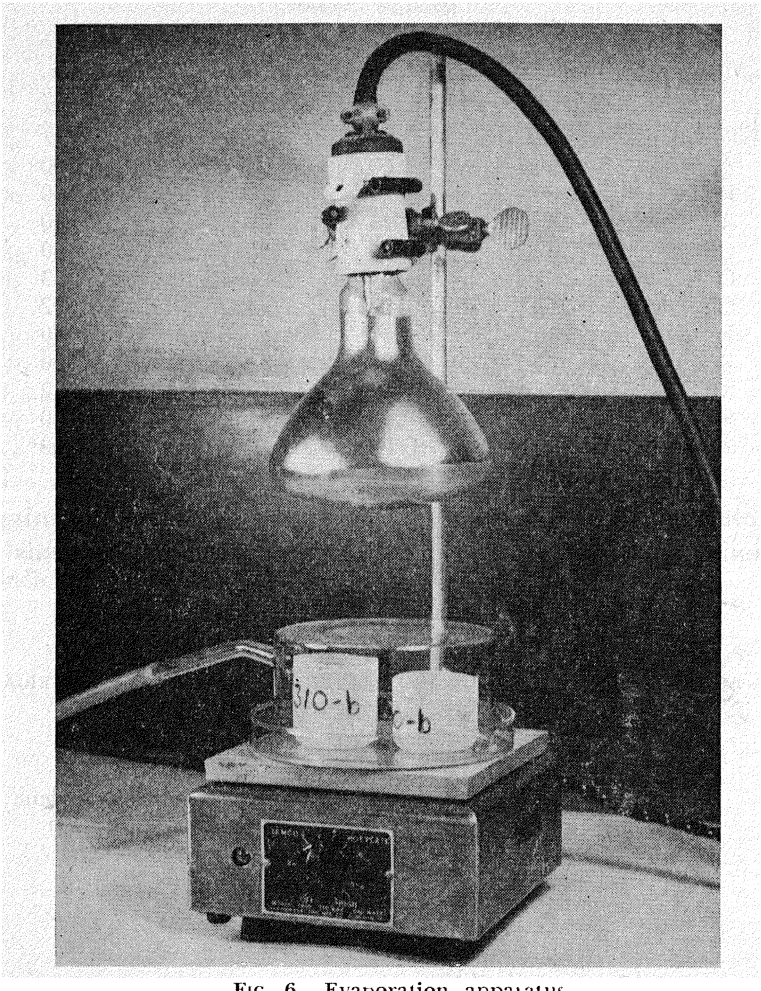


FIG. 6. Evaporation apparatus

watch glass and keeping them covered is adequate for most work in the microgram range. However, with millimicrogram quantities, more stringent precautions must be taken, particularly for some elements, including sodium,[105] potassium, magnesium, calcium, iron, copper, zinc, and aluminum. Long evaporation is one of the most hazardous steps. During work with millimicrogram amounts of cobalt, contamination occurred during evaporations, even with fluted

watch glasses covering the beakers at all times. This contamination was avoided by evaporating samples inside the chamber shown in Fig. 6, and the procedure was also made faster. Brophy and Strock [22] found it necessary to design a similar apparatus to prevent contamination during the analysis of trace elements in high-purity germanium.

Other Sources of Contamination. Whenever samples such as rocks, dry plants, or animal tissue must be ground, contamination is likely, even at the microgram level.[4,71] However, Barnett, et al.[14] report that, when samples of quartz and quartzite were ground between alumina plates, no aluminum contamination was observed spectrographically, only Mg (30 ppm) and Ti (1 ppm).[20]

Lamben [82] cautions biochemists about the use of Waring Blendors, since he found that copper contamination was a likely result. Maltby [93] found that some of the enamels used for graduation marks in English pipets which contain lead will dissolve off to produce erratic results in the determination of traces of lead.

