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CHROMATOGRAPHIC ANALYSIS

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A GENERAL DISCUSSION

ON

CHROMATOGRAPHIC ANALYSIS

22nd - 24th September, 1949

A GENERAL DISCUSSION on Chromatographic Analysis was held in the Department of Chemistry, Reading University (by kind permission of the Vice-Chancellor), on the 22nd, 23rd and 24th September, 1949. The President, Prof. Sir John Lennard-Jones, K.B.E., F.R.S., was in the Chair and over 250 members and visitors were present.

Among the distinguished overseas members and guests welcomed by the President were the following :—

Dr. J. Boldingh (Zwijndrecht, Holland), Prof. H. Brockmann (Göttingen), Prof. S. Claesson (Uppsala), Prof. T. Dillon (Galway), Miss H. M. Doery (Melbourne), Mr. J. Fahrenfort (Amsterdam), Dr. A. H. Gordon (Stockholm), Dr. Grifols-Lucas (Barcelona), Dr. F. A. Haak (Amsterdam), Dr. J. Th. Hackmann (Amsterdam), Dr. H. Hering (Paris), Miss T. Hofstee (Amsterdam), Dr. Klinkenberg (The Hague), Dr. E. Lederer (Paris), Dr. Stanford Moore (New York), Dr. J. van Ormondt (Delft), Dr. L. Sacconi (Florence), Dr. F. H. Spedding (Iowa), Prof. Arne Tiselius (Uppsala), Dr. A. E. R. Westman (Ontario), Prof. and Mrs. L. Zechmeister (California).

Contents

PAGE

General Introduction. By Arne Tiselius 7

I. Physicochemical Principles and Their Utilization

Introductory Paper.—By Stig Claesson 11

Theory of Chromatography—

VII. The General Theory of Two Solutes following Non-linear Isotherms. By E. Glueckauf 12

Chromatography with Several Solutes. By A. C. Offord and Joseph Weiss 26

Theory of Frontal Analysis and Displacement Development. By Stig Claesson 34

The Conservation Equation of Chromatography. By W. M. Smit . 38

GENERAL DISCUSSION.—Dr. B. Davison, Dr. F. G. Angell, Dr. E. Glueckauf, Dr. W. M. Smit, Prof. A. C. Offord, Dr. J. Weiss, Dr. J. F. Duncan 45

Adsorption and Some Constitutional and Steric Properties. By L. Zechmeister 54

Chromatography of Colourless Substances and the Relation between Constitution and Adsorption Affinity. By H. Brockmann . . 58

The Function of Adsorbent Activity in the Chromatographic Separation of Certain Anthraquinone Compounds. By A. Stewart . . 65

Equilibrium and Rate Studies of Cation-exchange with Monofunctional Resins. By D. K. Hale and D. Reichenberg 79

Cation Exchange with a Synthetic Phenolsulphonate Resin—

Part V. Kinetics. By T. R. E. Kressman and J. A. Kitchener . 90

Ion Exchange Studies—

II. The Determination of Thermodynamic Equilibrium Constants. By J. F. Duncan and B. A. J. Lister 104

Exchange Equilibria in Anion-exchange Resins: Porous Exchangers. By Robert Kunin and Robert J. Myers 114

Fully Swollen Alginate Gels as Permutites: Kinetics of Calcium-sodium Ion Exchange. By J. L. Mongar and A. Wassermann . . 118

Some Methods for Extending the Scope of Partition Chromatography. By Alfred A. Levi 124

PAGE

Partition Chromatography on Paper with Special Reference to Quantitative Separations. By A. H. Gordon 128

Separations Using Zeolitic Materials. By R. M. Barrer . . . 135

Modified Activated Carbon Adsorbents for Continuous Fractional Adsorption. By D. E. Weiss 142

GENERAL DISCUSSION.—Dr. A. J. P. Martin, Prof. L. Zechmeister, Prof. A. Tiselius, Dr. A. Klinkenberg, Mr. C. D. Cook, Dr. E. Glueckauf, Mr. B. A. J. Lister and Dr. J. F. Duncan, Dr. J. A. Kitchener, Mr. J. D. H. Strickland, Prof. C. W. Davies, Dr. J. F. Duncan, Dr. R. W. Richardson, Dr. A. Wassermann, Mr. D. K. Hale and Mr. D. Reichenberg, Dr. E. Heymann, Dr. D. J. Crisp, Dr. J. Boldingh, Prof. R. M. Barrer, Dr. R. L. M. Synge, Mr. A. Stewart, Mr. R. H. S. Robertson 151

Summarizing Paper.—By R. L. M. Synge 164

II. Applications

Introductory Paper.—By F. A. Robinson 168

II(A). Inorganic

Nature and Applications of Inorganic Alumina Chromatography. By George-Maria Schwab 170

The Mechanism of Inorganic Chromatographic Adsorption on Alumina. By Luigi Sacconi 173

Inorganic Chromatography on Cellulose—

Part III. The Use of Columns of Cellulose for the Separation and Determination of Metals. By F. H. Burstall, G. R. Davies and R. A. Wells 179

The Application to Qualitative Analysis of the Separation of Inorganic Metallic Compounds on Filter Paper by Partition Chromatography. By I. I. M. Elbeih, J. F. W. McOmie and F. H. Pollard . . . 183

Inorganic Capillary Analyses. By H. Flood 190

Separations on 8-Hydroxyquinoline Columns. By G. Robinson . . 195

Theory of Chromatography—

VIII. The Separation of Lithium Isotopes by Ion Exchange and of Neon Isotopes by Low-temperature Adsorption Columns. By E. Glueckauf, K. H. Barker and G. P. Kitt . . . 199

Large-scale Separation of Rare-earth Salts and the Preparation of the Pure Metals. By Frank H. Spedding 214

PAGE

Application of Ion Exchange to the Separation of Substances in Low Concentrations. By Edward R. Tompkins 232

GENERAL DISCUSSION.—Dr. B. A. J. Lister, Dr. L. Sacconi, Mr. R. A. Wells, Dr. F. H. Pollard, Dr. E. Glueckauf, Mr. G. Robinson, Prof. L. Zechmeister, Prof. A. Tiselius, Dr. W. M. Smit, Dr. R. Lessing, Mr. C. D. Cook, Prof. C. W. Davies 237

II(B). Organic and Biochemical

The Chromatography of Gases and Vapours. By C. S. G. Phillips . 241

Chromatography of Petroleum Hydrocarbons. By W. M. Smit . . 248

Chromatographic Fractionation of Black Oils. By A. S. C. Lawrence and D. Barby 255

Chromatography of the Carboxylic Acids. By Harold G. Cassidy and F. H. Max Nestler 259

Chromatographic Analysis of Fatty Oils. By K. A. Williams . . 264

The Application of Partition Chromatography to the Separation of the Sugars and Their Derivatives. By E. L. Hirst and J. K. N. Jones 268

The Chromatography of Proteins. The Effect of Salt Concentration and pH on the Adsorption of Proteins to Silica Gel. By Charles C. Shepard and Arne Tiselius 275

The Application of Chromatography to Amino Acids and Peptides. By Tudor S. G. Jones 285

Separation of Bases and Amino Acids by Displacement Chromatography on Ion-exchange Columns. By S. M. Partridge 296

Chromatography of Steroids and other Colourless Substances by the Method of Fractional Elution. By T. Reichstein and C. W. Shoppee 305

Partition Chromatography of the Tertiary Amine Salts of the Penicillins. By T. Leigh 311

Biologically Active Substances in Liver Extract. By E. Lester Smith 317

High Molecular Polymers Separation. By Stig Claesson 321

GENERAL DISCUSSION.—Dr. G. H. Beaven, Dr. A. S. C. Lawrence, Prof. A. Tiselius, Dr. A. J. P. Martin, Dr. E. Lederer, Prof. L. Zechmeister, Dr. R. Consden, Dr. J. A. V. Butler, Dr. A. J. Woiwod, Dr. K. W. Pepper 325

Summarizing Paper.—By A. J. P. Martin 332

Author Index 336

CHROMATOGRAPHIC ANALYSIS

GENERAL INTRODUCTION

By Arne Tiselius

Received 22nd September, 1949

It is probably well known to all those present how chromatography, although invented and applied for the first time as early as in 1906 by Tswett, was almost completely neglected for a period of 25 years, and how it was actually rediscovered in 1931 by Kuhn, Winterstein and Lederer, who applied it to the resolution of plant carotene into its components. Then followed a period of rapid development, the importance of which may perhaps best be illustrated by a quotation from Karrer, who in his Congress Lecture to the International Congress of Pure and Applied Chemistry in London in 1947 stated that " . . . no other discovery has exerted as great an influence and widened the field of investigation of the organic chemist as much as Tswett's chromatographic adsorption analysis. Research in the field of vitamins, hormones, carotinoids and numerous other natural compounds could never have progressed so rapidly and achieved such great results if it had not been for this new method, which has also disclosed the enormous variety of closely related compounds in nature."

As a matter of fact the progress in the field of chromatography during the last 5–10 years has been so striking both with regard to the method itself and to its scope of application that one is tempted to speak about a second discontinuity in the curve which would describe the development of this remarkable method and its importance in all fields of chemistry. The time chosen for this Discussion is therefore particularly suitable and I feel that we all would like to express our thanks to the Faraday Society for organizing a discussion of this matter just now. The number of communications in this field is rapidly increasing and there has hardly been time enough to compare experiences with alternative methods or to discuss the numerous new applications which now seem possible. This meeting should afford an opportunity for this and for other valuable discussions in the field.

Some of the most important recent developments are: the development of partition chromatography, the introduction of efficient ion-exchange materials suitable for chromatographic work, the introduction of improved methods for following the process of separation by continuous physico-chemical measurements or by automatic sample-collectors and the micro-chromatographic methods made possible by using filter paper as a medium for partition chromatography. The combination of chromatographic and tracer methods is only in its beginning but has tremendous possibilities. Although not strictly belonging to chromatographic methods in a narrow sense I would like to add to these examples the counter-current extraction methods developed by Craig.

If one would try to mention some particularly striking examples of newer applications, it is obvious that chromatography is no longer limited to the substances studied by organic chemists and biochemists. The recent successful separations of rare earths and fission products from the uranium piles are particularly good examples of the wide scope of the method. Even

if it is obvious that the separation and purification of organic substances of native or synthetic origin is the main object of most chromatographic work it is now clear that the particular application to the complex mixtures of breakdown products obtained by splitting proteins, polysaccharides, nucleic acids and other large molecules has already given chromatography a prominent place among the tools of the structural chemistry of these extremely important substances. Among the newer applications it should also be noted that some quite promising attempts have been made to apply chromatography to high polymers and to viruses and some other proteins. The adsorption analysis of gases and vapours also seems to have useful applications. Many other examples will be given in the papers presented at this Discussion. No doubt chromatography can now be said to be of essential importance for the whole field of chemistry, and no other separation method has such a wide field of application.

Despite this, one has still the impression that much work in this field is too empirical. That is natural enough, because if a separation is successful there is no immediate need for going deeper into the subject in that particular case. But if the separation is not successful there is every reason to do so. We are still far from the goal of placing chromatographic methods on a rational basis to the same extent as has been done, for example, with fractional distillation. The formal theory of the method has been worked out in detail, and we shall listen to some valuable contributions on this aspect in the first section of this Discussion. In my own laboratory we have found it useful to distinguish between three main types of chromatography, namely, frontal analysis, elution analysis and displacement analysis, as the conditions for the successful operation of a column are quite different in these three cases. The theoretical treatment of elution in partition chromatography, as worked out by Martin and Synge, is particularly illuminating since it describes the action of a partition column as a counter-current extraction arrangement with a very large number of theoretical plates. The fundamental characteristics of a column are of course the affinity between the solute and the particles of the column in the medium used for the separation, that is, *the adsorption or the partition isotherm*, or, with a common denominator, the " distribution " isotherm, and that naturally will enter into any theoretical treatment of the operations as the chief unknown variable. One of the great advantages of partition chromatography is that this function can be predicted reasonably well on the basis of known solubilities in the two phases. The situation is not quite as favourable in adsorption chromatography. It appears to be very difficult to prepare reproducible adsorbents if they are to be sufficiently powerful to be used in chromatographic columns. The influence of small impurities on such large active surfaces is naturally very great. In my opinion, however, one should not be too pessimistic about the possibilities here, because one has the impression that on the whole the *relative* magnitudes of adsorption affinity in a series of substances will be fairly constant, even if the capacity may vary from batch to batch. That is certainly true for a number of active charcoals, as shown, for example, by Claesson in his work on fatty acids where Traube's rule determines the variation of adsorption with molecular size in an homologous series. Similar regularities have been found with amino acids and sugars on charcoal. The change of affinity by changing the composition of the solvent is, of course, of the greatest importance in the " development " of chromatograms, and here the change from non-polar to polar solvents (or the reverse) has been a standard practice in chromatography for many years. Such effects can be predicted qualitatively, at least with a reasonable degree of certainty, and have been studied in detail, for example, by Trappe in his work on the " elu-

tropic series" in the elution of lipids. The ion-exchange resins, as, for example, Amberlite, Wofatite and Dowex, are really to a certain extent "adsorbents made to order" and have already proved to be extremely useful both for separation of inorganic ions, of amino acids and many other substances. On the other hand, these adsorbents show a specificity which is not only dependent upon electrochemical properties. It would be highly desirable to study such phenomena in detail, and to investigate the influence of the size of pores in these materials and the possibility of preparing very porous resins for use with larger molecules.

Speaking of adsorbents which can be made to have a certain specificity, F. H. Dickey has recently published a paper [1] in which silica gel was precipitated together with, for example, methyl orange. The resulting adsorbent, after removing the indicator, had a very marked specific affinity for this substance. If this principle would prove to be of general applicability, it would, of course, mean a great step forward.

Mixed solvents, obtained, for example, by the addition of a polar solvent to a non-polar, working with a polar adsorbent, are generally used for elution or for the development of chromatograms, as has already been mentioned. This is probably due to a displacement effect, as was already realized by Tswett in his first communication. The reverse effect also must exist, although it has not been studied in great detail. Thus addition of a second component to the medium must, under certain circumstances, promote the adsorption of the solute. This component may be adsorbed in a polymolecular layer, which may have an affinity for the solute, which the original adsorbent did not have. We are here on the borderline between adsorption and partition chromatography—in the latter case the layer has extended so much that one can speak of a separate phase. In discussing the various forms of partition chromatography and the eventual influence of adsorption in these, I believe it is well to keep in mind that such phenomena are likely to occur. There is also the possibility that the substance added to the medium will not appreciably change the properties of the adsorbent but will change the thermodynamic potential of the *solute* by change in electrolytic dissociation, by complex formation or by other effects which express themselves as a change in solubility of the solute. This is made use of in certain cases of elution by modification in pH, by addition of citrate or other complex-forming agents. But here also the reverse effect has been observed. Thus any agent which will decrease the solubility of a substance should increase its adsorption, provided that the adsorbent is not influenced. Some dyestuffs which are normally not adsorbed on filter paper will do so on addition of salting-out agents, for example, ammonium sulphate, and good chromatograms can be obtained by elution with water. Some proteins show this phenomenon too, especially on Celite and silica gel, and it has been used for the chromatographic separation of some viruses.

We thus have in our hands varying methods of modifying the adsorption affinity and specificity of adsorbents by addition of suitable substances. With the enormous number of alternative combinations possible it is natural that this field has as yet been very little explored, but it is well to remember that it is no longer necessary to feel oneself limited to the adsorption characteristics of the commercially available adsorbents as they are obtained from the manufacturer. However, for the application in chromatography it is essential not only to have adsorbents of satisfactory affinity and specificity, they must also have a reasonably high capacity and be capable of acting reversibly at a fairly fast rate. That often limits the choice quite considerably. For the elution procedure it is very essential to avoid the

[1] *Proc. Nat. Acad. Sci.* (*Washington*), 1949, **35**, 227.

unpleasant lagging behind and broadening of the zones which is usually called " tailing." The tailing may be due to a too slow establishment of equilibrium, but just as often it is the consequence of the adsorption or partition isotherm being curved which makes the higher concentrations of a zone travel faster than the lower. A great advantage of partition chromatography is the fact that the isotherm is generally almost linear. With adsorption this is not so often the case, at least not for strongly adsorbed substances. At low concentrations, however, many adsorption isotherms are linear, as required by the Langmuir theory. This is, of course, ultimately a question of the homogeneity of the surface with respect to affinities, but also a question of the space available and when saturation is approached the isotherm is bound to bend and become concave, both in adsorption and partition processes. In such cases it is evident that elution cannot separate two components A and B (A more strongly bound than B) if the lower concentrations of B would show a higher relative adsorption or partition than the higher concentrations of A. A great advantage of displacement and frontal analysis is that the stationary concentration of the zones is constant even in such a case and thus a separation can be realized, and very large columns may be used without any spreading out of the zones.

In the development of chromatographic technique during recent years there has been a tendency to use the so-called " liquid " chromatogram method for following the separation. This method has great advantages as it makes one independent of optical methods for observing the zones directly on the column. The new automatic sample-collectors of Moore and Stein have proved extremely useful for this purpose. The other alternative of continuous observation of some convenient physicochemical property related to the concentration of the percolate as it leaves the column has been studied particularly in my own laboratory and also in a number of other places. These methods are particularly useful in the study of displacement and frontal analysis when the number of observations must be very large to get the concentration-volume curve accurately reproduced. Some combination of both methods would seem to be the most ideal procedure, but it is highly desirable to increase the sensitivity of the optical (or other) methods applied to be able to deal with the very low concentrations used in most work in partition chromatography. This is also essential for the proper interpretation of frontal analysis curves, where one wants to use low concentrations in order to avoid displacement effects as far as possible.

The direct observation of zones on the column has many advantages, and several interesting suggestions for new procedures have been made for the observation of colourless substances. Ultra-violet absorption observation of columns in quartz tubes offers many interesting possibilities, but one is, of course, then always limited in the choice of the adsorbent. The filter-paper chromatography offers some problems of this kind—there it now seems particularly important to develop the method further in the quantitative direction.

Chromatography has so far been based only upon adsorption and partition phenomena, but it is obvious that any phenomenon which would—in a specific manner—influence the rate at which a zone of a substance travels through a column might be utilized for separation purposes. Electrophoresis and ionophoresis are related processes and have been utilized in a way analogous to chromatographic separation, using the column chiefly as a stabilizing medium to avoid gravitational or thermal disturbances. A combination of chromatography and electrophoresis has been tried by Strain. It seems as if electrophoretic separation in columns of ion-exchange resins would offer interesting possibilities, as it must be expected that the specific affinity

of the substances to the resin will strongly influence their migration. There is another possibility, which I have several times discussed with Dr. Martin and Dr. Synge, namely, to make use of differences in the frictional resistance in a gel. Such separations would mainly depend upon differences in molecular size, and would thus be very valuable in many cases, but offers great technical difficulties. Some of the separations observed in ionophoresis in gels may be due to such effects, in part at least.

I am afraid that in this Introduction I have dealt more with methods than with applications, but it is perhaps not necessary to this audience to exemplify further the possibilities of a method of such an enormously wide scope as chromatography. The various titles of the many papers to be presented at this Discussion and the presence here of representatives of all fields of chemistry and from many countries is a proof as good as any of this point. I shall be very happy if the remarks I have made here would stimulate the Discussion, to which we are all looking forward with the greatest interest.

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I. PHYSICOCHEMICAL PRINCIPLES AND THEIR UTILIZATION

INTRODUCTORY PAPER

BY STIG CLAESSON

Received 2nd September, 1949

The importance of chromatography is still rapidly increasing as can be clearly seen from the great number of very interesting contributions to this Discussion. Papers have been presented to this Section which have deepened our understanding of the chromatographic method and important advances have also been made in the experimental application of these ideas.

It is certainly true that well-designed experimental arrangements are of the utmost importance when difficult separations are attempted. Remarkable results can be achieved in this way which is evident from many contributions to this Discussion. A typical example of this kind of work is also Moore and Stein's experiments [1] on the separation of amino acids, some of the most carefully planned and beautiful experiments in chromatography ever published.

However, if a problem can be solved in different ways, the simplest way is always the best. A simple fraction collector is therefore often quite as useful as a more intricate apparatus for the continuous recording of the concentration of the effluent, and in many cases the inspection of the column with an ultra-violet lamp is quite satisfactory, particularly when the quenching of the fluorescence of the adsorbent is observed.[2 3]

The most important factor in all chromatographic work is, of course, the properties of the adsorbent in the column. Nothing can therefore ever

[1] Moore and Stein, *J. Biol. Chem.*, 1948, **176**, 337, 367; 1949, **178**, 53, 79.
[2] Sease, *J. Amer Chem. Soc.*, 1947, **69**, 2242.
[3] Brockmann and Volpus, *Ber*, 1947, **80**, 77.

compete in importance with the introduction of new and powerful adsorbent columns and it is also well known to everybody that the greatest progress in chromatography in recent years is due to the introduction of two new types of adsorbent, the partition column and the ion-exchange column.

It is therefore always of the greatest interest to follow the introduction of new principles for controlling the adsorption process either by changing the adsorbents or solvents used. Several very interesting papers dealing with such problems are found here.

In this connection a paper published by Dickey should be mentioned.[4] Following some ideas of Pauling he was able to show that silica gel precipitated in the presence of, e.g., propyl orange after washing had a greater adsorption affinity for that compound than for methyl, ethyl or butyl orange. If such results can be improved and extended they will be of such value that their importance can hardly be overestimated.

There are, however, two factors about which so little is known that they almost prevent progress in certain branches of chromatography. One is the problem of the connection between adsorption and chemical structure. Some progress has certainly been made in this field, but much still remains to be done before we can choose the adsorbents in a scientific way instead of using our intuition which is almost the best we can do to-day.