Finally, the frustrating aspects of contamination problems are illustrated by the report of a committee that is trying to set up standards for the lead content of British foods.[7]

SEPARATION METHODS

The field has been reviewed by Burriel-Marti and Ramirez-Muñoz,[28] Garrison and Hamilton,[48] Hudgens,[74] Specker and Hartkamp,[150] and Stevenson and Hicks.[152] (See also Elving [38] and Mellon.[97])

Precipitation. The classical method of separating microgram amounts of metals from the major biological elements, sodium, potassium, and calcium, is that of Mitchell [102] and co-workers,[108,142] using 8-hydroxyquinoline, tannin, and thionalide. They obtained excellent recoveries of microgram amounts of metals. This method or modifications of it have been used by others.[63,114,116,142,158]

At extremely small concentrations, gravimetric precipitations are incomplete, owing to the solubility of the precipitate.[52] Even coprecipitations may not be quantitative; for example, the coprecipitation of beryllium on calcium phosphate, which is complete at the microgram level, is only 50 to 70% complete for millimicrograms.[160]

Solvent Extraction. The situation is slightly different in the field of solvent extraction. Although an equilibrium is involved, repeated extractions are possible, and, barring complication, the coefficient of extraction should be constant down to extremely low concentrations, as indeed it is for some elements.[57]

However, for ferric chloride distributing between ether and water, the coefficient becomes rapidly less favorable as the iron concentration is decreased, and this method cannot be used to separate tracer iron.[48] Other similar difficulties are known, and are blamed on polymerization, adsorption on the liquid–liquid interface, or other such phenomena.[35, 36, 98] Nevertheless, extraction is of very great use for many elements.[3, 104, 115, 117, 118, 131, 145, 174]

A general method of extraction for the concentration of many elements has been given by Gorbach and Pohl,[52–54] as well as some information on the comparison between extraction and precipitation as methods. Apart from dangers inherent in precipitation, occlusion, and mixed crystal formation, they point out that even the most insoluble of precipitates has a limiting sensitivity due to its solubility (as mentioned above), whereas extraction methods have no such inherent limitations. They performed an experiment to compare the sensitivity of the extraction method with the precipitation sensitivity data given by Berg.[16] After placing 1μg of each of copper, iron, manganese, and vanadium into 1 liter of their double-distilled water, they added 1 ml of 3% 8-hydroxyquinoline in acetic acid, neutralized the solution, and extracted it with chloroform. Each phase was then evaporated to one drop, placed on a spectrographic electrode, sparked, and analyzed qualitatively. No lines were found in the spectrum of the water phase, but clear lines of the four elements were found in the spectrum of the 8-hydroxyquinoline phase. Their results are given in Table VI.

TABLE VI

COMPARISON OF PRECIPITATION AND EXTRACTION SENSITIVITIES FOR 8-HYDROXYQUINOLINE *

In parts per million

	Sensitivity by	
Element	Precipitation	Extraction
Copper	2	0.0002
Iron	1	0.0002
Manganese	2	0.0002
Vanadium	2	0.001

* After Gorbach and Pohl [52–54] and Berg.[16]

This certainly seems to indicate that extraction methods are far better for millimicrogram work than precipitation methods. However, no blanks are given, and they list no special precautions for obtaining

very pure water or during evaporation; so their figures may be low by a factor of 10 to 1000. Nevertheless, their data show the superior sensitivity of extraction over precipitation.

Studies were made on the separation of millimicrogram amounts of cobalt from an aqueous solution of blood ash, by dithizone extraction.[173] Samples that imitated the composition of the ash of 50 ml of whole blood and that contained 6 mμg of cobalt as Co^{60} were buffered at *p*H 8.0, with ammonium citrate and ammonia and brought to 10 ml volume. They were then extracted with successive 2-ml por-

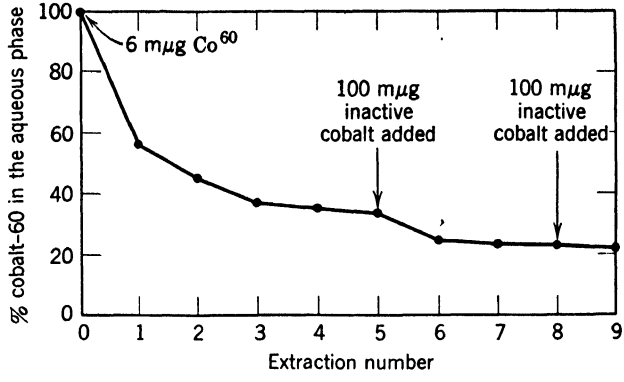


FIG. 7. Incomplete extraction of cobalt by dithizone.

tions of 0.05% dithizone in carbon tetrachloride. The concentration of cobalt in the aqueous phase was followed by counting its total gamma-ray activity. A plot of this activity against the numbers of extractions is given in Fig. 7, which shows that, even after five extractions, about 30% of the cobalt is left behind. This was found repeatedly. The addition of 100 mμg of inactive cobalt to the aqueous phase did not render the active cobalt more accessible to the dithizone. As is shown by Fig. 7, very little more of the Co^{60} was extracted. The addition of an oxidizing agent, $NaBO_3$, to the aqueous phase before the extraction made the cobalt completely unextractable by dithizone, which pointed toward cobaltic ion or a complex of cobaltic ion, such as $Co(NH_3)_6^{+3}$ as the unextractable, unexchanging species. However, exclusion of air and addition of reducing agents to the aqueous phase did not improve the per cent extraction. Such phenomena have been observed in other systems [119] and have often been associated with lack of exchange between different valencies or different complexes in solution, or with radiocolloid formation.[139]

Electrolytic Separations. Simultaneous electrolytic separations of many elements have generally been restricted to relatively large quantities of material.[28] Reviews of the field have been given by Ashley,[8] Haissinsky,[61] Rogers,[128] and Sannié and Poremski.[134] Some of the problems involved in using the method are incomplete separation of the metal from the cathode,[120] increasing nobility of metals with decreasing concentration,[126,127] solubility of the deposit, and interaction of major constituents of the solution with the traces to be separated.[123] Rogers [125] treats the effect of solution and electrode volume on separation efficiency.

Distillation. Under specific conditions distillation has been used in the estimation of millimicrograms of mercury in biological materials and in air,[73,76] and in the separation of volatile elements from rocks.[129] The carrier distillation method of spectrochemical analysis [143] and the double-arc method [5] are both techniques for separating volatile elements from a refractory base and simultaneously exciting their spectra. The Conway microdiffusion technique is also an important distillation-separation method. A good example of this technique is the determination of submicrogram amounts of cyanide in biological fluids by microdiffusion.[43] Distillation of ruthenium and other elements is much used in fission-product chemistry.[48]

Ion Exchange. Ion-exchange methods of separating minute amounts have come rapidly to the fore in recent years to become one of the chemist's most selective tools. Many reviews and several books have appeared covering their application to analytical chemistry.[21,80,81,109,132,137,138,159] Therefore, our discussion will be limited to those applications that bear directly on its use in the millimicrogram range. In ion exchange nothing comparable to coprecipitation occurs, amounts from micromicrograms to moles can be handled, quick separations are possible, and necessary reagents are few. These advantages give ion exchange a position of unique importance in separation and concentration, not only of traces and tracers, but also of different oxidation states and even of isotopes.[79,151]

The following difficulties are to be expected with ion-exchange resins.

1. Incomplete pickup of desired ions by the column.
2. Incomplete release of desired ions by the column.
3. Irreproducible behavior of the columns.
4. Contamination of solutions with ions held tightly by the resins and released slowly.

Heitmann and Schmidt [64] tested leakage and retention of traces of

metal, using Dowex 50, IR120, IRA 460, Permutit RS, and Permutit ES. They found that, when radioactive sodium was put on a column of cation resin at the rate of 15 μg per ml, only 0.005 to 0.43% of the added activity leaked through. Upon elution with 10% hydrochloric acid, 99.4 to 99.8% of the sodium was removed. In other units, only fractions of a millimicrogram leaked through to remain unseparated, and only fractions of a millimicrogram were unrecovered from the column.