The other factor which prevents the progress of chromatography is the lack of reproducible adsorbents of constant quality standardized in suitable ways. This difficulty could certainly be overcome by the chemical industry of to-day if the importance of this factor was made sufficiently clear to manufacturers of chemical products by a suitable group of scientists, e.g., those present at this Discussion.

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[4] Dickey, *Proc. Nat. Acad. Sci.*, 1949, **35**, 227.

THEORY OF CHROMATOGRAPHY

VII. The General Theory of Two Solutes following Non-linear Isotherms

BY E. GLUECKAUF

Received 2nd June, 1949

The movement of solutes in an ideal adsorbing column during a chromatographic separation depends on the adsorption isotherm, i.e., the amounts f_1 and f_2 adsorbed in equilibrium with the concentrations c_1 and c_2 of the two solutes.

Calculation leads to the fundamental relationship, $df_1/dc_1 = df_2/dc_2$. This results from the conservation of solute mass in an adsorbing column under the normal chromatographic conditions, and it requires that coexistent c_1 and c_2 are functions of each other only. The solution of the equation leads to a family of curves (characteristics) in a system with the co-ordinates c_1 and c_2. Co-existing concentrations of the two solutes occurring in the same chromatographic boundary lie on one such curve and this curve is uniquely determined by the composition of the original solution and by the solution used for developing the chromatogram. The sequence of concentrations in the column (i.e., the path followed in the diagram of characteristics) as well as any new concentration plateaux developing spontaneously, and the occurrence of sharp or diffuse boundaries, can be predicted by the application of a few simple rules, which are deduced. In this way a unified method is provided which can predict the chromatographic development of any binary mixture of which the binary adsorption isotherms are known. This applies both to solvent development and displacement development.

General Theory.—If a small amount of solvent dv containing an adsorbable solute of concentration c passes through a narrow section of the column weighing dx g., then conservation of mass requires that the amount of solute lost from the solvent during passage of this section must have increased the solute content of this section ($f(c)$dx), see Fig. 1.

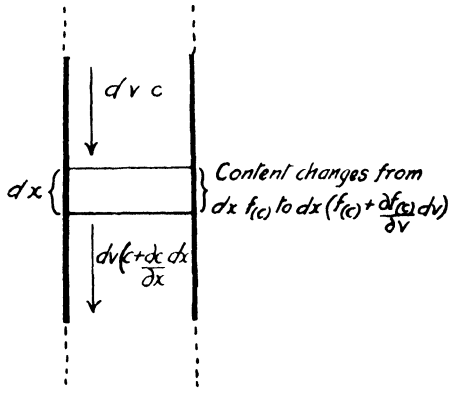

FIG. 1.—Balance of solute mass for a solution flowing through a column of sorbent.

This, as has been shown by Wilson,[1] must be true for every individual solute, and leads to the differential equation

$$\left(\frac{\partial c_i}{\partial x}\right)_v + \left(\frac{\partial f_i}{\partial v}\right)_x = 0 \quad . \quad . \quad . \quad . \quad . \quad (1)$$

In the further development of this function it must be taken into consideration that f_i is usually a function of the concentrations of all the solutes present in the solution :

$$f_i = f_i(c_1, c_2, \ldots . c_i) \quad . \quad . \quad . \quad . \quad . \quad (2)$$

For a sharp boundary, the conservation of mass requires that

$$\mathrm{d}v \,.\, \Delta c_i = \mathrm{d}x \,.\, \Delta f_i \,, \quad . \quad . \quad . \quad . \quad . \quad (3)$$

and as this applies to all the solutes present, the movement of such a sharp boundary can be expressed in terms of any of the solutes :

$$\frac{\mathrm{d}x}{\mathrm{d}v} = \left(\frac{\Delta c_1}{\Delta f_1}\right)_x = \left(\frac{\Delta c_2}{\Delta f_2}\right)_x = \text{etc.} \quad . \quad . \quad . \quad (4)$$

For a diffuse boundary, eqn. (1) can be transformed as was done by de Vault [2] :

$$\left(\frac{\mathrm{d}v}{\mathrm{d}x}\right)_{c_1} = \left(\frac{\mathrm{d}f_1}{\mathrm{d}c_1}\right)_{x,v} \quad . \quad . \quad . \quad . \quad (5a)$$

and

$$\left(\frac{\mathrm{d}v}{\mathrm{d}x}\right)_{c} = \left(\frac{\mathrm{d}f_2}{\mathrm{d}c_2}\right)_{x,v} \text{ etc.} \quad . \quad . \quad . \quad . \quad (5b)$$

For further discussion we confine ourselves to only two solutes and we assume that the isotherms f_1 and f_2 are such that

(I) for $c_1 = 0, f_1 = 0$,
for $c_2 = 0, f_2 = 0$

and

(II) that $\partial f_2/\partial c_1$ has the same sign as $\partial f_1/\partial c_2$.

The first assumption is obvious and the Gibbs-Duhem equation requires that the last assumption is always satisfied.

[1] Wilson, *J. Amer. Chem. Soc.*, 1940, **62**, 1583.
[2] De Vault, *ibid.*, 1943, **65**, 532.

Two situations are possible :

(i) either there is a functional relationship between c_1 and c_2 alone, not involving v and x. Then we have a relationship of the type,

$$c_1 = \varphi(c_2) \quad . \quad . \quad . \quad . \quad . \quad (6)$$

or (ii) there is no such relationship.

A mathematical analysis of the properties of the system (5*a*, *b*) shows that case (i) holds only for the very simplest boundary conditions, i.e., if a solution of constant concentration is fed into an adsorption column which itself contains the solutes in another constant concentration. Fortunately this is the rule in normal chromatographic procedure. One starts with the boundary conditions $c_1 = 0$, $c_2 = 0$ (empty column), then adds the solution to be separated into its constituents with the constant composition ($c_1 = c_1^\circ$, $c_2 = c_2^\circ$), and then develops this band of constant concentrations with another solution where again $c_1 = 0$ and $c_2 = 0$. As soon as we introduce special boundary conditions, e.g., an initial distribution of solutes in the column of a type which does not arise from feeding a solution of constant composition, we have case (ii). In these cases it will not be possible to find a simple dependence between the variations of x and v due to the fact that there is a varying $c_1 - c_2$ combination which does not permit the function df_i/dc_i to be expressed in terms not containing x and v themselves. For all practical purposes case (ii) will only arise when, in the rare case of isotherms convex against the c-axis (when a diffuse front is produced), the band is developed with a different solvent. It is not believed that these cases are sufficiently important to warrant an exceedingly involved discussion.

In the event that there is a functional relationship (6) between c_1 and c_2 (case (i)) eqn. (5*a*, *b*) become

$$\left(\frac{dv}{dx}\right)_{c_1 c_2} = \left(\frac{df_1}{dc_1}\right) \quad . \quad . \quad . \quad . \quad (7)$$

$$\left(\frac{df_1}{dc_1}\right) = \left(\frac{df_2}{dc_2}\right) . \quad . \quad . \quad . \quad . \quad (8)$$

and eqn. (8) makes it possible to derive this relationship between coexistent values of c_1 and c_2 (eqn. 6).

Partial differentiation of both sides of (8) leads to

$$\left(\frac{dc_1}{dc_2}\right)^2 + \frac{\frac{\partial f_2}{\partial c_2} - \frac{\partial f_1}{\partial c_1}}{\frac{\partial f_2}{\partial c_1}} \cdot \left(\frac{dc_1}{dc_2}\right) - \frac{\left(\frac{\partial f_1}{\partial c_2}\right)}{\left(\frac{\partial f_2}{\partial c_1}\right)} = 0 , \quad . \quad . \quad (9)$$

which form was first used by Offord and Weiss.[3] For any given c_1 and c_2, the quadratic eqn. (9) leads to two alternative values for dc_1/dc_2. In view of assumption II with respect to $f_1(c_1\ c_2)$ and $f_2(c_1\ c_2)$, the free term in this equation is negative. Consequently the two roots of the quadratic are both real, one being positive, the other negative.

Eqn. (9) makes it possible to construct the curves of c_1 as function of c_2 for any given isotherm and for any given starting point $c_1^\circ\ c_2^\circ$, the next points on the curve being given by

$$\left.\begin{aligned} c_2' &= c_2^\circ - \Delta c_2 \quad \text{and} \quad c_1' = c_1^\circ - \left[\frac{dc_1}{dc_2}\right]_{c_1^\circ c_2^\circ} \cdot \Delta c_2 , \\ c_2'' &= c_2' - \Delta c_2 \quad \text{and} \quad c_1'' = c_1' - \left[\frac{dc_1}{dc_2}\right]_{c_1' c_2'} \cdot \Delta c_2 , \end{aligned}\right\}$$

[3] Offord and Weiss, *Nature*, 1945, **155**, 725.

and so on. In this manner we can determine the two curves passing through the point c_1° c_2°, with positive slope, by using first the positive values of dc_1/dc_2, and with negative slopes, by using the negative values. Starting with all possible points c_1° c_2° in the quadrant $c_1 > 0$, $c_2 > 0$, we shall obtain two whole families of curves, covering the entire quadrant. These families of curves are known as the " families of characteristics " of the differential eqn. (8) (Fig. 2). Only such combinations of c_1 and c_2 can occur in the same chromatographic boundary which lie on the same characteristic.

(Instead of constructing the c_1-c_2 characteristics, it is sometimes more convenient to use the f_1-f_2 characteristics. The choice depends on the equation for the adsorption isotherm. All the general conclusions drawn for the c characteristics apply equally to the f characteristics, though the equations are obviously modified.)

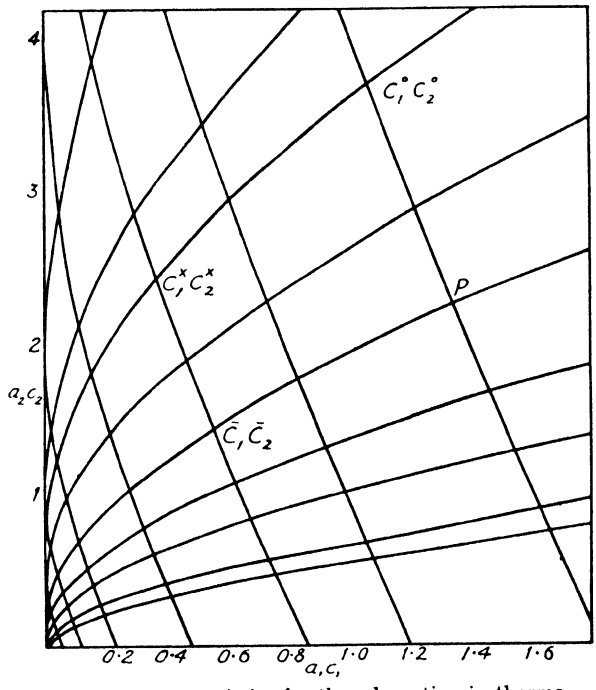

FIG. 2.—c_1-c_2 diagram of characteristics for the adsorption isotherms

$$a_1 c_1 = f_1 (f_1 + f_2)$$
$$a_2 c_2 = f_2 (f_1 + f_2)$$
for $\frac{a_2}{a_1} = 2$.

Only sets of concentrations lying on the same characteristic can coexist in the same chromatographic boundary.

The fact that the entire quadrant $c_1 > 0$, $c_2 > 0$ is covered by these curves is of importance, because it implies that the " envelope " of these curves, which itself represents a solution to the differential equation, lies outside this quadrant and therefore does not include cases where both concentrations have values above zero, which alone are of practical significance. Thus no ambiguity is possible.

It is also important to note that a particular characteristic is defined by only one concentration set c_1 c_2, so that, if a column is filled with a solution c_1° c_2° and then developed with a solution $\bar{c}_1$ $\bar{c}_2$, we cannot as a rule

expect that these two solutions lie on the same characteristic. Each solution will possess its own pair of characteristics, which is of some importance for the form of chromatographic bands of two solutes (Fig. 3).

Thus, when a solution is poured on a column which has previously been treated with another solution containing the same solutes at different concentrations, they will, as a rule, produce two separate boundaries, separated by a band of constant concentrations c_1^* c_2^*, which correspond to the point of intersection of two characteristics passing through the points c_1° c_2° and $\bar{c}_1$ $\bar{c}_2$, respectively.

But before discussing this in detail, it is necessary to return once more to eqn. (7) which concerns the v-x relationship, i.e., the movement of a point of given concentrations c_1 c_2.

We can write

$$\left(\frac{dv}{dx}\right)_{c_1 c_2} = \frac{df_1}{dc_1} = \frac{\partial f_1}{\partial c_1} + \frac{\partial f_1}{\partial c_2} \cdot \left(\frac{dc_2}{dc_1}\right) \quad . \quad . \quad . \quad (10)$$

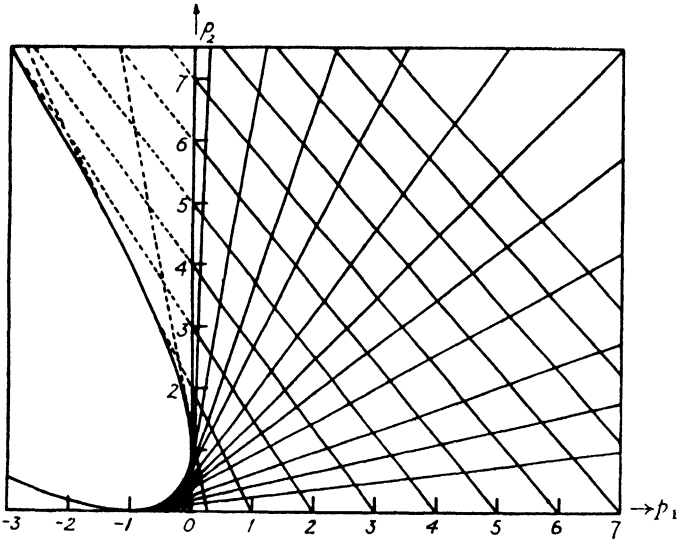

FIG. 3.—p_1-p_2 diagram of characteristics for any Langmuir isotherm showing the parabolic envelope of the linear characteristics.

If we take the case of a binary isotherm where $\partial f_1/\partial c_2$ is negative (which is almost invariably the case), then it follows that dv/dx for a given point c_1 c_2 is always smaller, and consequently the rate of movement dx/dv of a point c_1 c_2 is always larger for positive values of dc_2/dc_1 than for negative ones.

A concentration plateau c_1^* c_2^* in a chromatogram is only stable and growing when its forward point moves faster than its rear. It follows from this that *a concentration plateau c_1^* c_2^* is produced spontaneously in a boundary, if the boundary section in front of it travels along the positive characteristic of c_1^* c_2^* (i.e., towards c_1° c_2° in Fig. 2) while the rear travels along a characteristic where dc_1/dc_2 is negative (or zero)* (RULE 1).

This eliminates any ambiguity as regards the route which a boundary can take between two points, as the alternative concentration plateau at the point P (Fig. 2) cannot form spontaneously, if the solution $\bar{c}_1$ $\bar{c}_2$ follows the solution c_1° c_2°. (If, however, $\bar{c}_1$ $\bar{c}_2$ is in front of c_1° c_2°, then for the same reason the

When changes occur in a column along the characteristics of the equation

$$\frac{df_1\,(c_1\,c_2)}{dc_1} = \frac{df_2\,(c_1\,c_2)}{dc_2}, \quad . \quad . \quad . \quad (8)$$

then c_2 is always a function of c_1 defined by the original solution (c_1° c_2°) of the particular case. We can therefore write

$$\frac{dv}{dx} = \frac{df_1\,(c_1\,c_2)}{dc_1} = \frac{dF_1\,(c_1)}{dc_1}, \quad . \quad . \quad . \quad (11)$$

where F_1 is a new function of c_1 only, which fully corresponds to the adsorption isotherm $f(c)$ in the case of a single solute, with the only exception that the function F_1 depends also on the initial conditions c_1° c_2°. Counting v from the change-over to the new solution $\bar{c}_1$ $\bar{c}_2$ we can integrate to

$$x = v \Big/ \frac{dF_1\,(c_1)}{dc_1} = \frac{v}{F_1'} = v \Big/ \frac{df_1\,(c_1\,c_2)}{dc_1}, \quad . \quad . \quad (12a)$$

and

$$x = v \Big/ \frac{dF_2\,(c_2)}{dc_2} = \frac{v}{F_2'} = v \Big/ \frac{df_2\,(c_1\,c_2)}{dc_2} \quad . \quad . \quad . \quad (12b)$$

Once the transformation to the single solute equation has been made, the sequence of chromatographic band movements follows in a straightforward way, as has been described in previous publications.[4 5 6]

The question where a diffuse or a sharp boundary arises is again very similar to the case of a single solute as neither dc_1/dx nor dc_2/dx must become positive in a frontal boundary or negative in a rear boundary. It thus depends on F_1'' and on $F_2'' = \left[\frac{d^2F_2}{dc_2^2}\right]_v$ which type of boundary occurs. But, unlike the case of a single solute, it requires some knowledge of the characteristics to see under what conditions F_1'' and F_2'' are positive or negative.

Langmuir Isotherms.—The general case can be best understood by illustrating it with the Langmuir isotherm, which offers the advantage that eqn. (8) can be directly solved by integration, so that general expressions can be obtained for all functions, and for the movements of all points in the chromatographic band.

Writing the Langmuir isotherm in the form

$$f_1 = \frac{a_1 c_1}{1 + \beta a_1 c_1 + \beta a_2 c_2}, \quad . \quad . \quad . \quad . \quad (13a)$$

and

$$f_2 = \frac{a_2 c_2}{1 + \beta a_1 c_1 + \beta a_2 c_2}, \quad . \quad . \quad . \quad . \quad (13b)$$

where $a_2 > a_1$ and β are all some positive constants. It is convenient to introduce

$$\beta a_1 = b_1 \text{ and } \beta a_2 = b_2 \text{ and } a_2/a_1 = b_2/b_1 = K.$$

(As shown by Kemball, Rideal and Guggenheim [7] the use of different values of β_1 and β_2 offends the Gibbs-Duhem law.)

For these isotherms (8) reduces to

$$b_1(1 + b_2c_2)dc_1dc_2 + b_1b_2c_2(dc_1)^2 - b_1b_2c_1(dc_2)^2 - b_2(1 + b_1c_1)\,dc_1dc_2\,. \quad (14)$$

[4] Glueckauf, *Proc. Roy. Soc. A*, 1946, **186**, 35.
[5] Glueckauf, *J. Chem. Soc.*, 1947, 1321.
[6] Coates and Glueckauf, *J. Chem. Soc.*, 1947, 1309.
[7] Kemball, Rideal, and Guggenheim, *Trans. Faraday Soc.*, 1948, **44**, 952.

Putting for brevity

$$\frac{b_1 b_2}{b_2 - b_1} \cdot c_1 = p_1, \quad . \quad . \quad . \quad (15a)$$

and

$$\frac{b_1 b_2}{b_2 - b_1} \cdot c_2 = p_2, \quad . \quad . \quad . \quad . \quad (15b)$$

and dividing by $(\mathrm{d}p_2)^2$, we obtain

$$p_2 \left(\frac{\mathrm{d}p_1}{\mathrm{d}p_2}\right)^2 - \left(+ p_1 - p_2\right) \cdot \frac{\mathrm{d}p_1}{\mathrm{d}p_2} - p_1 = 0. \quad . \quad . \quad (16)$$

Differentiating again with respect to p_2

$$\left[2\, p_2 \cdot \frac{\mathrm{d}p_1}{\mathrm{d}p_2} - \left(1 + p_1 - p_2\right)\right] \cdot \frac{\mathrm{d}^2 p_1}{\mathrm{d}p_2^2} = 0. \quad . \quad . \quad (17)$$

Hence, either p_1 is a linear function of p_2 or

$$2\, p_2 \cdot \frac{\mathrm{d}p_1}{\mathrm{d}p_2} = 1 + p_1 - p_2 \quad . \quad . \quad . \quad . \quad (18)$$

Eliminating $\mathrm{d}p_1/\mathrm{d}p_2$ in (16) by means of (18), we obtain

$$(1 + p_1 - p_2)^2 + 4 p_1 p_2 = 0. \quad . \quad . \quad . \quad (19)$$

This function is a parabola which merely touches the quadrant $c_1 \geqslant 0$, $c_2 \geqslant 0$ at the point $c_1 = 0$, $c_2 = \frac{b_2 - b_1}{b_1 b_2}$, lying otherwise entirely outside this quadrant. It does not, therefore, apply to any real concentrations and need not concern us.

The only solutions of (16) where p_1 and p_2 are both positive arise therefore from

$$\frac{\mathrm{d}^2 p_1}{\mathrm{d}p_2^2} = 0, \quad . \quad . \quad . \quad . \quad . \quad (20)$$

where p_1 is a linear function of p_2. This leads to

$$p_1 = \lambda \cdot p_2 - \lambda/(1 + \lambda), \quad . \quad . \quad . \quad . \quad (21a)$$

or, returning to the concentrations,

$$c_1 = \lambda c_2 - \frac{(b_2 - b_1)}{b_1 b_2} \cdot \frac{\lambda}{(1 + \lambda)}. \quad . \quad . \quad . \quad (21b)$$

Here λ is an integration constant, depending on the given starting concentrations. In a system of c_1 and c_2 co-ordinates, eqn. (21*a* or *b*) represents straight lines which envelop the parabola of eqn. (19), and this makes it easy to construct the lines (21*a* or *b*) geometrically (Fig. 3). If we are given some point c_1 c_2 we can draw through this point the two tangents to the curve (19) and these tangents give us the two characteristics passing through the point c_1 c_2, which contain all the coexistent concentration sets. From the fact that the lines (21) are tangents to a convex curve, it follows that no two different lines possess the same value λ. Consequently λ, which is the slope of the lines (21*a*), can serve as a parameter to distinguish one characteristic from another.