Using the anion-exchange method of Kraus, a method was worked out for the separation of cobalt from biological materials and its determination in millimicrogram amounts. It will be discussed briefly in the light of the above considerations.[157]

Determination of Cobalt in Human Blood. It is known that cobalt occurs in human blood since vitamin B_{12}, a normal blood constituent, contains this element. The concentration of vitamin B_{12} in human blood has been measured by means of microbiological assays, and found to be in the parts per billion range. Some data have been given [169,176] indicating that the concentration of cobalt in human serum ranges from 4 to 14 ppb and Pohl and Demmel,[118] found 5.9 ppb in the serum of a cow. However, cobalt could not be found in this concentration range when human blood was analyzed by a spectrochemical method of adequate sensitivity. A separation method was therefore set up, and, after unsuccessfully trying to extract cobalt and encountering the difficulties described earlier, anion exchange and the separation method of Kraus and Moore [78] were utilized.

In brief, the method involved drawing a 50-ml sample of blood under carefully controlled conditions, separating it into plasma and cells if desired, dry ashing by the method described above, separating cobalt from the other elements by using a column of Dowex 1 as the chloride, and then determining it by a new spectrochemical method of very high sensitivity.

Figure 8 summarizes the procedure and emphasizes its freedom from complexity and attendant contamination hazards. Hydrochloric acid and the exchange resin are the only reagents. Evaporations were all carried out in the cover apparatus shown in Fig. 6.

The procedure on the ion-exchange column is shown in Fig. 9. The Dowex-1 resin column is washed with 30 ml of 0.01 *M* hydrochloric acid to remove absorbed elements remaining from the previous separation. Then it is washed with 5 ml of 9 *M* hydrochloric acid to condition it for the subsequent sample. The sample solution is transferred to the column reservoir and is allowed to flow through the column

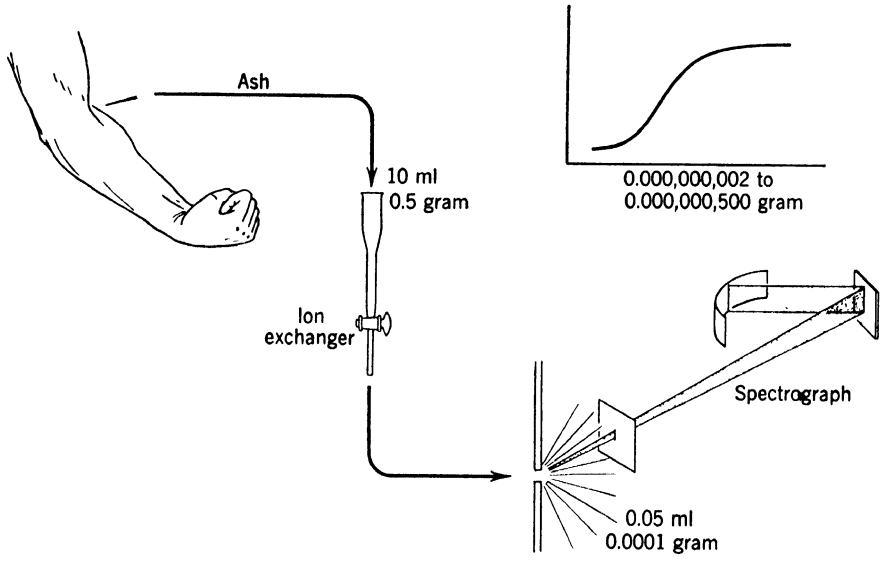


FIG. 8. Determination of cobalt in blood.