As two tangents pass through every point $c_1 > 0$, $c_2 > 0$, we can introduce the two λ values as parameters characterizing every mixture c_1 c_2. We take the positive λs as the parameter μ, and the negative ones as the parameter ν. The positive-slope family of characteristics has values of $\mu \geqslant 0$ while the negative-slope family has values of ν between 0 and -1.

If we are given a point $c_1\ c_2$, then μ and ν can be determined by solving (21*b*), for λ in

$$\lambda^2 + \lambda\left(1 - \frac{c_1}{c_2} - \frac{(b_2 - b_1)}{b_1\, b_2\, c_2}\right) - \frac{c_1}{c_2} = 0, \quad . \quad . \quad . \quad (21c)$$

and identifying the positive root of the quadratic with μ and its negative root with ν.

The values of μ as function of c_1/c_2 and $(b_2 - b_1)/b_1\ b_2\ c_2$ are shown in Fig. 2.[4]

From the equations of the two characteristics (see (21*b*)),

$$p_1 = \mu p_2 - \mu/(1 + \mu),$$

and

$$p_1 = \nu p_2 - \nu/(1 + \nu),$$

we can solve for p_1 and p_2, or c_1 and c_2.

$$c_1 = \frac{b_2 - b_1}{b_1\, b_2} \cdot \frac{-\mu \,.\, \nu}{(1 + \mu)(1 + \nu)}, \quad . \quad . \quad . \quad (22)$$

$$c_2 = \frac{b_2 - b_1}{b_1\, b_2} \cdot \frac{1}{(1 + \mu)(1 + \nu)}, \quad . \quad . \quad . \quad (23)$$

which gives us the values of c_1 and c_2, in terms of the parameters μ and ν. Both expressions are positive, as ν is negative and $> (-1)$.

We can also express $f_1(c_1\, c_2)$ and $f_2(c_2\, c_2)$ as functions of the parameters μ and ν:

$$f_1 = -\mu \,.\, \nu \frac{b_2 - b_1}{\beta b_1(\mu + K)(\nu + K)}, \quad . \quad . \quad . \quad (24)$$

$$f_2 = K \frac{b_2 - b_1}{\beta b_1(\mu + K)(\nu + K)}, \quad . \quad . \quad . \quad (25)$$

we then obtain the movement of a point of concentration $c_1\, c_2$:

$$\left[\frac{\Delta x}{\Delta v}\right]_{c_1\, c_2} = \frac{dc_2}{df_2} = \left[\frac{\partial c_2}{\partial \nu}\right]_\mu \Big/ \left[\frac{\partial f_2}{\partial \nu}\right]_\mu \quad . \quad . \quad . \quad (26)$$

along the positive characteristic,

and

$$= \left[\frac{\partial c_2}{\partial \mu}\right]_\nu \Big/ \left[\frac{\partial f_2}{\partial \mu}\right]_\nu \quad . \quad . \quad . \quad . \quad (27)$$

along the negative characteristic, by partially differentiating (23) and (25) with respect to ν or μ. Thus we obtain the simple functions:

$$\left[\frac{\Delta x}{\Delta v}\right]_{c_1\, c_2 \mu} = \frac{1}{a_2 K}\left(\frac{\mu + K}{\mu + 1}\right)\left(\frac{\nu + K}{\nu + 1}\right)^2 \quad . \quad . \quad . \quad (28)$$

(See Fig. 4 (*a*), A→B or D→C.)

$$\left[\frac{\Delta x}{\Delta v}\right]_{c_1 c_2 \nu} = \frac{1}{a_2 K}\left(\frac{\mu + K}{\mu + 1}\right)^2 \cdot \left(\frac{\nu + K}{\nu + 1}\right) \quad . \quad . \quad . \quad (29)$$

(See Fig. 4 (*a*), D→A or C→B.)

It is easy to see from this that the movement along the positive characteristic proceeds faster than along the negative one, as was already predicted generally for isotherms where $\partial f_1/\partial c_2$ and $\partial f_2/\partial c_1$ are negative.

The condition that we have a sharp boundary requires that dc/dx, deduced from (28) or (29), is positive at a falling concentration (e.g., when forming

the front of a band) and negative at a rising concentration (e.g., as rear of a band).

Using $$\left[\frac{dc}{dx}\right]_{\mu,v} = \left[\frac{\partial c}{\partial \nu}\right]_{\mu} \Big/ \left[\frac{\partial x}{\partial \nu}\right]_{\mu,v},$$

and $$\left[\frac{dc}{dx}\right]_{\nu,v} = \left[\frac{\partial c}{\partial \mu}\right]_{\nu} \Big/ \left[\frac{\partial x}{\partial \mu}\right]_{\nu,v},$$

as all variations must occur along one of the characteristics, we obtain by differentiating (22), (23), (28) and (29) for constant ν:

$$\left[\frac{dc_1}{dx}\right]_{\mu,v} = \frac{\mu \cdot (\nu + 1)}{D} = \text{always positive}, \quad . \quad . \quad (30)$$

where $D = 2\beta\ (\mu + K)(\nu + K) \cdot v/K$;

$$\left[\frac{dc_1}{dx}\right]_{\nu,v} = \frac{\nu \cdot (\mu + 1)}{D} = \text{always negative} \quad . \quad . \quad (31)$$

$$\left[\frac{dc_2}{dx}\right]_{\mu,v} = \frac{(\nu + 1)}{D} = \text{always positive} \quad . \quad . \quad (32)$$

$$\left[\frac{dc_2}{dx}\right]_{\nu,v} = \frac{\mu + 1}{D} = \text{always positive.} \quad . \quad . \quad (33)$$

For a single solute isotherm of the Langmuir type $[dc/dx]_v$ is always positive; we can, therefore, conclude from the above information (30–33) that the more strongly adsorbed solute II behaves in the presence of solute I, as far as type of boundary is concerned, in the same way as if solute I were not there at all. This means that *where the more strongly adsorbed solute c_2 increases along the column we have a diffuse boundary, while where it changes to a lower concentration we have a sharp boundary* (RULE 2). Fig. 4 illustrates the practical aspect of this rule for the properties of various boundaries.

The rate of movement of these sharp boundaries (s.b.) is given by

$$\left[\frac{\Delta x}{\Delta v}\right]^{s.b.} = \frac{\Delta c_1}{\Delta f_1} = \frac{\Delta c_2}{\Delta f_2},$$

and replacing the c and f values by μ and ν from eqn. (32–35) leads to

$$\left[\frac{\Delta x}{\Delta v}\right]^{s.b.}_{\mu} = \frac{1}{a_2 K} \left(\frac{\mu + K}{\mu + 1}\right) \left(\frac{\nu_1 + K}{\nu_1 + 1}\right) \left(\frac{\nu_2 + K}{\nu_2 + 1}\right) \quad . \quad . \quad (34)$$

(See Fig. 4 (*a*), B→A or C→D.)

$$\left[\frac{\Delta x}{\Delta v}\right]^{s.b.}_{\nu} = \frac{1}{a_2 K} \left(\frac{\mu_1 + K}{\mu_1 + 1}\right) \left(\frac{\mu_2 + K}{\mu_2 + 1}\right) \left(\frac{\nu + K}{\nu + 1}\right) \quad . \quad . \quad (35)$$

(See Fig. 4 (*a*), A→D or B→C.)

The subscripts for μ and ν refer to the two concentration sets which are connected by the sharp boundary (e.g., the points B and C in Fig. 4 (*a*)). The knowledge of the characteristics μ and ν thus enables us to derive, with the help of eqn. (28), (29), (34) and (35), the form and rate of movement of all boundaries in the column or in the eluate.

Returning to the families of characteristics in the $c_1 - c_2$ diagram, we see that the four cases mentioned in eqn. (28), (29), (34) and (35) do not describe all the possibilities which can occur if one solution is eluted by another. The two solutions following each other in these cases were exceptional in so far as both concentration sets lie on one characteristic. This, however, is not usually the case. If the two solutions following each other have no characteristic in common, and this is the rule, a new concentration plateau develops corresponding to that point of intersection of two charac-

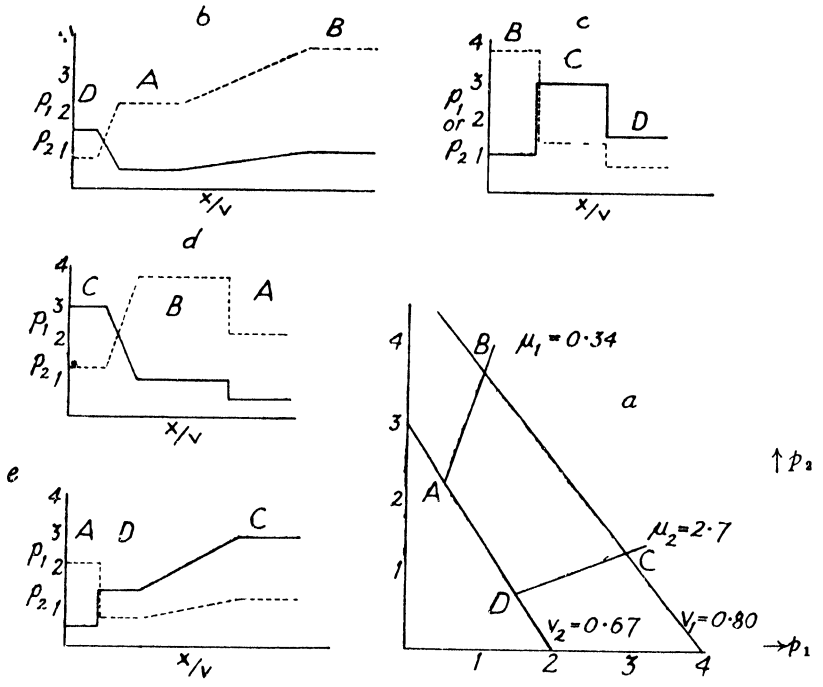

FIG. 4.—(*a*) Diagram of characteristics connecting two binary solutions, e.g., A and C, or B and D. μ and ν are the values of the gradients $[dc_1/dc_2]$ of the characteristics. Concentration changes of the two solutes (solute I ——, solute II - - -) in a column produced when

(*b*) Solution D elutes a solution B.
(*c*) ,, B ,, ,, ,, D.
(*d*) ,, C ,, ,, ,, A.
(*e*) ,, A ,, ,, ,, C.

teristics passing through the two points which is stable according to Rule 1, which states that the positive characteristic always moves in front of a negative one. This means that we can have the following four cases, which can be classified only according to the variation of μ and ν along the column :

Case	μ	ν	Reference for Fig. 4 (*a*)
I	decreasing	decreasing	D → B (see Fig. 4 (*b*))
II	increasing	increasing	B → D (see Fig. 4 (*c*))
III	decreasing	increasing	C → A (see Fig. 4 (*d*))
IV	increasing	decreasing	A → C (see Fig. 4 (*e*))

The resulting boundaries of the column chromatograms with their spontaneously developing concentration plateaux are shown in Fig. 4 (*b*)–(*e*). The rate of movement, i.e., relative position of the boundaries, has been obtained from the μ- and ν-values by means of eqn. (28), (29), (34) and (35).

It should be pointed out that, while normally the sharp boundaries do not follow exactly the curves of the characteristics, they do so in the case of Langmuir isotherms, because here, due to the linearity of the characteristics, dc_1/dc_2 is identical with $\Delta c_1/\Delta c_2$.

Formation and Development of a Two Solute Band.—During the formation of the original band, produced by feeding a solution c_1° c_2° into an empty column ($c_1 = 0$, $c_2 = 0$), we have clearly a case of type II (Fig. 4 (*c*)) with increasing μ and ν. An example is shown in Fig. 5 (*a*), the stable boundary course being represented S → Q → O. A new concentration plateau is created at Q, representing the pure frontal band of solute I (Fig. 5 (*b*)).

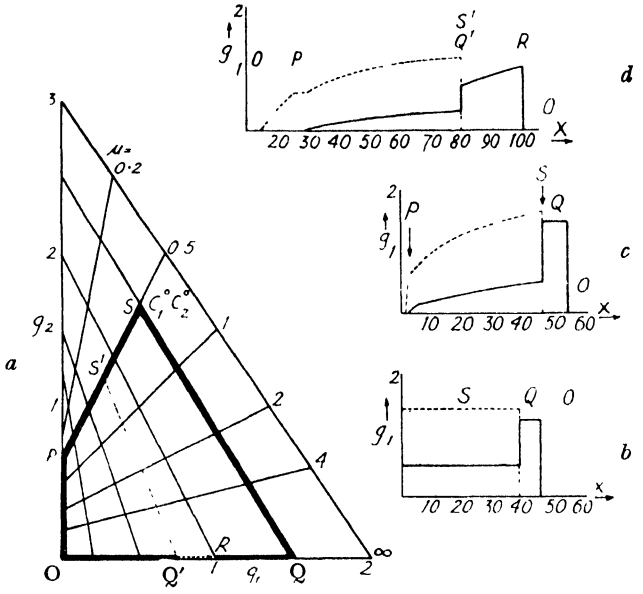

FIG. 5.—Formation and solvent-development of a chromatographic band of the original concentrations c_1° c_2°.

(*a*) As shown in the diagram of g_1-g_2 characteristics.

Distribution of solute in a column for the above case.

(*b*) Original band.

(*c*) and (*d*) Stages in the development of the band.

If, after the formation of the original band, we proceed to "develop" the chromatogram by the addition of further amounts v of pure solvent ($c_1 = 0$, $c_2 = 0$), we form a rear boundary of the type I (Fig. 4 (*b*)) with decreasing μ and ν, which in Fig. 5 (*a*) is represented by O → P → S.

This rear boundary forms a new concentration plateau, characterized by the point P in the f_1-f_2 (or g_1-g_2) diagram, which is the point at which f_1 becomes zero (Fig. 5 (*a*) and (*c*)). The actual movements of the various points with continuing development (v) follow directly from the application of the equations given in Tables I to V.[6]

Fig. 5 (*a*) gives the f_1-f_2 characteristics, which for a Langmuir isotherm are identical in form with the p_1-p_2 characteristics (eqn. (21*a*)), if one chooses the parameters,

$$g_1 = \frac{\beta b_1}{b_2 - b_1} \cdot f_1, \quad . \quad . \quad . \quad . \quad (36)$$

and

$$g_2 = \frac{\beta b_2}{b_2 - b_1} \cdot f_2. \quad . \quad . \quad . \quad . \quad (37)$$

The equations of motion as function of the parameters $\varphi = \left[\frac{\partial f_1}{\partial f_2}\right]_\psi$ for the positive and $\psi = \left[\frac{\partial f_1}{\partial f_2}\right]_\varphi$ for the negative characteristics are similar to the eqn. (28), (29), (34) and (35).

We obtain :

$$\left[\frac{\Delta x}{\Delta v}\right]_\varphi = \frac{1}{a_2 K}\left(\frac{1+\varphi}{\frac{1}{K}+\varphi}\right)\left(\frac{1+\psi}{\frac{1}{K}+\psi}\right)^2, \quad . \quad . \quad . \quad (38)$$

$$\left[\frac{\Delta x}{\Delta v}\right]_\psi = \frac{1}{a_2 K}\left(\frac{1+\varphi}{\frac{1}{K}+\varphi}\right)^2 \cdot \left(\frac{1+\psi}{\frac{1}{K}+\psi}\right), \quad . \quad . \quad . \quad (39)$$

$$\left[\frac{\Delta x}{\Delta v}\right]_\varphi^{s.b.} = \frac{1}{a_2 K}\left(\frac{1+\varphi}{\frac{1}{K}+\varphi}\right)\left(\frac{1+\psi_1}{\frac{1}{K}+\psi_1}\right)\left(\frac{1+\psi_2}{\frac{1}{K}+\psi_2}\right), \quad . \quad . \quad (40)$$

$$\left[\frac{\Delta x}{\Delta v}\right]_\psi^{s.b.} = \frac{1}{a_2 K}\left(\frac{1+\varphi_1}{\frac{1}{K}+\varphi_1}\right)\left(\frac{1+\varphi_2}{\frac{1}{K}+\varphi_2}\right)\left(\frac{1+\psi}{\frac{1}{K}+\psi}\right) \cdot \quad . \quad . \quad (41)^*$$

For the construction of bands in the column, these f_1-f_2 characteristics are somewhat more convenient, as the areas under the x-f curves (or x-g curves) represent the original masses of the solutes which must be constant. (When dealing with elution (c-v) curves the advantage is with the c_1-c_2 characteristics, for the same reason.)

Returning to our Rule 1, we expect that the plateaux of P and Q (Fig. 5) should be stable and growing, as here positive precedes negative characteristic. However, at the point S this situation is reversed and this plateau, which had been experimentally introduced, prior to the development with pure solvent, is therefore unstable and constantly diminishing. Eventually the point S will disappear altogether, thereby making Q also unstable, so that both plateaux S and Q will eventually disappear. S slowly recedes on the line SP towards P ; so we arrive at the diagram shown in Fig. 5 (*a*) by the sequence O → P → S′ → Q′ → R → O, which results in the chromatogram shown in Fig. 5 (*d*), which type persists until the constantly receding point S′ has merged with P, when Q′ coincides with O, i.e., when separation is complete.

Displacement Development.—The use of the characteristics does not only apply to ordinary solvent development, but also to development with a solution containing a third solute. A particularly simple case arises when

* Due to the fact that $\beta(f_1 + f_2) < 1$, it follows that $\frac{1}{K} > -\psi$.

the third solute is most strongly adsorbed, which leads to the so-called displacement development. In this case we get no ternary mixtures and we can, therefore, describe the situation by two adjoining maps of characteristics shown in Fig. 6. The original concentrations (c_1° c_2°) are represented by the point E, the concentration of the developing solute (c_3°) by point A. The connection between A and E, and E and O under Rule 1, goes via the points B, D and F, all of which form stable plateaux in the chromatogram, while the original plateau E, where a negative characteristic precedes the positive one, is unstable and diminishes in length (Fig. 7 (*a*) and (*b*)). Eventually E will disappear completely and henceforward the path from D to O goes via the point G (Fig. 7 (*c*)). This change makes the previously formed plateaux D and F unstable, and they eventually disappear. The end of the chromatographic separation is then reached, leaving the plateaux A, B and G stable, all of which represent pure species of solute (Fig. 7 (*d*)).

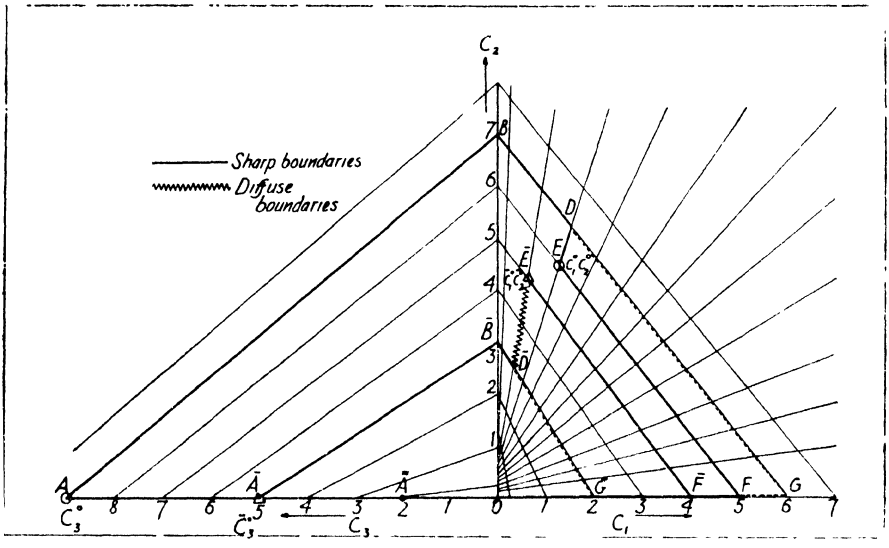

FIG. 6.—Development of a binary band with a solution containing a third solute, as shown in the diagram of c_1-c_3 and c_2-c_3 characteristics.

Example: $b_1 = \frac{1}{2}$, $b_2 = 1$, $b_3 = 2$.

We can immediately see from Fig. 6 what would happen if the developing concentration c_3°, ($\bar{A}$), is not high enough. In this case a diffuse boundary arises between the plateaux $\bar{D}$ and $\bar{E}$ (see also Fig. 6) which disappears eventually due to the instability of plateau $\bar{E}$ and the separation ends with the plateaux $\bar{A}$, $\bar{B}$, $\bar{G}$.

Furthermore, if c_3° is smaller than indicated by the point $\bar{\bar{A}}$ where the enveloping parabola (not shown) touches the abscissa, the only way from $\bar{\bar{A}}$ to E leads via O, which means that the third solute fails to make contact with the other solutes and then there is no longer any displacement development.

Actually, all these cases have already been discussed individually,[4] but the use of the " characteristics " makes it possible to give a unified picture of all possible chromatographic processes involving the separation of two solutes.

Separation of Three Solutes.—With a certain amount of algebraical discomfort, the theory can be extended to ternary solutions. We obtain then a three-dimensional system of characteristics, and changes caused by solvent development proceed by variation of one characteristic, while keeping the other two constant. However, as no new chromatographic features are derived from this extension, its discussion may well be postponed.

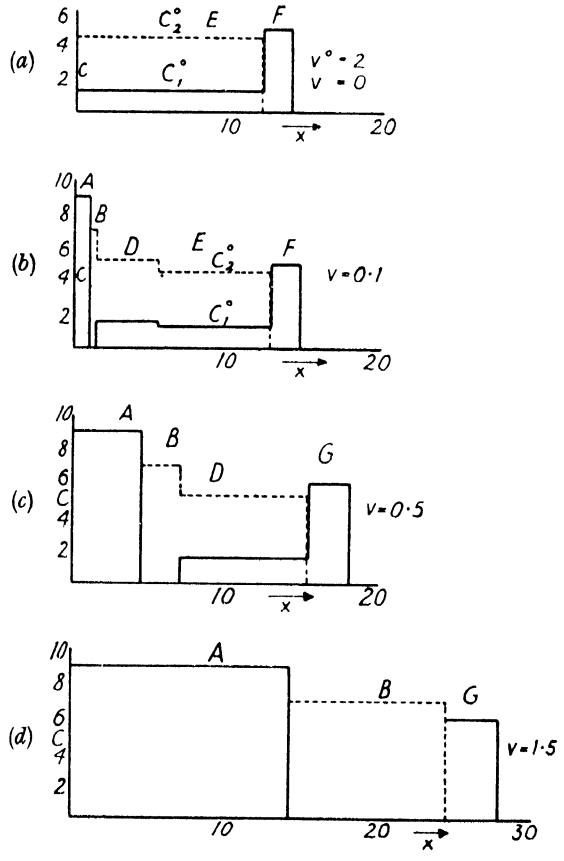

FIG. 7.—Concentration diagram in the column, deduced from Fig. 6, line A-B-D-E-F-O, etc.

(*a*) Original two solute band (solute I ——, solute II - - - -).

(*b*) (*c*) Stages in the development with a third solute.

(*d*) Complete separation.

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Atomic Energy Research Establishment,
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CHROMATOGRAPHY WITH SEVERAL SOLUTES

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The theory of chromatography of a single solute has been given previously by de Vault and by Weiss. The present investigation has for its object a theory of chromatography for two or more solutes.

The treatment is based on the differential equations:

$$\frac{\partial c_1}{\partial x} + \frac{\partial q_1}{\partial v} = 0, \qquad \frac{\partial c_2}{\partial x} + \frac{\partial q_2}{\partial v} = 0$$

connecting the concentrations c_1 and c_2 with the amounts adsorbed per unit length q_1 and q_2 respectively under equilibrium conditions, where v is the volume and x the distance along the column. We first investigate under what initial conditions these equations will have a solution which is a function of v/x and using this result determine solutions in a few cases.

We find that two very different phenomena may result. (i) In one case definite bands are formed in which the concentrations are constant. These constant values depend on the original bands and they can be determined in terms of the initial concentrations. (ii) In other cases bands of varying concentrations are formed. A typical example of the first type occurs when a uniform band of one or more solutes is developed with a solution of a different solute which is more strongly adsorbed than any of the solutes present initially (as illustrated, for instance, by Tiselius' displacement method).

The second type of chromatogram may occur when a band of two solutes at constant concentrations is developed with pure solvent or when a band consisting of a single solute is developed with a solution of a second solute which is much less strongly adsorbed than the first solute. Several examples are discussed and various criteria for distinguishing these cases are given.

The theory of chromatography of a single solute has been given by de Vault,[1] and by Weiss.[2] Subsequently a treatment for two or more solutes was briefly indicated by the present writers[3] and in some special cases was given by a number of authors.[4]

The object of this paper is to give a general theory for the chromatography of two or more solutes. We begin with a general discussion and then give examples for two and three solutes.

I. General Discussion.—The amounts adsorbed per unit cross-section at the level x of the band after the passing of a volume v are denoted by $q_1(x, v)$ and $q_2(x, v)$. It is assumed that q_1 and q_2 depend only on the concentrations c_1 and c_2 of the solutes (1) and (2) and that the adsorption equilibrium is practically always established.

If q_1, q_2, c_1 and c_2 are continuous functions with continuous partial derivatives, considerations of conservation of mass imply that:

$$\frac{\partial c_1}{\partial x} + \frac{\partial q_1}{\partial v} = 0 \qquad . \quad . \quad . \quad . \quad (1.1)$$

and

$$\frac{\partial c_2}{\partial x} + \frac{\partial q_2}{\partial v} = 0 \qquad . \quad . \quad . \quad . \quad (1.2)$$

[1] De Vault, *J. Amer. Chem. Soc.*, 1943, **65**, 532; also Wilson, *ibid.*, 1940, **62**, 1583.
[2] Weiss, *J. Chem. Soc.*, 1943, 297.
[3] Offord and Weiss, *Nature*, 1945, **155**, 725.
[4] Cf. Glueckauf, *Proc. Roy. Soc. A*, 1946, **186**, 35; *J. Chem. Soc.*, 1947, 1308, 1321.

However, we shall not assume that c_1 and c_2 are continuous functions of x and v. This is based on results obtained by one of us and by others [1 2] in the discussion of the chromatography of a single solute. At first sight it may seem somewhat artificial to admit discontinuous functions, but it must be borne in mind that c_1 and c_2 represent certain mathematical functions which satisfy eqn. (1.1) and (1.2). These functions will, in general, represent the concentrations only approximately because they were derived under the assumption of equilibrium conditions and with the neglect of any diffusion and convection effects. Thus though the concentrations may be continuous, it may well be that they can be best approximated by discontinuous functions. Experience in the chromatography of a single solute shows that this is actually the case.

Let us suppose now that there is a certain front of discontinuity AB. Let $q_{1(I)}$ and $q_{2(I)}$ be the amounts adsorbed per unit length in the band immediately above the level AB and $q_{1(II)}$ and $q_{2(II)}$ be the corresponding quantities in the band immediately below AB. We shall suppose that the concentrations remain constant as the level AB moves down the tube. Let $c_{1(I)}$, $c_{2(I)}$, $c_{1(II)}$ and $c_{2(II)}$ be the corresponding concentrations. Suppose that as the result of the addition of a volume δv the level of discontinuity moves down the tube a distance δx to a level A'B'. (see Fig. 1).

Then the decrease in the amounts of the substances (1) and (2) in the section (AB)(B'A') will be

$$\left\{ q_{1(II)}-q_{1(I)} \right\} \delta x \text{ and } \left\{ q_{2(II)}-q_{2(I)} \right\} \delta x,$$

and these quantities will be equal to the *increases* in the amounts of solute in the small volume δv which are

$$\left\{ c_{1(II)}-c_{1(I)} \right\} \delta v \text{ and } \left\{ c_{2(II)}-c_{2(I)} \right\} \delta v$$

respectively, and hence

$$\left\{ q_{1(II)}-q_{1(I)} \right\} \delta x - \left\{ c_{1(II)}-c_{1(I)} \right\} \delta v = 0, \quad . \quad . \quad (2.1)$$

$$\left\{ q_{2(II)}-q_{2(I)} \right\} \delta x - \left\{ c_{2(II)}-c_{2(I)} \right\} \delta v = 0. \quad . \quad . \quad (2.2)$$

Since δv and δx are not zero and since these equations must hold simultaneously, we get

$$\frac{\delta v}{\delta x} = \frac{q_{1(II)}-q_{1(I)}}{c_{1(II)}-c_{1(I)}} = \frac{q_{2(II)}-q_{2(I)}}{c_{2(II)}-c_{2(I)}} \quad . \quad . \quad . \quad (2.3)$$

Allowing δx and δv to tend to zero, we deduce that

$$\frac{dv}{dx} = \frac{q_{1(II)}-q_{1(I)}}{c_{1(II)}-c_{1(I)}} = \frac{q_{2(II)}-q_{2(I)}}{c_{2(II)}-c_{2(I)}} \quad . \quad . \quad . \quad (3)$$

This is a differential equation giving the rate at which a level of discontinuity AB moves down the tube, in terms of the ratio of the discontinuities in the amounts adsorbed and the concentrations.

We shall now make some general assumptions concerning q_1 and q_2. We shall suppose that they depend only on c_1 and c_2 and that they are regular functions of c_1 and c_2; that is to say, that they can be expanded in a Taylor series in c_1 and c_2. Thus,

$$q_1 = \sum_{\substack{m=0 \\ n=0}}^{\infty} a_{m,n} c_1^m c_2^n, \qquad q_2 = \sum_{\substack{m=0 \\ n=0}}^{\infty} b_{m,n} c_1^m c_2^n.$$

Since obviously q_1 is zero if c_1 is zero and q_2 is zero if c_2 is zero, we must have $a_{0,n} = b_{m,0} = 0$ for all m and n.
This implies that

$$\left(\frac{\partial q_1}{\partial c_2}\right)_{c_1=0} = \left(\frac{\partial q_2}{\partial c_1}\right)_{c_2=0} = 0 \quad . \quad . \quad . \quad . \quad (4)$$

Thus the eqn. (1) become

$$\frac{\partial c_1}{\partial x} + \frac{\partial q_1}{\partial c_1}\frac{\partial c_1}{\partial v} + \frac{\partial q_1}{\partial c_2}\frac{\partial c_2}{\partial v} = 0, \quad . \quad . \quad . \quad (5.1)$$

$$\frac{\partial c_2}{\partial x} + \frac{\partial q_2}{\partial c_1}\frac{\partial c_1}{\partial v} + \frac{\partial q_2}{\partial c_2}\frac{\partial c_2}{\partial v} = 0. \quad . \quad . \quad . \quad (5.2)$$

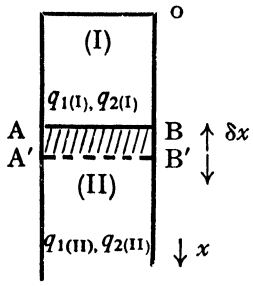

FIG. 1.—Front of discontinuity AB separating the regions (I) and (II).

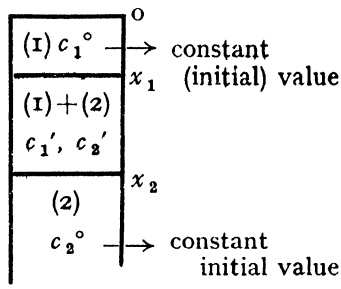

FIG. 3.—Uniformly distributed solute (2) partly developed with a solution of a more strongly adsorbed substance (1).

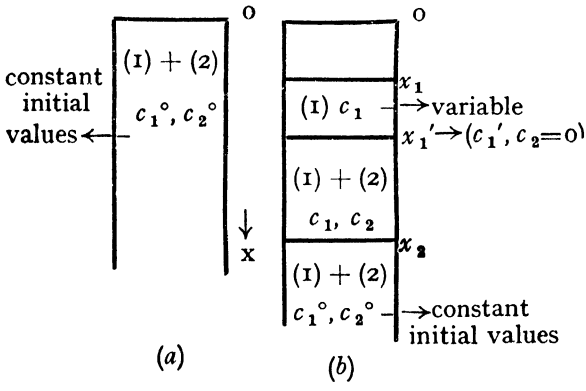

FIG. 2.—(*a*) 2 Solutes: initial band.
(*b*) 2 Solutes: after partial development.

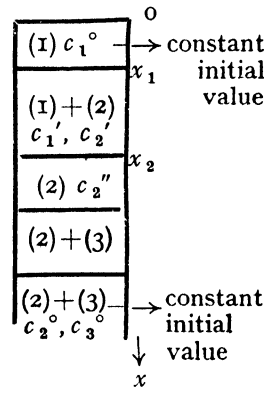

FIG. 4.—Originally uniform mixture of two substances (2) and (3) partly developed with a solution of a third substance (1) which is more strongly adsorbed.

One could solve any problem involving two solutes and without discontinuities if one knew a solution of these equations under sufficiently general conditions. However, no such solution is known. The classical

solution * assumes that c_1 and c_2 are regular functions of x and v and this hypothesis is difficult to apply, since the initial conditions as usually stated in nearly *every* problem in chromatography require c_1 and c_2 to be discontinuous for $x=0$ and $v=0$.

There is, however, an important class of problems where it is possible to obtain solutions of these equations. It can be shown that under certain very special initial conditions the solutions of these equations will be approximately functions of (v/x). The initial conditions for this are that when $x=0$, c_1 and c_2 are both constant for all v and that when $v=0$ the distributions q_1 and q_2 of solute in the tube are constant for all x.

Now it happens that usually under the experimental conditions in chromatography these conditions are in fact often satisfied to a first approximation. For instance, they are fulfilled when an (infinite) band containing adsorbed material from a solution of two solutes of constant concentrations is developed with pure solvent or with a solution of a third substance. In practice where the columns are of finite length it is reasonable to expect that the subsequent development would be the same since it is determined by the state of affairs at the *top* of the chromatogram. It would therefore seem that a solution of eqn. (5) under such initial conditions would furnish all that a practical chromatographer would desire. However, another difficulty now arises. It appears that under such initial conditions eqn. (5) need not have a unique solution and so *no* mathematical argument can be fully conclusive and any results obtained will have to be tested by experiment.

Let us proceed with the discussion of eqn. (5) on these lines. Substituting $y=(v/x)$, and assuming that c_1 and c_2 depend on y as defined above, one gets

$$\left(\frac{\partial q_1}{\partial c_1} - y\right)\frac{dc_1}{dy} + \frac{\partial q_1}{\partial c_2}\frac{dc_2}{dy} = 0, \quad . \quad . \quad (6.1)$$

$$\frac{\partial q_2}{\partial c_1}\frac{dc_1}{dy} + \left(\frac{\partial q_2}{\partial c_2} - y\right)\frac{dc_2}{dy} = 0. \quad . \quad . \quad (6.2)$$

In general, the solution of these equations will be

$$\frac{dc_1}{dy} = \frac{dc_2}{dy} = 0.$$

The necessary and sufficient conditions for them to have a solution other than this one is

$$\begin{vmatrix} \left(\frac{\partial q_1}{\partial c_1} - y\right) & \frac{\partial q_1}{\partial c_2} \\ \frac{\partial q_2}{\partial c_1} & \left(\frac{\partial q_2}{\partial c_2} - y\right) \end{vmatrix} = 0. \quad . \quad . \quad (7.1)$$

This corresponds to the quadratic equation

$$y^2 - y\left(\frac{\partial q_1}{\partial c_1} + \frac{\partial q_2}{\partial c_2}\right) + \frac{\partial q_1}{\partial c_1}\frac{\partial q_2}{\partial c_2} - \frac{\partial q_1}{\partial c_2}\frac{\partial q_2}{\partial c_1} = 0, \quad . \quad (7.2)$$

which has the roots

$$\alpha\ (c_1, c_2) = \tfrac{1}{2}\left[\left(\frac{\partial q_1}{\partial c_1} + \frac{\partial q_2}{\partial c2}\right) + \left\{\left(\frac{\partial q_1}{\partial c_1} - \frac{\partial q_2}{\partial c_2}\right)^2 + 4\frac{\partial q_1}{\partial c_2}\frac{\partial q_2}{\partial c_1}\right\}^{\frac{1}{2}}\right], \quad (8.1)$$

$$\beta\ (c_1, c_2) = \tfrac{1}{2}\left[\left(\frac{\partial q_1}{\partial c_1} + \frac{\partial q_2}{\partial c_2}\right) - \left\{\left(\frac{\partial q_1}{\partial c_1} - \frac{\partial q_2}{\partial c_2}\right)^2 + 4\frac{\partial q_1}{\partial c_2}\frac{\partial q_2}{\partial c_1}\right\}^{\frac{1}{2}}\right], \quad (8.2)$$

* See, for example, Goursat, *Leçons sur l'intégration des équations aux derivés partielles du premier ordre* (1891), p. 2.

and thus eqn. (7) may be written

$$(y-\alpha)\ (y-\beta) = 0 . \quad . \quad . \quad . \quad (9)$$

Whenever (7) is satisfied (6) gives

$$\frac{dc_2/dy}{dc_1/dy} = \frac{(y-\partial q_1/\partial c_1)}{\partial q_1/\partial c_2},$$

that is,

$$\frac{dc_2}{dc_1} = \frac{(\alpha-\partial q_1/\partial c_1)}{(\partial q_1/\partial c_2)} . \quad . \quad . \quad . \quad (10.1)$$

or

$$\frac{dc_2}{dc_1} = \frac{(\beta-\partial q_1/\partial c_1)}{(\partial q_1/\partial c_2)} . \quad . \quad . \quad . \quad (10.2)$$

according to which solution of (7) we take. In either case,

$$\alpha = dq_1/dc_1 \text{ and } \beta = dq_1/dc_1 \quad . \quad . \quad (11)$$

and similarly

$$\alpha = dq_2/dc_2,$$

so that in a *variable* band we have

$$dq_1/dc_1 = dq_2/dc_2 . \quad . \quad . \quad . \quad (12)$$

II Examples

(i) Chromatogram formed from two solutes and developed with pure solvent.—As we have already remarked our special conditions are satisfied in this case and we may apply the general theory developed above.

Let us first consider the problem that arises when a solution of two solutes is poured on to a column of adsorbent material, which is initially free of solutes. The first question to be decided is : *can a variable band be found in these circumstances?* If this does occur then eqn. (10.1) and (10.2) hold throughout the band. Moreover it is clear that dc_2/dc_1 will be positive since c_1 and c_2 will either increase together or decrease together. Further, it is to be expected that the presence of one solute will hinder the adsorption of the others so that $\partial q_1/\partial c_2$ and $\partial q_2/\partial c_1$ will be negative and their product $(\partial q_1/\partial c_2)\ (\partial q_2/\partial c_1)$ positive. Hence from the eqn. (8) one obtains for the discriminant :

$$\left\{\left(\frac{\partial q_1}{\partial c_1}-\frac{\partial q_2}{\partial c_2}\right)^2 + 4\ \frac{\partial q_1}{\partial c_2}\ \frac{\partial q_2}{\partial c_1}\right\}^{\frac{1}{2}} > \left|\frac{\partial q_2}{\partial c_1}-\frac{\partial q_2}{\partial c_2}\right| \quad . \quad (13)$$

and so it follows from eqn. (8) that $(\alpha - \partial q_1/\partial c_1)$ is *positive* and $(\beta - \partial q_1/\partial c_1)$ is *negative.* Consequently, of the two eqn. (10), (10.1) gives a negative value for dc_2/dc_1 and (10.2) a positive value. Therefore, throughout this band we must have

$$\frac{dc_2}{dc_1} = \frac{(\beta-\partial q_1/\partial c_1)}{\partial q_1/\partial c_2} , \quad . \quad . \quad . \quad (14.1)$$

or

$$\beta = \frac{\partial q_1}{\partial c_1} + \frac{\partial q_1}{\partial c_2}\frac{dc_2}{dc_1} = \frac{dq_1}{dc_1} . \quad . \quad . \quad . \quad (14.2)$$

But then (9) implies that

$$y = v/x = \beta , \quad . \quad . \quad . \quad . \quad (15.1)$$

or

$$v/x = dq_1/dc_1 . \quad . \quad . \quad . \quad . \quad (15.2)$$

If now the level x is fixed and the volume v increased, c_1 and c_2 will increase. But with normal isotherms dq_1/dc_1 is a *decreasing* function of c_1 (i.e., with all ordinary adsorption isotherms as c_1 increases dq_1/dc_1 decreases) and so

consequently the above equation can never be satisfied. Hence in this case there can be *no variable band.*

(ii) Development of a column consisting of a uniform mixture of two solutes (1) and (2) with pure solvent.—We shall assume that when development occurs it will be a *continuous* phenomenon and so throughout the developed bands of the two solutes eqn. (7) holds. Let us now suppose that

$$(\partial q_1/\partial c_1)_{c_1=c_2=0} > \left(\frac{\partial q_2}{\partial c_2}\right)_{c_1=c_2=0}. \quad . \quad . \quad (16)$$

This corresponds to treating the solute (1) as the more strongly adsorbed one. Then from eqn. (4) and (8) one has

$$\alpha(c_1, 0) = (\partial q_1/\partial c_1)_{c_2=0} \text{ and } \beta(c_1, 0) = (\partial q_2/\partial c_2)_{c_2=0}$$

and so (7) reduces to

$$\left\{y-(\partial q_1/\partial c_1)_{c_2=0}\right\}\left\{y-\left(\frac{\partial q_2}{\partial c_2}\right)_{c_2=0}\right\} = 0. \quad . \quad (17)$$

But (16) implies that for small c_1,

$$(\partial q_1/\partial c_1)_{c_2=0} > (\partial q_2/\partial c_2)_{c_2=0}, \quad . \quad . \quad . \quad (18)$$

and so since $y=(v/x)$ is a decreasing function of x (i.e., large for x small) we must have

$$(v/x) = (\partial q_1/\partial c_1)_{c_2=0} . \quad . \quad . \quad . \quad (19)$$

On the other hand when $c_2 > 0$, clearly dc_2/dc_1 is positive and so as above,

$$\frac{dc_2}{dc_1} = \frac{(\beta-\partial q_1/\partial c_1)}{\partial q_1/\partial c_2}, \quad . \quad . \quad . \quad (20)$$

and this implies that

$$v/x = \beta, \quad . \quad . \quad . \quad . \quad (21)$$

or, in other words, that for $c_2 > 0$ it is the second factor in (7) which vanishes, while for $c_2=0$ it is the first factor. But by our hypothesis the development is a *continuous* phenomenon and this means that eqn. (7) must hold for *all* c_2. If now we imagine c_2 tends to zero in (7), then since for $c_2 > 0$ the second factor is zero and for $c_2=0$ the first factor is zero, it follows that at the point where c_2 *just* vanishes, both factors must be zero and so at this level,

$$(\partial q_1/\partial c_1)_{c_2=0} = (\partial q_2/\partial c_2)_{c_2=0} . \quad . \quad . \quad (22)$$

Eqn. (22) determines the concentration c_1' of the solute (1) at the level at which c_2 in zero and the level x_1' at which this occurs is given by

$$v/x_1' = (\partial q_1/\partial c_1)_{c_1=c_1', c_2=0} = (\partial q_2/\partial c_2)_{c_1=c_1', c_2=0}. \quad . \quad (23)$$

Let us examine eqn. (22). The two functions $z_1=(\partial q_1/\partial c_1)_{c_2=0}$ and $z_2=(\partial q_2/\partial c_2)_{c_2=0}$ are both usually decreasing functions of the same variable c_1.