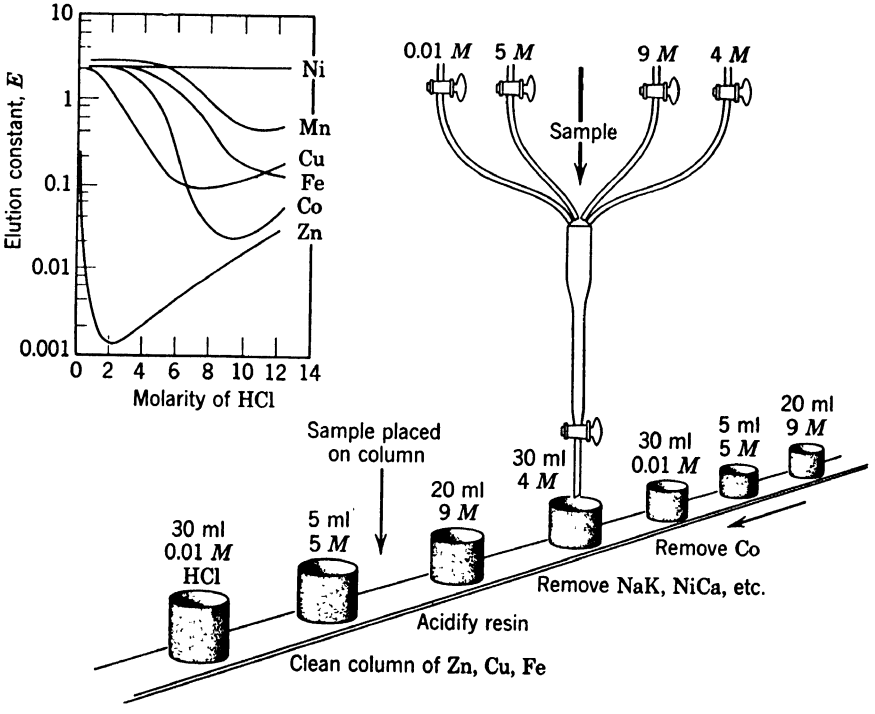


FIG. 9. Dowex-1 separation of cobalt. (From Kraus and Moore[78])

at a rate regulated between ¾ and 1 ml per minute. Using the same rate of flow, the column is next washed with 20 ml of 9 *M* hydrochloric acid. This frees the column of unabsorbed ash constituents, including the alkali metals, alkaline-earth metals, nickel, and other materials. Thirty milliliters of 4 *M* hydrochloric acid is then added to the reservoir and is allowed to flow through the column at the same rate. The cobalt is eluted by this acid, which is collected in a clean

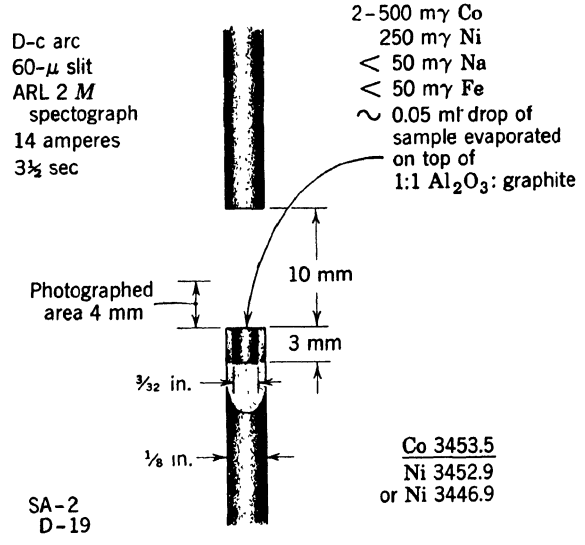


FIG. 10. Details of spectrographic method for the determination of cobalt.