The first function z_1 denotes the rate of change of the amount adsorbed of solute (1) with concentration of this solute in the absence of solute (2). This quantity, for all ordinary isotherms, decreases as the concentration c_1 increases. The second function z_2 represents likewise the rate of change of adsorbed amount of solute (2) with concentration c_2, for very low values of c_2 and in a solution which also contains the solute (1) at a certain concentration c_1. Thus the curves for z_1 and z_2 are essentially different in character: z_2 will normally decrease as c_1 increases but the decrease may be expected to be small compared with the corresponding decrease in z_1. This is the case if the "strength" at which solute (2) is adsorbed at very

low concentrations is not greatly affected by the presence of solute (1) in the solution. If z_1 and z_2 decrease, but z_1 decreases more rapidly than z_2, there will be a point at which $z_1=z_2$ (since initially z_1 is the larger). That is a c_1' for which eqn. (22) is satisfied.

Hence, in this case, when the column consisting of solutes (1) and (2) corresponding to constant concentrations c_1^0 and c_2^0 (Fig. 2 (*a*)) is developed with a volume v of solvent at *the top* of the tube there will be a *clear band* (from o to x_1, Fig. 2 (*b*)).

If

$$(\partial q_1/\partial c_1)_{c_1=c_2=0} > (\partial q_2/\partial c_2)_{c_1=c_2=0},$$

then at a level x_1' given by

$$v/x_1' = (\partial q_1/\partial c_1)_{c_1=c_2=0}, \quad . \quad . \quad . \quad (24)$$

a band of solute (1) will occur the concentration at any level x (in the region from x_1 to x_1'; Fig. 2 (*b*)) being given by

$$v/x = (\partial q_1 \partial/c_1)_{c_2=0}, \quad . \quad . \quad . \quad . \quad (25)$$

the concentration c_1 of solute (1) within this band increases until it reaches a critical value c_1' given by eqn. (22).

After this, there follows a mixed band (x_1' to x_2) where the concentrations c_1 and c_2 are connected by the differential eqn. (12) subject to the conditions that when $c_1=c_1'$, $c_2=0$. This development continues to a certain level where a sharp change in the concentrations occurs and we arrive at the undeveloped point of the band with the concentrations at their original values of c_1^0 and c_2^0 (Fig. 2(*b*)).

Various other solutions may be possible in this case, depending on the characters of the isotherms, but the above solution appears to be of particular interest.

There is one further point of some importance. The chromatogram we have described is that obtained when an infinite band is developed. In practice the band will be of finite length of mixed solutes (1) and (2) followed usually by a band of the less strongly adsorbed solute (2). On developing with (pure) solvent the nature of the development at the top of the tube is clearly independent of the length of the band. The solution we have given then enables us to calculate the volume of solvent required to obtain complete separation. Writing

$$Q(c_1) = q_1(c_1, 0),$$

then the amount of solute (1) in the band between the levels x_1 and x_1' (see Fig. 2(*b*)) is

$$\int_{x_1}^{x_1'} Q(c_1)\mathrm{d}x = \int_0^{c_1'} Q(c_1)\frac{\mathrm{d}x}{\mathrm{d}c_1}\,\mathrm{d}c_1, \quad . \quad . \quad (26.1)$$

and on integration by parts this becomes

$$\left[Q(c_1)x\right]_0^{c_1'} - \int_0^{c_1'} xQ'(c_1)\mathrm{d}c_1 = x_1'Q(c_1') - \int_0^{c_1'} xQ'(c_1)\mathrm{d}x,$$

bearing in mind that $Q(0) = 0$.

Now throughout this band $\frac{v}{x} = Q'(c_1)$, where v the volume added is, of course, a constant, and so the above expression becomes

$$v\left\{\frac{Q(c_1')}{Q'(c_1')} - c_1'\right\}. \quad . \quad . \quad . \quad . \quad (26.2)$$

Now suppose that the total amount of solute (1) present was m_1. Then, the tube being of unit cross-section, the volume v_s required to obtain total separation will be

$$v_s = m_1 \left\{ \frac{Q(c_1')}{Q'(c_1')} - c_1' \right\}^{-1}, \quad . \quad . \quad (27)$$

where c_1' is given by (22).

(iii) An infinite column of uniformly distributed solute is developed with a solution of a more strongly adsorbed substance.—Our simple equations are valid in this case. We shall suppose, as usual, that solute (1) is the more strongly adsorbed. In any mixed band dc_2/dc_1 is negative and this implies that:

$$\frac{v}{x} = \alpha(c_1, c_2) = \tfrac{1}{2}\left[\left(\frac{\partial q_1}{\partial c_1}+\frac{\partial q_2}{\partial c_2}\right)+\left\{\left(\frac{\partial q_1}{\partial c_1}-\frac{\partial q_2}{\partial c_2}\right)^2 + 4\,\frac{\partial q_1}{\partial c_2}\frac{\partial q_2}{\partial c_1}\right\}^{\frac{1}{2}}\right]. \quad (28)$$

If $\left(\frac{\partial q_1}{\partial c_2}\right)\left(\frac{\partial q_2}{\partial c_1}\right)$ is small relative to the remaining terms, this implies that

$$\frac{v}{x} = \frac{\partial q_1}{\partial c_1} + \frac{4\,(\partial q_1/\partial c_2)(\partial q_2/\partial c_1)}{|\partial q_1/\partial c_1 - \partial q_2/\partial c_2|}. \quad . \quad . \quad (29)$$

Suppose a volume v of a solution of solute (1) is added, then as we go down the tube $(\partial q_1/\partial c_1)$ increases as c_1 decreases. Also for $c_2=0$, $\partial q_1/\partial c_1$ is zero but not zero for $c_2 > 0$. Hence the second term also increases (the product $(\partial q_1/\partial c_2)(\partial q_2/\partial c_1)$ being positive). Therefore both terms on the right-hand side increase which is impossible since with increasing x the left-hand side decreases. Hence we are led to a contradiction and must conclude that in this case there can be *no variable* band. Therefore the only solution possible is a *discontinuous* one. To satisfy eqn. (3) there must be *two discontinuities* separated by a mixed band. If c_1' and c_2' denote here the concentrations of the solutes in the mixed band then,

$$\frac{q_1(c_1^0, 0) - q_1(c_1', c_2')}{(c_1^0 - c_1')} = \frac{q_2(c_1', c_2')}{c_2'}, \quad . \quad . \quad (30.1)$$

and

$$\frac{q_1(c_1', c_2')}{c_1'} = \frac{q_2(0, c_2^\circ) - q_2(c_1', c_2')}{(c_2^\circ - c_2')} \quad . \quad . \quad (30.2)$$

are the two simultaneous equations for c_1' and c_2'. If x_1 denotes the level of the top of the mixed band and x_2 the level of the bottom (Fig. 3) after a volume v has been added, then by eqn. (3), since $x=0$ when $v=0$,

$$\frac{v}{x_1} = \frac{q_2(c_1', c_2')}{c_2'} \quad . \quad . \quad . \quad . \quad (31.1)$$

$$\frac{v}{x_2} = \frac{q_1(c_1', c_2')}{c_1'} \quad . \quad . \quad . \quad . \quad (31.2)$$

(iv) Band consisting of a uniform mixture of two substances (2) and (3) which is developed with a solution of a third substance (1).—In this case we have, in general, a cubic equation to solve and so a detailed analysis would be somewhat complicated. We shall consider only the case when the substance (1) is more strongly adsorbed than the other two substances and even then we shall only instance two cases which might arise.

(*a*) If the adsorption isotherms of the three substances are represented by q_1, q_2 and q_3, and if

$$\frac{q_1(c_1^0, 0, 0)}{c_1^0} > \left(\frac{\partial q_2}{\partial c_2}\right)_{c_1=c_2=c_3=0} > \left(\frac{\partial q_3}{\partial c_3}\right)_{c_1=c_2=c_3=0} \quad (32)$$

then there is no interaction between (1) and the mixture of the components (2) and (3). A band of solute (1) is formed at the top and the mixture (2) and (3) is developed as in Example (ii).

(*b*) If

$$\left(\frac{\partial q_2}{\partial c_2}\right)_{c_1=c_2=c_3=0} > \frac{q_1(c_1^0, 0, 0)}{c_1^0} > \left(\frac{\partial q_3}{\partial c_3}\right)_{c_1=c_2=c_3=0} \quad (33)$$

then at the top of the tube we shall have a chromatogram corresponding to Example (iii). Altogether there may be *five* bands. In the *top band* the substance (1) at concentration c_1^0. In the *second band* solutes (1) and (2) corresponding to concentrations c_1' and c_2'. In the *third band*, (2) at concentration c_2''. The *fourth band* may be a mixed band as in Example (i) and in the *fifth band*, (2) and (3) are present at their original concentrations c_2^0 and c_3^0 (see Fig. 4).

The values of c_1', c_2' and c_2'' are given by the following equations:

$$\frac{q_1(c_1^0, 0, 0) - q_1(c_1', c_2', 0)}{(c_1^0 - c_1')} = \frac{q_2(c_1', c_2', 0)}{c_2'}, \quad (34.1)$$

$$\frac{q_1(c_1', c_2', 0)}{c_1'} = \frac{q_2(0, c_2'', 0) - q_2(c_1', c_2', 0)}{c_2'}, \quad (34.2)$$

$$\frac{q_2(0, c_2^0, c_3^0)}{(c_2^0 - c_2'')} = \frac{q_3(0, c_2^0, c_3^0)}{c_3^0}. \quad (34.3)$$

The levels x_1 and x_2 corresponding to the top and bottom of the first mixed band are then given by

$$\frac{v}{x_1} = \frac{q_2(c_1', c_2', 0)}{c_2'}. \quad (35.1)$$

$$\frac{v}{x_2} = \frac{q_1(c_1', c_2', 0)}{c_1'}. \quad (35.2)$$

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THEORY OF FRONTAL ANALYSIS AND DISPLACEMENT DEVELOPMENT

BY STIG CLAESSON

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A simple theoretical model, which is useful for practical applications of chromatography, is discussed for frontal analysis and displacement development.

It can be said that the theory for chromatographic separation has been developed along two different lines. One group of workers has tried to use as simple theoretical models as possible in order to get simple but still useful results for the actual calculations when separating complex mixtures. Another

group of workers has tried to work out a very detailed theory for the very complex phenomena which take place in a column. Due to mathematical difficulties this type of work has mostly been carried out for systems with one or at most two solutes. Such results are usually of little use in practical applications of chromatography, but they are of utmost importance when one is trying to get a deeper understanding of the chromatographic process in general or to reach the limit of resolving power as in isotope separation.

In this paper the " simple " theory which is useful for practical applications of chromatography will be discussed for two rather important special cases, namely, frontal analysis and displacement development.

Frontal analysis, introduced by Tiselius [1] in 1940, is the simplest possible arrangement for chromatography. The solution containing one or several solutes is forced through the column with adsorbent, previously washed with pure solvent. The concentration of the effluent is determined and plotted as a function of the volume which has passed the filter. In this way characteristic curves are obtained showing one step for every solute. In Fig. 1 is seen a schematic curve for two solutes. The first step contains

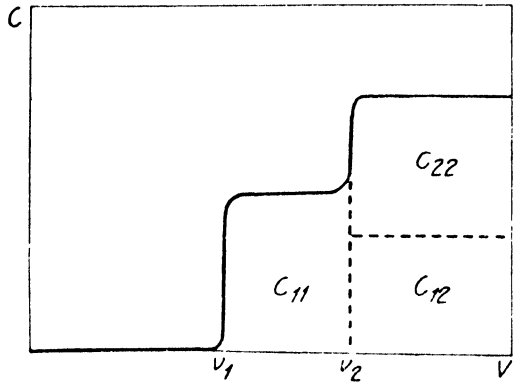

FIG. 1.

solute 1, the second step solute 1 and 2 and so on. The volume which has passed when a particular solute i breaks through is called the retention volume for component i. This volume includes the small volume necessary to replace the solvent in the column at the beginning of the experiment. After subtracting this small volume we get the corrected retention volume v_i. The corrected retention volume per gram adsorbent is called specific retention volume v_i^0.

Frontal analysis has the great advantage that the adsorbed substance does not have to be eluted again and this method can therefore be used even in the case of irreversible adsorption. That is of great value as it is often difficult to find adsorbents with sufficient selectivity which will give off the adsorbed substance quantitatively. The disadvantage is, however, that the heights of the steps are not proportional to the concentrations of the corresponding solutes in the original solution. The reason for that is adsorption displacement which means that when solute 2 is passing down the column and adsorbed, it will knock off some already adsorbed molecules of solute 1 from the adsorbent and consequently the concentration c_{11} of solute 1 in step 1 is higher than in the original solution. We introduce the general notation $c_{i,j}$ for the concentration of solute i in step j.

[1] Tiselius, *Arkiv. Kemi, Min. Geol. B*, 1940, **14**, No. 22.

For quantitative work it is therefore necessary to calculate the composition of the solution from the observed retention volumes and the heights of the steps. In order to get simple results of practical value for systems with many solutes it is necessary to make simplified assumptions. We assume instantaneous equilibrium and perfectly plane fronts. The first treatment along these lines was given by de Vault [2] and further discussed by the present author.[3 4]

In the following we will restrict the discussion to systems with only two solutes. Extension of the theory to an arbitrary number of solutes is quite simple [2-4] but will not bring forward anything new in principle.

For a system with only one solute of concentration c_{11} the amount adsorbed $a_1^\circ = f_1(c_{11})$ per gram adsorbent in the column is evidently $v_1^\circ c_{11}$ and we have

$$v_1^\circ = \frac{f_1(c_{11})}{c_{11}} \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

For two solutes we get in the same way:

$$a_1^\circ = f_1\,(c_{12},\, c_{22}) = v_2^\circ\, c_{12} - (v_2^\circ - v_1^\circ)\, c_{11}, \quad . \quad . \quad (2)$$

$$a_2^\circ = f_2\,(c_{12},\, c_{22}) = v_2^\circ c_{22}. \quad . \quad . \quad . \quad (3)$$

If we now introduce a suitable equation for the adsorption isotherm these expressions can be simplified considerably. The simplest results are obtained from Langmuir's isotherm

$$a_1^\circ = k_1 c_{12}/(1 + l_1 c_{12} + l_2 c_{22}), \quad . \quad . \quad . \quad (4)$$

$$a_2^\circ = k_2 c_{22}/(1 + l_1 c_{12} + l_2 c_{22}). \quad . \quad . \quad . \quad (5)$$

By dividing eqn. (2) and (3) we get

$$c_{12} = c_{11} \frac{\left(1 - \frac{v_1}{v_2}\right)}{\left(1 - \frac{k_1}{k_2}\right)} \quad . \quad . \quad . \quad . \quad . \quad (6)$$

We have thus obtained a very simple formula for the calculation of the correct concentration c_{12} from the observed value c_{11}. It should be noted that k_i is the retention volume for solute i at infinite dilution (from eqn. (1)).

In case of several solutes eqn. (6) will take the form

$$c_{i(m+1)} = c_{im} \frac{1 - \frac{k_i}{k_m} \cdot \frac{v_m}{v_{m+1}}}{1 - \frac{k_i}{k_{m+1}}} \quad . \quad . \quad . \quad . \quad (7)$$

It is consequently possible to calculate all concentrations in this way provided that we know the k values, which have to be determined from experiments with pure components or by other suitable methods.

It must be borne in mind, however, that this displacement effect is not primarily dependent on the actual displacement process in the column. Changes of concentration of this type will occur in all systems where a separation is caused by differences in mobility between the different solutes and where these mobilities depend on the concentrations. These "displacement" effects which are a consequence of the conservation of the mass of the solute are also well known both in electrophoresis [5] and in

[2] De Vault, *J. Amer. Chem. Soc.*, 1943, **65**, 532.
[3] Claesson, *Arkiv. Kemi, Min. Geol. A*, 1946, **23**, No. 1.
[4] Claesson, *Arkiv. Kemi, Min. Geol. A*, 1946, **24**, No. 7.
[5] Dole, *J Amer Chem. Soc.*, 1946, **42**, 769.

ultracentrifugation where their importance has been particularly pointed out by Ogston and Johnston.[6]

To make this clear we may consider a cell without adsorbent where two substances with the mobilities s (cm./sec.) and concentrations c indicated in Fig. 2 have been partly separated. If we consider two plane surfaces P_1 and P_2 in the position indicated in the figure the amount of solute 1 entering per second through P_1 is $s_{12}c_{12}$ and this amount is used up in moving the lower boundary the distance s_{11} and the upper boundary s_{22}. We consequently get

$$s_{12}c_{12} = s_{11}c_{11} + s_{22}(c_{12} - c_{11}), \quad . \quad . \quad . \quad (8)$$

or

$$c_{12} = c_{11} \frac{\left(1 - \frac{s_{11}}{s_{22}}\right)}{\left(1 - \frac{s_{12}}{s_{22}}\right)} . \quad . \quad . \quad . \quad (9)$$

It is therefore evident that as soon as the mobility is dependent on the concentration ($s_{11} \neq s_{12}$) there will be a sudden change in the concentration at the boundary. The condensed formula (9) which is valid for all types of experiments will again take the form (6) if we include the substance taken up by the adsorbent and remember that the mobilities are inversely proportional to the retention volumes.

In fact, as eqn. (9) only contains ratios of mobilities it will be sufficient to assume that ratios of mobilities are independent of the total concentration in order to get simple results. That is true both for electrophoresis and ultracentrifugation.

It can be demonstrated [4] in the same way that eqn. (7) will be unchanged even if more complicated isotherms than Langmuir's are used as long as they have the same property as that isotherm, namely, that the ratio of the amounts adsorbed from a mixture is independent of the total concentration. That means that the isotherms for the different solutes can be of the form

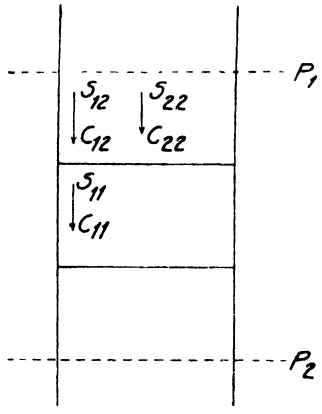

FIG. 2.

$$a_i = k_i c_i r\,(c_1 c_2 \ldots c_n), \quad . \quad . \quad . \quad (10)$$

where r is a quite arbitrary function as long as it is the same for all the solutes.

When the theory for frontal analysis has been developed the corresponding simple theory for displacement development is almost self-evident. In displacement development introduced by Tiselius [7] in 1943 the substances to be separated are adsorbed at the top of the column. A solution of a substance with stronger adsorption than that of the unknown mixture is then forced through the column. This " developer " (d) will then displace the different substances (1, 2) which also will displace each other. A diagram where the concentration of the effluent is plotted against the volume will therefore look as in Fig. 3, and the different components (1 and 2) will appear pure in the effluent. When a stationary state has been reached it is clear that all the boundaries will move down the column with the same rate as the front of the developer. As all the zones only contain one solute we

[6] Ogston and Johnston, *Trans. Faraday Soc.*, 1946, **42**, 769.
[7] Tiselius, *Arkiv. Kemi, Min. Geol A*, 1943, **16**, No. 18.

can apply eqn. (I) and as the specific retention volume then can be regarded as a measure of the rate we get

$$\frac{f_1(c_1)}{c_1} = \frac{f_2(c_2)}{c_2} = \ldots = \frac{f_d(c_d)}{c_d}, \quad . \quad . \quad . \quad . \quad (II)$$

where $f_d(c_d)$ is the adsorption isotherm for the developer. Consequently if the concentration of the developer is kept fixed in all experiments a particular solute i always has to be of the same concentration c_i in order to fulfil eqn. (II). The height of a step is therefore independent of the amount of substance present and is a constant which can be used for identification of a substance. As the area of a step is proportional to the

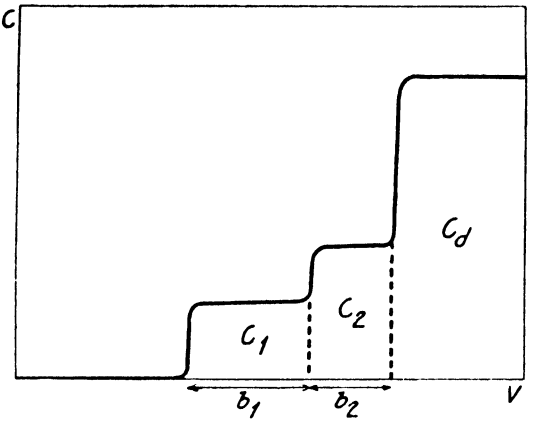

FIG. 3.

amount of substance present and its height is constant it is obvious that the length of a step (b) is proportional to the amount of substance. We therefore get a qualitative analysis by measuring the heights of the steps and a quantitative by measuring their lengths.

Displacement development is therefore a very elegant way of carrying out chromatographic separations as it can be used for preparative, qualitative and quantitative purposes at the same time. It has, however, the drawback, common to all methods using reversible adsorption, that it may be difficult to find a developer which will displace the unknown mixture quantitatively.

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THE CONSERVATION EQUATION OF CHROMATOGRAPHY

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The conservation equation of chromatography thus far used does not account for the volume occupied by the adsorbed phase. When the equation of conservation of matter is applied to the process of adsorptive percolation and this volume is accounted for, a general equation is obtained which may be more correct. This is shown by applying this equation to the equilibrium theory. Several effects occurring in chromatography which cannot be explained by the de Vault equation may now be interpreted, at least qualitatively. Moreover, the equation obtained reveals the possibility of a new method of adsorptive percolation.

In the development of the mathematical description of the process of adsorptive percolation two different methods of approach may be distinguished. One may start with the assumption of instantaneous equilibrium between solution and adsorbent (equilibrium theory) or one may dismiss the idea of complete equilibrium and treat the problem as a kinetic one (kinetic theory). However, in both cases the law of conservation of matter supplies the initial equation, which in both cases must be fundamentally the same. The equations obtained thus far give a ready explanation of the phenomena occurring during development. But when applying these equations in explanation of other processes of adsorptive percolation like the continuous introduction method (frontal analysis) or the elution method (displacement method), difficulties are encountered.

Moreover, the occurrence of so-called adsorption azeotropes,[1] especially with closely related compounds, needs further interpretation. Some experience gained with the adsorptive percolation of hydrocarbons suggested that the failures mentioned might be caused by the use of an approximate conservation equation.

Symbols

The following symbols are used in this paper :—

a, interstitial volume of an adsorption column per unit of length (ml./cm.).
b, index referring to a sharp boundary.
C, concentration of solute expressed as units of weight of solute per unit of volume of solution (g./ml.).
C_A, C_B, concentrations of substance A and substance B, respectively.
d, density (g./ml.).
$f_n(C_N)$, adsorption isotherm of component N. It also represents the weight of solute adsorbed per unit of weight of adsorbent at the concentration C_N (g./g.).
$f'_n(C_N)$, first derivative of the adsorption isotherm ($df_n(C_N)/dC_N$).
m, weight of adsorbent contained in the column per unit of length (g./cm.).
q, weight of solute adsorbed per unit of length of a column (g./cm.).
$f(q)$, volume occupied by the adsorbed *phase* (solute + solvent) per unit of length if a quantity q of solute is adsorbed (ml./cm.).
S, solvent.
V, volume of liquid forced into the column (ml.).
v, interstitial volume of dry adsorbent present in the column per unit of weight (ml./g.).
x, distance from the top of the column (cm.).
0, 1 (when combined with C) indices indicating zero and unit concentration respectively.
1, 2, 3 (when combined with f) indices indicating that the functions f_1, f_2 and f_3 are different functions.

1. The Conservation Equations used thus far.—The first conservation equation was given by Wilson [2] :

$$(\partial C/\partial x)_V + (\partial q/\partial V)_x = 0 , \quad . \quad . \quad . \quad (1)$$

where C is the concentration of the solute, x the distance from the top of the column, q the weight of solute adsorbed per unit of length and V the volume of liquid poured into the adsorption column. This equation has been corrected by de Vault [3] for the interstitial volume a per unit of length of the adsorption column.

De Vault arrives at the equation :

$$(\partial C/\partial x)_V + a(\partial C/\partial V)_x + (\partial q/\partial V)_x = 0 . \quad . \quad . \quad (2)$$

[1] Hirschler and Amon, *Ind Eng. Chem.*, 1947, **39**, 1565.
[2] Wilson, *J. Amer. Chem. Soc.*, 1940, **62**, 1583.
[3] De Vault, *J. Amer. Chem. Soc.*, 1943, **65**, 532.

The same equation has been used implicitly or explicitly by other authors [4] whether they adopted the equilibrium or the kinetic standpoint, for the equilibrium or the kinetic conditions are introduced subsequently when the relation between C and q is inserted. Eqn. (2) is based on the assumption that the interstitial volume a is constant. This is the point where introduction of a correction might be useful. The adsorbed phase occupies a certain fraction of the original interstitial volume thus causing the liquid to flow at a greater linear rate than when the adsorbed phase is not present.

As the quantity of material adsorbed is a function of the concentration the *free* interstitial volume, that is, the volume available to the *flowing* liquid, must be a function of the concentration.

2. Derivation of the Corrected Conservation Equation.—To introduce the correction for the volume of the adsorbed phase the conservation equation will be derived once more for a solution containing a single solute. The following assumptions, which do not differ from those usually applied, have been made. Within an adsorption band the concentration is a continuous function of x. Within a cross-section of infinitesimal length dx, $(\partial C/\partial x)_V$ is considered constant. The same applies to $(\partial q/\partial V)_x$ when an infinitesimal volume of liquid dV is introduced into the column.

When an infinitesimal volume dV is introduced into the column an equal volume has to pass through a cross-section at x, where x is a point within the adsorption band. As the concentration of the solution entering the cross-section at x and the concentration of the solution leaving the cross-section at $(x + dx)$ differ by

$$(\partial C/\partial x)_V \, dx,$$

the amount of solute present in this section of the column is increased by

$$(\partial C/\partial x)_V \, dx.dV.$$

This amount is distributed between the liquid phase and the adsorbed phase present in the section. The volume of the liquid phase present in the section is equal to

$$(a - f(q))dx,$$

where $f(q)$ means the volume of the adsorbed phase per unit of length within the section considered. Thus the amount of solute present in the liquid phase is equal to

$$(a - f(q))C dx.$$

The amount of solute present in the adsorbed phase is equal to $q dx$, where $q_n = mf_n(C_N)$. Thus the total amount of solute present in the section is equal to

$$\{(a - f(q))C + q\}dx.$$

If an infinitesimal volume dV is forced through this section, the increase of solute may therefore also be represented by

$$\frac{\partial}{\partial V} [\{a - f(q)\}C + q]_x \, dx.dV.$$

According to the law of conservation of matter the amount of solute introduced by the volume dV should be equal to the increase of the amount of solute present in the section. Thus

$$\left(\frac{\partial C}{\partial x}\right)_V dx.dV + \frac{\partial}{\partial V} [(a - f(q))C + q]_x dx.dV = 0, \qquad (3)$$

or

$$(\partial C/dx)_V + \{a - f(q)\}(\partial C/\partial V)_x - \{\partial f(q)/\partial V\}_x C + (\partial q/\partial V)_x = 0. \qquad (3)$$

[4] Martin and Synge, *Biochem. J.*, 1941, **35**, 1941. Weiss, *J. Chem. Soc.*, 1943. **145**, 297. Thomas, *Ann. N.Y. Acad. Sci.*, 1948, **49**, 161.

This is the corrected conservation equation of chromatography in its general form for a solution containing a single solute.

As to $f(q)$, it should be borne in mind that it is not correct to put

$$f(q) = q/d,$$

where d means the density of the adsorbed solute. For besides the solute the solvent is adsorbed too. As to this point it might be useful to stress that adsorption isotherms of pure gases and so-called isotherms of solutions containing a single solute usually have a different meaning. The adsorption isotherm of a pure gas shows the relation between the concentration (pressure) of the gas and the weight adsorbed.

However, the so-called adsorption isotherm of a solute shows the relation between the concentration and the weight of solute adsorbed *preferentially*. This relation might be better called a preferential adsorption isotherm. To avoid confusion it may be stated here that in this paper the adsorption isotherm of a solute means the relation between the *total* amount of solute adsorbed per unit of weight of adsorbent and the concentration of the solution with which it is in equilibrium.

3. Application of Eqn. (3) to the Equilibrium Theory.—Attempts will now be made to derive a fundamental equation of chromatography on the basis of the equilibrium theory for a liquid mixture of two substances A and B. Which of the two is considered as solvent does not matter. For the sake of convenience it is assumed that A and B have equal densities and no volume change occurs on adsorption or mixing. If the densities of A and B are represented by d and the concentration is expressed as g./ml., we may put

$$C_A + C_B = d.$$

Suppose the adsorption column considered contains m g. adsorbent per unit of length. The adsorption isotherm of A when diluted by B is represented by $f_1(C_A)$. At equilibrium,

$$q = mf_1(C_A)$$

(for non-equilibrium another relation between q and C should be introduced but this will not be discussed). If $f_2(C_B)$ represents the adsorption isotherm of B when diluted by A we may put

$$f(q) = \{f_1(C_A) + f_2(C_B)\}m/d.$$

If v is the interstitial volume of the dry adsorbent per unit of weight,

$$a = mv.$$

Substitution in eqn. (3) gives

$$(\partial C_A/\partial x)_V + m[\{v - f_1(C_A)/d - f_2(C_B)/d\}(\partial C_A/\partial V)_x - \{(\partial f_1(C_A)/\partial V)_x + (\partial f_2(C_B)/\partial V)_x\}C_A/d + (\partial f_1(C_A)/\partial V)_x] = 0.$$

On further substitution, viz.,

$$(\partial C_A/\partial x)_V = -(\partial V/\partial x)_{C_A}(\partial C_A/\partial V)_x,$$

$$(\partial f_1(C_A)/\partial V)_x = (\partial f_1(C_A)/\partial C_A)(\partial C_A/\partial V) = f_1' C_A(\partial C_A/\partial V)_x$$

and $$(\partial f_2 C_B/\partial V)_x = (\partial f_2(C_B)/\partial C_B)(\partial C_B/\partial V) = -f_2' C_B(\partial C_A/\partial V)_x,$$

the following equation is obtained:

$$-(\partial V/\partial x)_{C_A}(\partial C_A/\partial V)_x + m[v - f_1(C_A)/d - f_2(C_B)/d - \{f_1'(C_A) - f_2'(C_B)\}C_A/d + f_1'(C_A)](\partial C_A/\partial V)_x = 0,$$

or

$$-(\partial V/\partial x)_{C_A} + [vd - f_1(C_A) - f_2(C_B) + C_B f_1'(C_A) + C_A f_2'(C_B)]m/d = 0.$$

Thus

$$(\partial x/\partial V)_{C_A} = \frac{d}{m[vd - f_1(C_A) - f_2(C_B) + C_B f_1'(C_A) + C_A f_2'(C_B)]} \cdot \quad (4)$$

De Vault,[3] whose paper has been very useful to the author, assumed the interstitial volume to be constant and obtained

$$(\partial x/\partial V)_{C_A} = \frac{1}{m[v + f_1'(C_A)]} \cdot \quad . \quad . \quad . \quad (4a)$$

According to the initial assumptions, eqn. (4) holds good only within the adsorption band. In the same way, as de Vault did, it can be shown that at a boundary of an adsorption band eqn. (4) may lead to physical impossibilities, thus indicating the occurrence of a discontinuity (sharp boundary).

If V is increased at a constant rate, $(\partial x/\partial V)_{C_A}$ may be called the rate of transport of the concentration C_A. If $(\partial x/\partial V)_{C_A}$ increases with increasing concentrations, thus if

$$\partial\{(\partial x/\partial V)_{C_A}\}/\partial C_A \text{ is positive,}$$

it means that the highest concentration of A possible will move at the greatest rate. Consequently it will overtake all lower concentrations of A which might be in front of it. Thus a sharp front boundary will be finally formed and the lowest concentration possible will be trailing behind forming a diffuse boundary. If, however,

$$\partial\{(\partial x/\partial V)_{C_A}\}/\partial C_A \text{ is negative,}$$

the inverse will be obtained.

A further calculation, which will not be extended here, shows that if adsorption occurs according to the Langmuir theory of adsorption a leading front boundary of A will be formed if

$$f_1(C_A)/C_A > f_2(C_B)/C_B$$

holds good at all concentrations. This simple condition only means that the concentration of A in the liquid phase decreases if adsorbent is added to any mixture of A and B. If the adsorption isotherms of A and B are of the Freundlich type and

$$f_1(C_A)/C_A > f_2(C_B)/C_B,$$

a leading front boundary will also be formed, at least at low or high concentrations. At medium concentrations no general conclusion can be drawn. Thus it may be assumed, if

$$f_1(C_A)/C_A > f_2(C_B)/C_B,$$

a sharp front boundary of A will generally be formed.

As has already been stated, eqn. (4) only holds good within an adsorption band. When a sharp boundary occurs, whether in front or in the rear, another conservation equation has to be applied to the boundary. For either in front or at the rear of such a boundary, no trace of the substance considered should be present.

Therefore the conservation equation of a sharp boundary is

$$C\mathrm{d}V = \{(a - f(q))C + q\}\mathrm{d}x, \quad . \quad . \quad . \quad (5)$$

where C applies to the concentration in the boundary. If eqn. (5) is applied to the mixture of A and B and equilibrium is instantaneously established the following equation holds good for a sharp boundary:

$$\left(\frac{\partial x}{\partial V}\right)_{C_{A_b}} = \frac{d}{m\left\{vd + C_{B_b}\left(\frac{f_1(C_{A_b})}{C_{A_b}} - \frac{f_2(C_{B_b})}{C_{B_b}}\right)\right\}}, \quad . \quad (6)$$

where the subscript b indicates that the concentration refers to that in the sharp boundary.

For the diffuse boundary (zero concentration) the following equation is obtained:

$$\left(\frac{\partial x}{\partial V}\right)_{C_{A_0}} = \frac{1}{m\{v+f_1'(C_{A_0})-f_2(C_{B_1})/d\}}, \quad . \quad . \quad (7)$$

where the subscripts 0 and 1 indicate zero and unit concentration d respectively. Applying de Vault's theory,

$$\left(\frac{\partial x}{\partial V}\right)_{C_{A_b}} = \frac{1}{m(v+f_1(C_{A_b})/C_{A_b})} \quad . \quad . \quad . \quad (6a)$$

and

$$\left(\frac{\partial x}{\partial V}\right)_{C_{A_0}} = \frac{1}{m(v+f_1'(C_{A_0}))} \quad . \quad . \quad . \quad (7a)$$

are obtained.

4. Discussion.—Eqn. (4) represents the rate of transport of a certain concentration of A within the adsorption band. But the corresponding concentration of B should attain the same value. Thus

$$(\partial x/\partial V)_{C_A} = (\partial x/\partial V)_{C_B},$$

if $C_B = d - C_A$. Eqn. (4) meets this condition whereas eqn. (4*a*) does not, unless

$$f_1'(C_A) = f_2'(C_B),$$

which as a rule cannot be true.

As has already been shown, a sharp leading boundary of A will be formed if

$$f_1(C_A)/C_A > f_2(C_B)/C_B.$$

Consequently

$$(\partial x/\partial V)_{C_{A_b}} \leqslant \frac{1}{mvd} \text{ (eqn. (6)).}$$

This means that the rate of transport of any boundary is always smaller than the rate of transport of the liquid front, which of course is equal to $1/mvd$. Both rates become equal if $C_B = 0$, i.e., $C_A = d$. If, however,

$$f_1(C_A)/C_A < f_2(C_B)/C_B,$$

the front boundary will be diffuse and a sharp boundary will be present at the rear. In this case the rate of transport of the boundaries is always greater than the rate of transport of the liquid front, unless $C_A = d$. Thus it may be stated if

$$f_1(C_A)/C_A > f_2(C_B)/C_B,$$

substance A will always lose on the liquid front, whereas B has a tendency to overtake the liquid front until its concentration d has become equal to unity. This may serve as a suitable interpretation of the phenomena which obtain when a solution containing a single solute is introduced into the dry column (continuous introduction method). If a mixture of A and B is introduced into a dry column, A will move at a slower rate than the liquid front.

Therefore pure B will appear in front of the adsorption band and its quantity will increase if introduction is continued. Since at the top of the column the concentration of A is equal to the initial concentration and this is the highest concentration of A possible under these conditions, it will overtake all lower concentrations of A which might be in front of it. Thus within the adsorption band the concentration of A is equal to the initial concentration and the adsorption band will move at a constant rate.

Elution (displacement) may now be explained too. Suppose two substances A and B are present in an adsorption column and a new solvent S is added at the top of the column. Further

$$f_3(C_S)/C_S > f_1(C_A)/C_A > f_2(C_B)/C_B.$$

According to the above deductions, any A or B which happens to become diluted by S will move at a greater rate than the liquid front, whereas the rate of S should be smaller than (or, when undiluted, equal to) the rate of the liquid front. Therefore S will remain undiluted and act like a piston forcing A and B in front of it. The same applies to A as compared to B. Therefore the final result of elution will be three adjacent adsorption bands containing S, A and B at unit concentrations. As to the developer method, little further explanation seems to be necessary. If more than one solute is present and separation of the solutes has been obtained already, the rates of transport of the different solutes are established by eqn. (6) and (7) and thus the distance between the adsorption bands will usually increase on further development and at the same time the bands will be broadened.

The mere process of separation of a mixture of solutes is more complicated because of mutual alteration of the adsorption isotherms. Nevertheless, it may be accepted that the separation itself occurs qualitatively along the same lines. As a rule when the developer method is applied a sharp front boundary occurs. Experimentally asymmetric adsorption bands are usually found, the highest concentration being near the front.

Eqn. (6) and (7) reveal another possibility which may not as yet have been recognized. Suppose

$$f_1(C_A)/C_A < f_2(C_B)/C_B.$$

An adsorption band of A when present will move at a greater rate than the liquid front. But if the whole column has previously been wetted with B, the substance A cannot reach the liquid front before leaving the column. If a mixture of A and B is introduced into a column previously wetted with B, the lowest concentration of A will move at the greatest rate. Thus an adsorption band with a diffuse front and a sharp rear boundary will be formed immediately. This band is transported through the column if thereupon pure B is added to the column at the top. The band is broadened during this procedure but its rate is conditioned by eqn. (6) and (7). If still another solute, say, D, is present which is also adsorbed less strongly than B it will behave in the same way, but it may be that both limits of the rate of transport of the adsorption band of D are smaller than those of B. Thus this method, which is supposed to be called development with an eluent, may cause separation.

According to former conceptions this method would be impossible because B, the eluent, should not allow A or D to become adsorbed. However, a very simple experiment showed the present conception to be correct. A mixture of cetane and cetene could be partly separated by silica gel previously wetted with benzene, using benzene as developing liquid. It is well known that benzene may act as an eluent (displacer) for cetane and cetene when adsorbed on silica gel. The advantage of this method is that the total volume of " developer " necessary to collect all the solutes at the bottom of the column is smaller than the interstitial volume of the column. This means a saving of time. However, the separating efficiency of this method may be, in many cases, low.

The occurrence of rates of transport greater than the rate of the liquid front is also demonstrated in an earlier paper,[5] though not mentioned explicitly. Rates of transport up to about 1·3 times the rate of the liquid front have been found with chloroform and dodecylbenzene.

A few words may be added as to the occurrence of so-called adsorption azeotropes as described by Hirschler and Amon.[1] When an adsorption azeotrope occurs

$$f_1(C_A)/C_A - f_2(C_B)/C_B$$

[5] Smit. *Anal. chim. Acta*, 1948, **2**, 671.

changes sign at a certain concentration of, say, A. At concentrations lower than the azeotropic concentration the sign may be positive, and when the mixture is introduced into the column pure B appears in front of the adsorption band containing A. Beyond the azeotropic concentration pure A will appear in front, and B is contained within the adsorption band.

At the azeotropic concentration

$$f_1(C_A)/C_A - f_2(C_B)/C_B = 0.$$

The rate of transport of A and B both become equal to the rate of the liquid front and no separation occurs. So the conclusion derived from eqn. (6) and (7) agrees with experiment. At the same time it is clear that the occurrence of adsorption azeotropes is limited to substances having comparable adsorption affinities.

No attempt has been made to solve the differential equations, nor to develop formulæ for more than two substances. As de Vault already stated, this becomes very complicated. Moreover, as real adsorption isotherms are used in our equation, the quantitative solution is of no use as the real adsorption isotherms are not available. The main purpose of this paper has been to arrive at a rather simple formula which permits a qualitative explanation of the different methods of percolation, but on the other hand it might show that the quantitative deductions made thus far have to be handled with care.

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Koninklijke/Shell-Laboratorium,
Amsterdam.

GENERAL DISCUSSION *

Dr. B. Davison (*A.E.R.E., Harwell*) (*communicated*): I want to point out that for the case of Langmuir isotherms the method described by Dr. Glueckauf in his paper can be easily generalized to any number of solutes simultaneously present in solution. For simplicity I shall speak of three solutes only.

The basic equations are

$$\frac{df_1}{dc_1} = \frac{df_2}{dc_2} = \frac{df_3}{dc_3}, \quad . \quad . \quad . \quad . \quad . \quad (1)$$

in which

$$f_j = \frac{a_j c_j}{1 + b_1 c_1 + b_2 c_2 + b_3 c_3}. \quad . \quad . \quad . \quad . \quad (2)$$

Putting

$$p_j = b_j c_j\,; \; P = p_1 + p_2 + p_3 \quad . \quad . \quad . \quad (3)$$

and re-writing (1) in terms of p_j's and P, differentiating again using P as independent variable, and comparing with (3) we get †

either

$$p_1 p_2 p_3 \left[\frac{1}{a_1 p_1} \left(\frac{dp_1}{dP} \right)^2 + \frac{1}{a_2 p_2} \left(\frac{dp_2}{dP} \right)^2 + \frac{1}{a_3 p_3} \left(\frac{dp_3}{dP} \right)^2 \right] = 0, \quad . \quad (4)$$

* On four preceding papers.

† This derivation presupposes that none of c_j's is constant and P is not constant either. But assuming all a_j's to be different, one can easily show that P = const. is irreconcilable with eqn. (1) and can occur only in the regions where all concentrations are constant; consequently the eqn. (1) is not applicable. If one of the concentrations is constant, one can similarly show that either P is constant, or the concentration in question is zero and we have one solute less.

or $$\frac{d^2p_1}{dP^2} = \frac{d^2p_2}{dP^2} = \frac{d^2p_3}{dP^2} = 0,$$

and consequently p_1, p_2 and p_3 are linear functions of P.
Hence

$$p_j = m_j P + n_j, \quad . \quad . \quad . \quad . \quad (5)$$

in which m_j and n_j are some constants.

To satisfy (4) it is necessary that some of the concentrations are negative, which is impossible; thus (5) is the only solution of (1) having a physical significance. To determine the line (5) passing through the given initial concentrations (c_1°, c_2°, c_3°) one may proceed as follows.