polyethylene cup. For spectrochemical analysis, nickel (0.25 μg) is added in the same amount to each sample as an internal standard; then the polyethylene cup containing the solution of cobalt (plus internal standard) is placed in the cover apparatus and evaporated by heating with an infrared lamp. When the liquid volume has decreased to about one drop, it is transferred with a polyethylene pipet to the top of an electrode and dried there. The details of the spectrographic method are given in Fig. 10, and the working curve obtained by employing synthetic standards is given in Fig. 11. The results of the analyses of nine whole bloods and of two plasmas averaged 0.2 and 0.07 ppb, respectively. Experiments using 9, 18, or 27 mμg of cobalt showed complete recovery of cobalt added to the original sample either as the chloride, or in vitamin B_{12}, to within the precision of the method (10% standard deviation). See Table VII. About 5% of the cobalt was reproducibly lost in the column step, possibly due to leak-

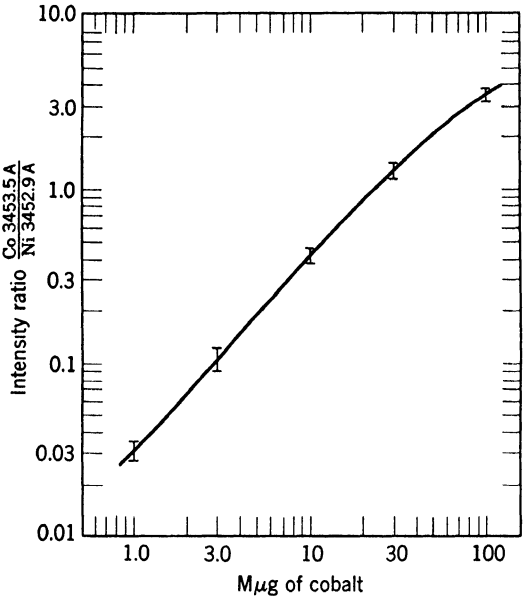


FIG. 11. Working curve for spectrographic determination of cobalt.

age, along with the 10^7 or 10^8 times excess of sodium, potassium, and other ions from which it was being separated. No losses could be detected in any other step when radioactive Co^{60} was used as a tracer,

TABLE VII

TESTS OF COBALT RECOVERY ON SAMPLES WITH ADDED VITAMIN B_{12}

	Millimicrograms of Cobalt			
Sample Number	Originally Present	Added	Total	Found
1	14.5	9.0	23.5	24
2	14.5	9.0	23.5	22
3	8.4	9.0	17.4	18
4	8.4	18.0	26.4	26
5	20.0	27.0	47.0	39
6	4.4	9.0	13.4	11

and no contamination could be discovered when large excesses of the reagents were analyzed spectrochemically. The over-all blank of the method was about 5.0 mμg.

Kraus and Nelson [79] have summarized the adsorption from hydrochloric acid of 65 elements and stated that in most cases desired sep-

arations are possible. Their statements give probably the best available picture of the clear superiority of ion-exchange resins as a general separatory tool for analytical chemistry.

DETERMINATION OF MILLIMICROGRAM QUANTITIES

The preceding chapters of this symposium have certainly thoroughly covered the determination of trace amounts of metals, and other reviews have been given.[124] However, a few examples will be discussed here as a summary, from the point of view of determining millimicrogram quantities of many metals simultaneously and to mention several miscellaneous methods.

In emission spectroscopy, the highest sensitivity for a large number of elements is given by the copper-spark method of Bachelder, Conway, Nachtrieb, and Wildi,[9] and Nachtrieb.[107] This method is extremely well suited to treatment of separated groups of elements not containing alkali metals. However, traces of sodium or potassium reduce its sensitivity tremendously. The cathode-layer arc technique [102] is probably the next most sensitive *general* method.

Flame spectrometry is an extremely sensitive and precise tool, and the list of elements to which it can be simultaneously applied is growing.[11, 66, 162] Mass spectrometry shows great promise as an extremely sensitive method of determining many elements together. It does not suffer from some of the disadvantages of optical spectroscopy and is not restricted to the metallic elements. Double-focusing spectrometers are necessary, however, and are not yet readily available.[75]

By activation analysis, many elements can be simultaneously determined. However, the separation and purification processes which follow the initial activation are slow compared to spectroscopy or spectrometry.[84, 95, 147, 154, 175]

Micrqspectrophotometry, coulometry, polarography, and other methods are applicable in the millimicrogram range but do not have the ability to determine many elements simultaneously. Other methods of determination which show promise of both high sensitivity and ability to handle many elements together include X-ray fluorescence [18, 111] and atomic absorption spectra.[168]

There are several methods for determinations or separation which are not in the millimicrogram range but warrant mention because of their ingenuity. Erkelens [40] has given a method for paper chromatography of heavy metals and their quantification while still on the paper.