Expressing n_j's in terms of m_j's and the initial concentrations and then substituting (5) into (1), gives

$$a_1 - \frac{b_1 f_1^\circ}{m_1} = a_2 - \frac{b_2 f_2^\circ}{m_2} = a_3 - \frac{b_3 f_3^\circ}{m_3}, \quad . \quad . \quad (6)$$

in which f_j° are the values of f_j for initial concentrations and, according to (3), $m_1 + m_2 + m_3 = 1$. Denoting the common value of the expressions (6) by S, expressing m_j's in terms of S and substituting into $m_1 + m_2 + m_3 = 1$ we have

$$\frac{b_1 f_1^\circ}{a_1 - S} + \frac{b_2 f_2^\circ}{a_2 - S} + \frac{b_3 f_3^\circ}{a_3 - S} = 1. \quad . \quad . \quad . \quad (7)$$

This is a cubic equation for which it can be shown that all roots are real and different. Thus there are three characteristics of type (5) passing through each initial concentration. If $a_1 < a_2 < a_3$ and the three roots of (7) are $S_1 < S_2 < S_3$ it can also be shown that

$$0 < S_1 \leqslant a_1 < S_2 \leqslant a_2 < S_3 \leqslant a_3. \quad . \quad . \quad . \quad (8)$$

If S_1, S_2 and S_3 are known, the corresponding c_1, c_2 and c_3 are given by

$$c_j = \frac{1}{b_j} \frac{a_i a_k}{(a_j - a_i)(a_j - a_k)} \frac{(a_j - S_1)(a_j - S_2)(a_j - S_3)}{S_1 S_2 S_3} \quad (i \neq j\,;\, k \neq j\,;\, i \neq k). \quad (9)$$

If we use S_j's as the unknown functions rather than the concentrations (to facilitate manipulation) de Vault's partial differential equations become

$$\frac{\partial S_j}{\partial x} + \frac{S_1 S_2 S_3}{a_1 a_2 a_3} \cdot S_j \cdot \frac{\partial S_j}{\partial v} = 0. \quad (j = 1, 2, 3) \quad . \quad . \quad (10)$$

Each characteristic (5) is obtained by varying the corresponding S_j keeping the other two constant. From (10) one can easily derive that the speed of propagation of any particular combination of concentrations in the region of variable S_j is given by

$$\left(\frac{dx}{dv}\right)_{c_1 c_2 c_3} = \frac{a_1 a_2 a_3}{S_1 S_2 S_3} \frac{1}{S_j}. \quad (S_j \text{ variable}) \quad . \quad . \quad (11)$$

From (8) and (11) we see that the regions with S_1 variable propagate at the fastest, and the regions with S_3 variable propagate at the slowest rate. It also follows from (11) that if the value of S_j at the rear of the region with variable S_j is greater than its value in the front part of this region ($S_{jR} > S_{jF}$) then the rear part propagates slower than the front part, and we have a diffuse boundary. Similarly for $S_{jR} < S_{jF}$ a self-sharpening boundary is obtained.

From formulæ (9) we can also derive

$$\frac{\partial c_i}{\partial S_j} = \frac{c_i a_i}{S_j (S_j - a_i)}, \quad . \quad . \quad . \quad . \quad (12)$$

which together with (8) predicts which concentrations increase and which decrease in each particular region. For instance, since all $\frac{\partial c_i}{\partial S_1}$ ($i = 1, 2, 3$) have the same sign either all three concentrations increase or all three decrease in the most forward region (S_1 variable)—this generalizes Glueckauf's Rule 1. Also since all $\frac{\partial c_3}{\partial S_j}$ ($j = 1, 2, 3$) have the same sign, the concentration of the most adsorbed solute always increases along the column $\left(\left(\frac{\partial c_3}{\partial x}\right)_v > 0\right)$ for a diffuse

boundary, while for a sharp boundary it decreases. This generalizes Glueckauf's Rule 2. The conclusions are illustrated on the diagrams.

Fig. 1*a* (continuous line) gives the S diagram for the case when one set of three concentrations $c_1°$, $c_2°$, $c_3°$ is developed by another set of three concentrations $\bar{c}_1$, $\bar{c}_2$, $\bar{c}_3$ of the same solutes, and these $c_j°$, $\bar{c}_j$ are such that all $S_j° > \bar{S}_j$ (all three

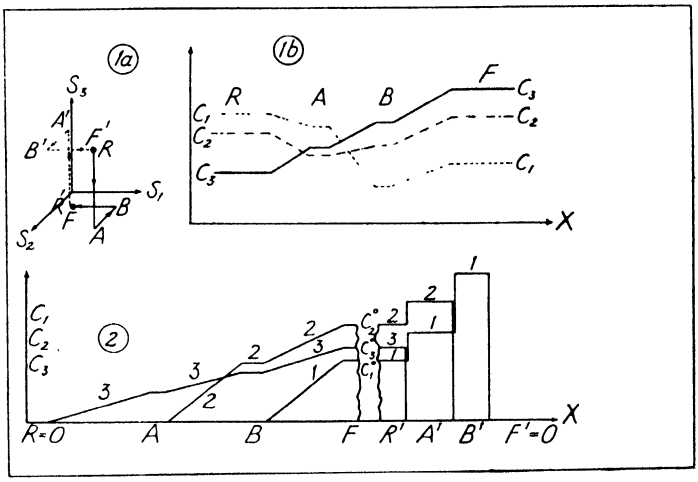

FIG. 1*a*, *b* and 2.

boundaries are diffuse) Fig 1*b* gives schematically the corresponding concentration diagram. The case of three diffuse boundaries will arise, for instance, if a set of three concentrations is developed by clear solvent. In this latter case the concentration diagram reduces to that represented in Fig. 2 (rear part). The dotted line in Fig. 1*a* gives the S diagram for the case when, on the contrary, $\bar{c}_1$, $\bar{c}_2$, $\bar{c}_3$ are developed by $c_1°$ $c_2°$ $c_3°$. The corresponding concentrations diagram for $\bar{c}_1 = \bar{c}_2 = \bar{c}_3 = 0$ is given in the front part of Fig. 2.

Dr. F. G. Angell (*Stockton-upon-Tees*) said · From the standpoint of a practical chromatographer it is worth while exploring, or if necessary experimentally determining, the adsorption isotherms of two substances on a particular adsorbent before proceeding with any chromatographic separation. According to Dr. Glueckauf's paper, conditions for sharp boundaries in adsorption chromatography have been uniquely defined and provided that equilibrium is established under experimental conditions it would appear that much time and labour could be spared and more chances of success accrue by such a procedure than by the more customary empirical method of trial and error with various adsorbents.

Dr. E. Glueckauf (*A.E.R.E., Harwell*) said : Dr. Smit mentions the need for further knowledge about chromatography in the case of adsorption azeotropes. Actually we can predict fairly well what will happen in such cases provided that the binary isotherms are at least approximately known. Let us assume, as an example, that these isotherms are of the exponential type :

$$a_1 c_1 = q_1(q_1 + q_2)^{(n_1-1)} \text{ etc.}^1$$

where at low concentrations solute 2 is the more strongly adsorbed, and where $n_2 > n_1 > 1$.

Such a system gives adsorption azeotropes whenever the total adsorption density

$$(q_1 + q_2) = \frac{a_2}{a_1} \exp\left(\frac{1}{n_2 - n_1}\right) .$$

[1] See Glueckauf, *J. Chem. Soc.*, 1947, 1302.

The q_1–q_2 characteristics (see preceding paper by Glueckauf) of co-existing concentrations have approximately the form shown in Fig. 1 (only one positive characteristic being shown). The essential difference from the normal type of characteristic is that the positive characteristic is convex against the point $q_1 = 0$, $q_2 = 0$.

Applying the rules given in the paper earlier in this Discussion we can see that nothing abnormal will happen during solvent development, if the original starting point $q_1^\circ q_2^\circ$ lies below the azeotropic line DZE. If lying above this line, the following complications will occur: at first, solute 2 will separate both in rear and front, with enrichment of solute 1 in the centre of the mixed band. Due to the spreading of the band during solvent development, the maximum concentration of the mixed band moves along the line BZA. After Z has been passed the formerly sharp front of the mixed band becomes a diffuse one; solute 1 enters the frontal band of solute 2, and eventually overtakes it. After a somewhat complex state of affairs (which can in detail be deduced from the diagram of characteristics), we arrive at the shape of the normal band distribution with a frontal band of pure solute 1. This procedure wastes a good deal of time and adsorbent in the process; which could be saved by working from the start at lower adsorption densities, i.e., with more dilute solutes. One can compare this process with the distillation of binary azeotropes, after the addition of a third inert solvent.

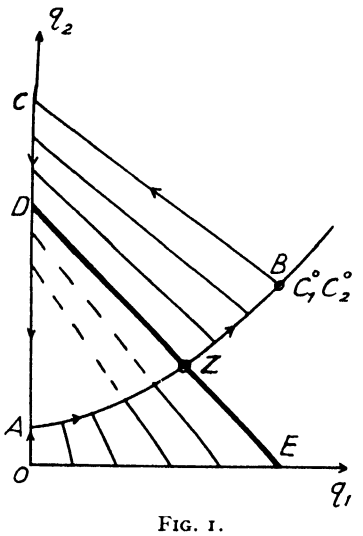

FIG. 1.

Dr. W. M. Smit (*Amsterdam*) (*communicated*): In stating that there is a need of further knowledge on the behaviour of adsorption azeotropes it has not been my intention to say that it is not yet possible to give a qualitative prediction of what will happen in an adsorption column if an adsorption azeotrope occurs.

When "more strongly adsorbed" stands for "moving at a lower rate" a qualitative prediction of the phenomena which will occur in the case of adsorption azeotropes is even possible without using any formula. By simple reasoning we may come to the same conclusions as arrived at by Dr. Glueckauf.

However, the adsorption azeotropes have been mentioned with a different purpose, viz., to demonstrate the need of correcting the conservation equation.

If a solution of azeotropic concentration is poured into an adsorption column no separation occurs and the adsorption front will move at the same rate as the liquid front. The equations for the rate of transport of the front of an adsorption band derived from the conservation equations of Wilson or of de Vault (see paper on the conservation equation) lead to conclusions which do not tally with this simple fact.

De Vault's equation only gives the right value for the rate of transport of the adsorption front if the amount adsorbed at the azeotropic concentration is zero, which cannot be true. The same obtains for the rate of transport of the adsorption front of a pure solvent containing no solutes.

Another point is that Dr. Glueckauf refers to ternary systems (a solvent with two solutes) whereas the literature quoted by me deals with binary systems (a solvent with one solute).

Finally, an interesting feature is that in the case of a binary system de Vault's conservation equation will lead to the same results as the corrected equation if $f(C_A)$ in de Vault's equation is replaced by another function $F(C_A)$ where $F(C_A)$ stands for the amount of substance A adsorbed *preferentially*. But this

implies that $F(C_A)$ cannot be represented by an adsorption isotherm of the Langmuir type, the Freundlich type or the exponential type as given by Dr Glueckauf. For $F(C_A)$ should at least meet the following condition: $F(C_A) = 0$, for $C_A = 0$ and $C_A = 1$. It may be a point for further investigation to determine how the equations for solutions containing more than one solute are affected by using the corrected conservation equation.

Dr. B. Davison (*A.E.R.E., Harwell*) said: The treatment of the problem given by Prof. A. C. Offord and Dr. J. Weiss in their paper is, in many respects, inadequate. In particular, when confronted with a situation when at the first sight there are several alternatives, they invariably choose the alternative which a more detailed analysis shows to be inacceptable. This can be most clearly seen if we re-examine the examples considered in their paper.

Let me first draw attention to formula (23). It will be noticed that the value of c_1' given by this formula depends only upon the form of q_1 and q_2 as functions of c_1 and c_2, and not upon the concentrations which were initially present.

For instance for the Langmuir isotherms

$$q_1 = \frac{a_1 c_1}{1 + b_1 c_1 + b_2 c_2} \quad ; \quad q_2 = \frac{a_2 c_2}{1 + b_1 c_1 + b_2 c_2},$$

the formula (23) leads to

$$c_1' = \frac{1}{b_1} \frac{a_1 - a_2}{a_2}.$$

It is rather difficult to visualize how this is possible, in particular if the initial concentration c_1° of the first solute is very much smaller than the value of c_1' given by (23). Indeed if $a_2 \ll a_1$ i.e., if solute 2 is little adsorbed, impossibly high values of c_1' would be postulated by eqn. (23). Eqn. (23) also leads to the interesting value (26·2) for the content of solute 1 in the pure rear band, which can exceed the total amount of solute 1 introduced into the column.

This suggests that we should re-examine the derivation of (23). The formula (23) was derived from the assumption that (7) should hold for all values of $y = v/x$. But (7) was derived as the condition for the eqn. (6) to have other solutions than $dc_1/dy = dc_2/dy = 0$. Thus all we can say is that in the vicinity of any particular value of v/x either (7) should hold, or both concentrations should be constant.

Let us see what will happen if we introduce a constant concentration band between the regions of $c_2 > 0$ and $c_2 = 0$, c_1 variable. Then at one boundary of this region

$$v/x = \left(\frac{\partial q_2}{\partial c_2}\right)_{c_2=0},$$

at the other

$$v/x = \left(\frac{\partial q_1}{\partial c_1}\right)_{c_2=0},$$

and the impossible eqn. (23) (or, at any rate, the equation with which one finds a great difficulty to reconcile oneself) disappears. This can be taken as a proof that, at least for Langmuir isotherms, there will necessarily be a constant concentration band separating the regions $c_2 > 0$ and $c_2 = 0$, c_1 variable. In the light of these remarks formulæ (26) must be modified (and perhaps also some other formulæ).

I also want to draw attention to formulæ (30) which postulates two discontinuities separated by a mixed band. For the sake of clarity I shall apply them to the case of Langmuir isotherms, where their inadequacy becomes very obvious. According to the assumption that solute 1 is more strongly adsorbed, $a_1 > a_2$.

Now if the postulated mixed band is to be formed spontaneously between two discontinuities, the boundary between the mixed band and the original solute should move faster than the boundary between the mixed band and the pure solution of the developing solute. In the notation of formula (31) it follows that

$$x_2 > x_1, \; v/x_2 < v/x_1$$

and, according to (31),

$$\frac{q_1(c_1', c_2')}{c_1'} < \frac{q_2(c_1', c_2')}{c_2'}.$$

For Langmuir isotherms, this becomes

$$\frac{a_1}{1 + b_1 c_1' + b_2 c_2'} < \frac{a_2}{1 + b_1 c_1' + b_2 c_2'},$$

$$\text{i.e., } a_1 < a_2$$

contrary to the assumption that solute 1 is more strongly adsorbed.

To resolve this contradiction let us re-examine the derivation of (30). Formula (30) is an application of formula (2·3)

$$\frac{q_{1(II)} - q_{1(I)}}{c_{1(II)} - c_{1(I)}} = \frac{q_{2(II)} - q_{2(I)}}{c_{2(II)} - c_{2(I)}} \quad . \quad . \quad . \quad . \quad (a)$$

derived from the conservation of material of both solutes. It is essential for the derivation of this formula that both solutes be present at the boundary. If, for instance, the solute 1 is not present on either side of the boundary, then the eqn. (2·1) disappears and we can no longer use equation (*a*).

If it is known beforehand that on one side of the boundary both solutes are present, then, of course, the formula (2·3) is necessarily satisfied. But if on one side of the boundary only one solute, say, solute 2, is present, and we want to determine the concentrations on the other side of the boundary, then there are two possibilities.

Either these concentrations will be determined by eqn (*a*) or only the solute 2 is present and $c_1' = 0$. In the example under consideration we have thus three possibilities for the concentrations between the two discontinuities, namely

(*a*) $c_1' = 0$ (*b*) $c_2' = 0$ (*c*) c_1' and c_2'

determined by the eqn (30).

We have already seen that the possibility (*c*)—the one chosen by Offord and Weiss—leads, at least in the case of Langmuir isotherms, to contradiction.

The possibility (*b*), i e., that of $c_2' = 0$, also leads, at least in the case of Langmuir isotherms, to the same contradiction ($x_1 = x_2(1 + b_1 c_1)$ and hence $x_1 > x_2$).

But the possibility (*a*), i e., that of $c_1' = 0$, leads to $x_2 = x_1(1 + b_2 c_2)$ and hence $x_2 > x_1$ as it ought to be. Thus the possibility (*a*) is the only acceptable one

I should also point out that if one eliminates from Offord and Weiss's paper all the inacceptable choices of alternatives, and uses the logically acceptable ones, one arrives at the same results as have been already published by Glueckauf.

Dr. E. Glueckauf (*A E R E, Harwell*) said: I wish to call Prof. Offord's attention to eqn. (17) which is a simple quadratic for y In view of eqn. (12), q_1 and q_2 are both functions of c_1 and c_2 only, and

$$(\partial q_1/\partial c_1)_{c_2=0} \text{ and } (\partial q_2/\partial c_2)_{c_2=0}$$

have thus values independent of y.

I ask Prof. Offord why he postulates (see eqn. 23) that the two roots of the quadratic (17) should be equal, when this is absolutely impossible. This answer is essential, because the rest of paragraph II is based on eqn. (23).

In order to avoid confusion in future research, I wish to remove any impression that there are two alternative theories or even two alternative ways of approach in this matter. Actually the fundamental approach is very similar as can be seen from the identity of a number of differential equations, and logical treatment of these equations would lead anybody to the same results, as were obtained in my researches during the last five years.

However, the meaning which these equations take on after integration, and the physical limits imposed by the fact that we are dealing with physical realities, have sometimes escaped the authors, and *of several mathematically possible solutions they have repeatedly chosen the physically impossible ones.* Thus we are confronted with concentration bands which, beginning with a zero-existence, have a negative growth-rate; with other bands which can contain more solute than has been put into the column.

In paragraph I, the treatment of a sharp boundary between two binary solutions (i.e., eqn. (2·3), (3), and Fig. 1) is inadequate and misleading. In paragraph II, all equations after (21) are erroneous, including Fig 2. In paragraph III all conclusions after eqn. (29) are wrong, including Fig. 3. In paragraph IV all conclusions are wrong, including Fig. 4.

I must apologize to Prof. Offord and Dr. Weiss for stating this so bluntly, the reason being that the Fig. 2, 3, 4 look so extremely plausible to the chemist who uses chromatography as a technical tool. The disturbing effects of using a finite grain-size and of non-equilibrium phenomena, which have *not* been taken into account in their papers, result in exactly those phenomena which Offord and Weiss claim to have found for an "ideal" column by means of their wrong interpretations. These disturbing phenomena cause a smearing-out of the flat region, missing at X_1' in their Fig. 2, and they cause variable mixed boundaries under the conditions of Fig. 3 and 4. But *these* phenomena though shown in the Figures are *not* represented by the equations of Offord and Weiss.

Prof. A. C. Offord (*London*) (*partly contributed*): In spite of the remarks of Dr. Glueckauf I must emphasize that our approach to the problem is fundamentally different from his and any points of resemblance in the two theories are largely a matter of accident.

One of the main problems treated in both contributions is the development of a chromatogram consisting of a band of two substances by pure solvent. Dr. Glueckauf starts with the simultaneous partial differential equations

$$\frac{\partial c_i}{\partial x} + \frac{\partial f_i}{\partial v} = 0 \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where $i = 1, 2$ and f_1 and f_2 are functions of c_1 and c_2 only. These equations are subject to the initial conditions that $c_1 = c_2 = 0$ when $x = 0$, and that $c_1 = c_1^\circ$ and $c_2 = c_2^\circ$ when $v = 0$. He then gives what is claimed to be a solution of these equations, which is broadly of the following form. The first quadrant is divided into five sectors by four straight lines (i) (ii) (iii) and (iv) issuing from the origin, and the functions c_1 and c_2 are given by various expressions, different for each sector. This solution has, however, no mathematical validity, for the partial derivatives of the functions c_1 and c_2 do not exist on the lines (i) (ii) (iii) and (iv). In fact, these differential equations have no solutions under the boundary conditions stated.

Our contribution was a continuation of the work of Weiss [2] on the problem for a single solute and it has much in common with this earlier investigation. The mathematical treatment for the single solute (see the Appendix [2]) hinges on the fact that we are not dealing with a sharp discontinuity at the top of the band, but with a continuous tailing-off. Indeed, an explicit form was assumed for this tail off (see eqn. (A 13)). The conclusion arrived at was, however, that, in the case of isotherms with pronounced curvature, the major term, giving the concentration in the rear part of the elute, was in fact independent of the initial shape assumed for the top of the band. Hence, in spite of the fact that in its final form the result appears to depend on the displacement relations only, it was only by making use of the notion of a diffused initial boundary (created by the presence of diffusion) that the problem could be given a tangible mathematical form.

Unfortunately the same treatment cannot be applied in the case of two solutes, but none the less similar considerations apply. The main point remains, that the boundary conditions $c_1 = c_2 = 0$ when $x = 0$, and $c_1 = c_1^\circ$, $c_2 = c_2^\circ$, when $v = 0$, have neither mathematical nor physical validity, and it is essential, not only for physical but also for mathematical reasons to treat this boundary as a diffused boundary which it most certainly is. If we fail to do this we do not arrive at an "ideal" problem, as asserted by Dr. Glueckauf, but at one which has no meaning.