The developed chromatogram is placed in a bell jar which is evacuated and filled with H_2S^{35}. The unreacted H_2S^{35} is washed out of the paper. The remaining metal sulfides are then presumably quantified by routine counting techniques. Determinations down to 1 μg have been achieved, and further refinements should bring this method into the millimicrogram range. Ishimori and Takashima [77] analyzed tap water, silicate rocks, and alkali chlorides for potassium by precipitating $K_2NaCo(NO_2)_6$ in the usual way with a reagent containing Co^{60}. The precipitate is counted instead of weighed, and 1 μg can be reached. Another such promising method is the ring furnace of Weisz.[170] This device is a combination of quantitative spot testing and chromatography. Reagents are placed in the center of a paper disk which is supported around its circumference by a brass ring. This ring is hot enough to evaporate solvents which migrate to it from the center of the ring. Thus microchromatographic separations can be carried out upon one drop of test solution, and quantifying tests can be applied to the separated rings in situ or after removal from the ring furnace.

Although slightly off the track, because it concerns separation and detection rather than determination, the work of Seely [144] is interesting. A microscope slide is covered with gelatin containing the usual spot-testing reagents such as rubeanic acid or 2,2¹-bipyridyl. When atmospheric particles weighing 10^{-10} to 10^{-15} gram fall on this surface, they react and a ring of colored product diffuses out from the particle. The characteristic color can be observed under the microscope, and metal identifications can be made. Mixed particles give two or three concentric colored rings, owing to differences in diffusion rate of the various ions through the gelatin, constituting chromatographic separation on the submillimicrogram scale!

As the concentrations and amounts that analysts handle decrease even further, the importance of separation techniques must continue to increase. While the ideal separation method may never be found, the developments of the last two decades have certainly been impressive strides in that direction. Unfortunately, the problem of contamination will always be with us, but here also the recent advances have been large. Taken together, the progress in these two fields has opened the way for development and application of more and more sensitive methods of trace analysis, thus providing information for ever wider fields of scientific endeavor.

DISCUSSION

Discussion Leader
Bert L. Vallee
Harvard Medical School and
Peter Bent Brigham Hospital

It is difficult if not impossible to add to the thorough exposition of Dr. Thiers on contamination hazards and concentration techniques.

There is, however, one phase of the problem which has received little or no emphasis here, but which I consider to be of the utmost importance: the *interpretation* of trace-analysis data obtained on complex substances as exemplified by biological matter and other natural products.

The analysis of such material ultimately aims at the appreciation of the functional significance of the constituents. Thus, while it is a basic prerequisite to appreciate the elemental composition of matter, it must be borne in mind that a given element may be present as a part of many different organic and/or inorganic moieties. The determination of *total* inorganic sulfur in petroleum, for example, is no guide to the various sulfur-containing compounds present, their organic structure, properties, and possible effects on the various procedures to which the crude material is subjected. Nor can it be predicted, a priori, that this element postulated maybe to affect catalytic cracking, is truly the one at fault, though its relative abundance compared with, let us say, vanadium, would lead to an erroneous neglect of this latter element.

Inorganic analysis, of which trace-metal analysis is a special case, is not a guide to organic structure and function. Erroneous extrapolation of the data with regard to the significance of a given constituent, which is often based on relative abundance in a crude preparation, is easily as vexing and certainly as significant a problem as contamination from extraneous sources.

Considerations such as these, often overlooked in favor of methodology, deserve emphasis to retain our perspective with regard to the reasons for and utilization of analytical data.

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