It should have been explained that our treatment only applies when the isotherms have a pronounced curvature. The results we give are not true for

[2] *J. Chem. Soc.*, 1943, 297.

linear isotherms, where the phenomenon of diffusion plays a very much more marked role. Unlike Dr. Glueckauf, we can lay no claim to a definitive mathematical theory valid in all cases, and, in our communication, we considered only a few problems which we regarded as of particular interest. In the case of the special problem under consideration, we remarked that various other solutions were possible depending on the particular isotherms. This applies to the particular example selected by Dr. Davison, where the more strongly adsorbed substance is present only in weak concentration. Our solution does not apply in this case. If one substance is present only as a trace, then another mathematical treatment is possible and we hope to return to this problem later.

Dr. Glueckauf's main objection arises from our treatment of eqn (7) in our paper [3] leading to eqn (23), which is vital for all further consideration. This treatment is, however, necessary if the eqn (1) are to have a solution (with continuous partial derivatives) valid over the part of the chromatogram where the faster moving solute first appears. Eqn. (22) is not incompatible with the differential eqn (12), because in our treatment there is no initial condition associated with (12) and so this equation does not determine c_2 in terms of c_1

Dr. J. Weiss (*Newcastle*) said: As we have pointed out in our paper the problem is in certain respects not fully defined and it is essential to take into account the experimental fact that—on account of diffusion and convection —there is *never* a sharp boundary at the top of the band. By paying attention to this fact, diffusion has been taken into account implicitly in our theory This is not the case in Glueckauf's treatment and it is an important point in our theory

As we have stated, we have assumed that the development of a chromatogram of two solutes with the solvent is, in general, a continuous process. We have assumed this in view of the experimental results of various workers as there is no evidence to show that one obtains under these conditions a band of constant concentration as postulated by Glueckauf. This is in fact demonstrated clearly even by the experimental work of Coates and Glueckauf.[4] Fig. 2 and 4 of this paper are very instructive and I would like to recommend anyone interested in this problem to consult these Figures and to see for themselves that the "constant band" predicted by this theory is a figment of imagination These authors have also presented there some theoretical curves. However, in order to show even a rough agreement between theory and experiment they had to choose the arbitrary constants in the theoretical equations in such a way that the "constant band"—which is the main point in Glueckauf's theory—is practically eliminated, i e, is reduced to such small proportions that it falls well within the region of the experimental error

I shall be only too pleased to accept Glueckauf's theory if he can produce any experimental evidence in favour of it. that he obviously cannot, and he explains by "disturbing effects" while our theory can at least give an account of the main experimental features.

We find it hard to understand also what Dr. Glueckauf says in the last paragraph of his remark. Diffusion phenomena might be expected to have the effect of spreading out the band and so making the flat region which he predicts more pronounced.

Dr. J. F. Duncan (*A.E.R.E., Harwell*) (*communicated*) In order to make an experimental test of the theory developed by Dr. E. Glueckauf, the case shown in Fig. 4 (*d*) of his paper was simulated with the following solutions:

Solution 1: 0·025 N $NaNO_3$, 0·1 N KNO_3, 0·125 N HNO_3.
Solution 2: 0·15 N $NaNO_3$, 0·06 N KNO_3, 0·04 N HNO_3.

Assuming the mass action law to obtain we have

$$K_{Na+} = \frac{C_{Na_R}}{C_{H_R}} \times \frac{C_{H_S}}{C_{Na_S}},$$

[3] This Discussion.
[4] *J. Chem. Soc.*, 1947, 1308.

from which

$$\frac{C_{Na_R}}{C_R} = \frac{K_{Na^+}(C_{Na_S}/C_S)}{1 + (K_{Na} - 1)(C_{Na_S}/C_S)} , \quad . \quad . \quad . \quad (1)$$

where the terms have the same meanings as in our paper. Eqn (1) has the form of a Langmuir equation. Hence

$$b_1 = (K_{Na^+} - 1)/C_S ,$$

and similarly, for potassium

$$b_2 = (K_{K^+} - 1)/C_S .$$

Assuming [5] $K_{Na^+} = 1{\cdot}52$, and putting $K_{K^+} = 2{\cdot}00$ (a reasonable value, estimated from the results of other workers) we get

$$p_{Na^+} = 5C_{Na_S} \text{ and } p_{K^+} = 5C_{K_S}$$

Hence for solution 1, $p_{Na^+} = 0{\cdot}125$, $p_{K^+} = 0{\cdot}5$,

and for solution 2, $p_{Na^+} = 0{\cdot}75$, $p_{K^+} = 0{\cdot}03$.

This represents the case when a solution A in the column is followed by a solution C, the characteristic diagram being similar to that shown in Fig. 4 (*a*). The result of the experiment showed quite clearly that an intermediate concentration plateau forms, with both concentrations higher than A, in the manner shown in Fig 4 (*d*).

Dr. J. Weiss (*Newcastle-upon-Tyne*) (*communicated*). Dr. Davison has confined his assertions to a special form of the Langmuir isotherm for two solutes, viz.,

$$q_1 = \frac{a_1 c_1}{1 + b_1 c_1 + b_2 c_2} , \text{ etc.},$$

and, furthermore, he makes a somewhat arbitrary choice of the constants. It is obvious that our theory holds, as stated, only when q_1 and q_2 are functions of c_1 and c_2 through the whole course of the development (when both these solutes are present) However, in the Langmuir equations q_1 and q_2 are functions of c_1 and c_2 only if $b_1 c_1$ and $b_2 c_2$ are both of the order of 1. It is well known that if this is not the case the equations go over into either (*a*) the linear isotherms, if $b_1 c_1$ and $b_2 c_2 \ll 1$, or (*b*) q_1 and q_2 become constant (independent of c_1 and c_2), if $b_1 c_1$ and $b_2 c_2 \gg 1$. It is clear that this imposes considerable restrictions on the application of this form of the Langmuir isotherm Thus it clearly cannot be applied to the displacement development (Tiselius' case) because when the more strongly adsorbed component is present in excess according to this simple form of the Langmuir isotherm the amount adsorbed would become independent of the concentration of this solute. These facts have not been taken into account by Dr. Davison and thus his remarks are hardly of any significance.

Dr. E. Glueckauf (*A.E.R.E., Harwell*) (*communicated*): Two matters seem clear from the paper and the contributions of Dr. Offord and Dr. Weiss: (i) that the former has little experience in the application of boundary conditions to hyperbolic differential equations which govern the process concerned (which, by the way, is in close analogy to the propagation of waves in channels); (ii) that the latter tries unsuccessfully to sidetrack the issue by counter attacks which, being without substance, can be easily answered.

No reply has been made concerning Dr. Davison's explicit criticism of eqn (30), which invalidates the whole of their para. 3 and 4, and it must be assumed that Offord and Weiss concur.

Dr. Offord's explanation " that their treatment only applies when the isotherms have a pronounced curvature " and when the more strongly adsorbed substance is present at higher concentrations is not given expression in any of the boundary conditions used in their paper and it is difficult to escape the impression that it has been an afterthought. In particular, Dr. Offord omits to say that, when

[5] Duncan and Lister, *Chem. and Ind.*, 1949, 24.

he limits application to isotherms of pronounced curvature, and excludes cases where the more adsorbed material (1) is in the minority (which, in terms of the Langmuir isotherm, is equivalent to the conditions $b_1c_1 + b_2c_2 \gg 1$ and $c_1^\circ > c_2^\circ$), his result approximates more and more to that of my theory. This must be so, because his mathematically correct solution is the point (see my Fig. 3) where the parabolical envelope touches the ordinate at $p = 1$. It required exceptionally special assumptions (which the authors do not state in their paper and which do not frequently occur under experimental conditions) before Offord and Weiss's solution would apply even approximately.

Dr. Weiss emphasizes that "in *their* paper, the problem is in certain respects mathematically not fully defined." The reason for this is that they have omitted to introduce an essential boundary condition, namely, the concentration of the original solution c_1° and c_2°. This omission is caused by the fact that they impose the condition of continuity of the partial derivatives—a condition which is quite legitimate, and in fact necessary in the elliptic differential equations, but has no justification in equations of the hyperbolic type And having imposed such a condition they are no longer able to satisfy the initial conditions given by c_1° c_2°

That chromatographic conditions (in particular the value of c_2 at $c_1 = 0$) depend on the original concentrations c_1° c_2° has been shown experimentally and very clearly indeed in the Fig (4)[6] which Dr. Weiss has examined in so careful and unbiased a manner. The "constant band" which is by no means the main point of my theory, but merely follows from it for certain isotherms, is not and should not be predominant in this case, where the exchange constants a_1 and a_2 differ as little as they do. I also wish to draw attention to the communication by Dr. Duncan concerning experimental data. Dr. Weiss, who has *never* published any chromatographic experiment, has stated "that he will be only too pleased to accept Glueckauf's theory if he can produce any experimental evidence in favour of it." I therefore hope that Dr. Weiss will be pleased to do so.

[6] Coates and Glueckauf, *J Chem Soc.*, 1947, 1313

ADSORPTION AND SOME CONSTITUTIONAL AND STERIC PROPERTIES

BY L. ZECHMEISTER

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The concept "anchoring group" is proposed and some pertinent experimental approaches are indicated A report is submitted on the separation of *cistrans* stereoisomers, and the dependence of the adsorption affinity on molecular morphology is discussed

Although it is not assumed that a single, correct picture can be given for the adsorption and geometrical orientation of organic molecules on a solid surface, the following concept may serve as a basis of discussion.

Two essential features can be postulated for the simple and stable fixation of an organic solute on an adsorbent: first, its molecules should fit into the cavities of a certain size located on the active surface; and second, adequately oriented forces should be operative between the adsorbent and the adsorbed molecule. These two postulates are, of course, closely interrelated.

Very little is known about the geometrical orientation of adsorbed molecules, but one may speculate that the manner of orientation would influence, among others, the relative behaviour of closely related compounds. If the

orientation be roughly parallel to the surface, it could be perhaps expected that, within a homologous series, a maximum of the adsorption affinity would be reached at a certain chain length. On the other hand, it may be considered that, in the case of a roughly vertical orientation of the adsorbed molecules with reference to the surface, a continuous increase or decrease in the adsorption affinities would be observed by passing stepwise from lower members of the series to some higher ones.

Whatever the orientation may be in a given system, it seems reasonable to assume that not each section of the adsorbed molecule will be equally responsible for the fixation process. On the contrary, we propose that a decisive part will be played by certain atomic groups which are conveniently designated as "anchoring groups." This concept is similar to that in which it is presumed that the fixation of a drug to bacteria takes place by the intermediary of a haptene group (or groups). In favourable instances an anchoring group could be located experimentally by showing that a modification of that particular section of the molecule causes an unusually sharp change in the adsorption affinity.

Perhaps a single example will demonstrate the general lines along which we think that laboratory work may help in collecting material for a more satisfactory discussion of such problems. It was found that the introduction of a methyl group in the α-position to the sulphur atom of α-terthienyl markedly increased the adsorption affinity; and that, upon dimethylation, this effect was about doubled. On the other hand, Kofler reported that the adsorbability of some tocol derivatives decreased upon methylation near the phenolic OH group, although this substitution had but little effect in a more remote position to the hydroxyl mentioned. In the case of thiophene rings the introduced methyl group seems to have been involved in the fixation process but the anchoring group of the tocol derivative was probably the phenolic hydroxyl whose function is sterically hindered by the presence of adjacent methyl groups.

While manifold possibilities are open for experimentation in the direction just outlined, our problem changes its character entirely when we pass from chemical to stereochemical considerations. Then, in a way, the situation appears to be simplified, since in stereochemistry the dependence of the adsorption affinity on the overall shape of the molecules is to be considered, and not the presence or absence of certain functional groups. The compounds which are suitable for such investigations should possess numerous stereo-isomeric forms whose respective morphological types should vary as widely as possible. This postulate is unequally fulfilled in various stereo-isomeric sets; for example, the epimerization of a sugar would not essentially alter the overall shape of its molecules.

The conditions for studies of this kind are especially favourable in the field of the natural and synthetic polyenes, which compounds possess a long, conjugated carbon-carbon double-bond system in an open chain. Their structure is "morphologically sensitive" to spatial variations. Although the ordinary or all-*trans* form (Fig. 1) which shows a rod-like general shape is not greatly modified by a single *trans*→*cis* rotation which occurs near an end of the system, it undergoes a radical change and is converted into a V-like pattern when such a spatial re-arrangement takes place at or near the centre (II). On the other hand, in the course of continued *trans*→*cis* rotations, after having passed through several bent forms, a straightening-out takes place. Thus, the molecules of the resulting poly-*cis* compound show a rod-like overall shape (III) which is morphologically similar to that of the all-*trans* molecules (I).

As is well known, the respective all-*trans* forms of the polyene hydrocarbons ($C_{40}H_{56}$), α-carotene, β-carotene, γ-carotene, and lycopene, can

L3925

be easily converted into a mixture of stereo-isomers containing mainly compounds with mono-*cis* and di-*cis* configurations. Such a mixture is resolved chromatographically, whereupon some insight into the respective configurations can be gained by means of appropriate spectroscopic methods.

Whereas any manner of bending of an all-*trans* carotene molecule causes a displacement of the main extinction maxima towards shorter wavelengths in the visible spectral region, the simultaneously occurring alteration in the adsorption affinity does not follow such a simple rule. It was found for each of the three carotenes, for example, that a stereo-isomer which very probably contains a single, peripherally-located *cis* double bond, is adsorbed above the corresponding all-*trans* compound in the Tswett column. In contrast, those spatial forms of the carotenes which possess a centrally-located *cis* double bond (and in some instances another *cis* bond) show considerably weaker adsorbability than the all-*trans* isomer. Thus, we have the following chromatographic sequence:

(Top)	Peripheral mono-*cis* carotene All-*trans* carotene	} essentially straight types.
(Bottom)	Central mono-*cis* carotene, etc.	: essentially bent type.

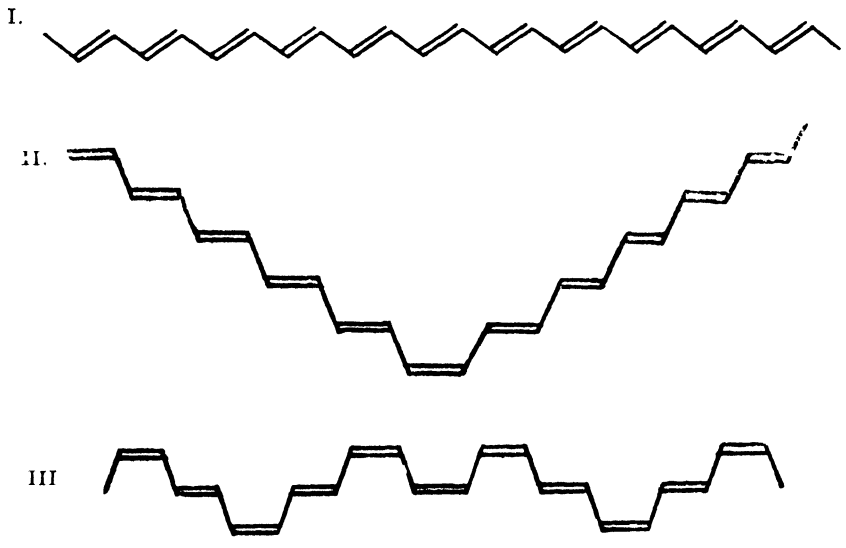

Fig. 1.

Typical spatial forms of a long, conjugated double bond system in an open chain : I, all-*trans* ; II, central mono-*cis* ; and III, poly-*cis* (penta-*cis*).

It should be noted in this connection that the adsorption affinity of a monohydroxy-β-carotene, viz., all-*trans* cryptoxanthin ($C_{40}H_{55}.OH$), undergoes the changes just described when stereo-isomerized *in vitro* ; hence, in this instance the presence of a single hydroxyl group has no decisive influence on the relative adsorbabilities of the respective spatial forms.

In contrast, each *cis* isomer so far observed of the analogous dihydroxy compounds, viz., zeaxanthin and lutein ($HO.C_{40}H_{54}.OH$), is located in chromatograms above the corresponding all-*trans* zone. Thus, the outstanding features of this *trans*→*cis* isomerization are weakening of the colour and an

increase in the adsorption affinity. One could suppose that in these stereochemical sets both hydroxyl groups would participate in the anchoring process, and that the decrease of the distance between them, as caused by bending of the molecule, would promote fixation. However, it is also possible that such spatial forms of the dihydroxycarotenes do exist which show decreased adsorption affinities, as compared with the all-*trans* form; but for some reason they do not appear in appreciable quantities in the stereoisomeric mixtures which can be obtained by current methods.

Some representatives of a different type of stereo-isomeric hydrocarbons, viz., poly-*cis* γ-carotenes and poly-*cis* lycopenes, which so far we were unable to prepare in the laboratory, could be isolated from some plant materials. They contain four to seven of their double bonds in *cis* configuration. If we pass from an all-*trans* compound to either of its poly-*cis* forms, then the remarkable weakening in the colour runs parallel with a substantial decrease in the adsorption affinity. A similar parallelism is also observed within the subclass of the poly-*cis* lycopenes, whose chromatographic sequence and spectral sequence are identical. With reference to both of these physical characteristics the individual differences are much smaller within the class of the poly-*cis* isomers than between either poly-*cis* form and all-*trans* lycopene.

Before closing these considerations, we should stress that the described variations in the adsorption affinity which are a function of morphological changes, are of the same order of magnitude as analogous effects caused by reasonably-chosen structural conversions. For example, a solution containing several spatial forms of both α-carotene and β-carotene gave the following chromatographic sequence on calcium hydroxide, when developed with petroleum ether:

(Top)	*Neo*-β-carotene *V*
	Neo-α-carotene *U*
	All-*trans* β-carotene
	Neo-α-carotene *V*
	Neo-β-carotene *B*
	Neo-β-carotene *E*
	Neo-α-carotene *W*
	Neo-β-carotene *F*
	All-*trans* α-carotene
(Bottom)	*Neo*-α-carotene *B*

Evidently, the weaker adsorption affinity of all-*trans* α-carotene (containing 10 conjugated double bonds) as compared with that of all-*trans* β-carotene (11 such bonds) can be compensated and even overruled by a suitable adjustment of the molecular form.

Furthermore, an increase of the adsorption affinity several times stronger than that caused by the reaction, α-carotene → β-carotene, is obtained when we convert a poly-*cis* compound into the corresponding all-*trans* form.

Finally, the author wishes to express his appreciation to his colleagues, (Mrs.) A. Chatterjee, R. B. Escue, F. Haxo, R. M. Lemmon, A. L. LeRosen, W. H. McNeely, J. H. Pinckard, A. Polgár, A. Sandoval, W. A. Schroeder, J. W. Sease and B. Wille.

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CHROMATOGRAPHY OF COLOURLESS SUBSTANCES AND THE RELATION BETWEEN CONSTITUTION AND ADSORPTION AFFINITY

By H. Brockmann

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A method for the chromatographic separation of colourless substances based on the use of fluorescent adsorbents is described Relations between adsorption affinity and chemical constitution of azobenzene and stilbene derivatives are demonstrated The dependence of adsorption affinity on the nature of the adsorbent and solvent was investigated Water soluble salts like copper sulphate or zinc sulphate were found to be useful adsorbents for the chromatographic separation of azobenzene compounds.

Chromatography of Colourless Substances.[1]—The adsorption zones of colourless substances can be followed by the use of fluorescent adsorbents. Ultra-violet light of a wavelength absorbed by the adsorbed substance is employed. The part of the column containing adsorbed substance then appears as a dark band. Adsorbents can be made to fluoresce by the addition of small quantities of irreversibly adsorbed fluorescing compounds. Morin is very satisfactory for alumina, magnesium oxide and calcium carbonate, and berberine for silica. Salicylic acid and 2-hydroxynaphthalene-3-carboxylic acid can also be used.

Experimental.—A very convenient method for the preparation of a fluorescent column consists in the mixing of the adsorbent with 2 % to 5 % of finely powdered luminous paint. This method is generally applicable.

Calcium sulphate dihydrate, prepared by the precipitation of calcium chloride with the calculated quantity of sulphuric acid at 80°–90° C can be converted into a valuable adsorbent for colourless compounds, and also, incidentally, an excellent one for coloured ones Heating at temperatures between 150° and 200° C yields three different products : the hemihydrate has an activity equivalent to that of alumina IV, the soluble anhydrous salt can be obtained in two grades, one equivalent to alumina III and the other to alumina II. The product obtained by heating above 250° is only slightly active and is of no practical value. The large adsorption affinity of the soluble anhydrite is very likely to be due to numerous faults in the crystal lattice.

The fluorescent chromatograms were illuminated with light of 366 mμ from a mercury vapour lamp and an appropriate filter. For compounds which absorb at shorter wavelengths, a Chlorfilter was used with windows of UGS glass (Schott, Jena) which transmits only the Hg line at 253 7 mμ. In our most recent experiments on the separation of colourless compounds, the following apparatus was used. Light from an electric arc (iron electrodes) was passed through a slit 35 mm. long and 3–4 mm wide, a quartz lens 5 cm. thick, and a quartz or rock salt prism 50 mm. high The resulting line spectrum (length of lines 50 mm.) was projected onto a fluorescent screen ; after focusing, the fluorescent screen was replaced by a cardboard sheet on which the position of the spectral lines in the ultra-violet spectrum had been marked. The adsorption column, contained in a quartz tube, was placed in front of the cardboard and moved until the optimum contrast was obtained making the bands of the chromatogram clearly recognizable. It is advisable to employ a combination of adsorbent and fluorescent compound which does not continue to emit light after illumination (luminous paint, N4 green I, Francke, Frankfurt, was used).

[1] Brockmann and Volpers, *Ber.*, 1947, **80**, 77 ; 1949, **82**, 95. Sease, *J. Amer. Chem. Soc.*, 1947, **69**, 2242.

Appearance of Zones.—There are two ways in which the zones can be made apparent: (i) in the case of adsorbents rendered fluorescent by adsorbed morin or berberine, the zones become apparent through extinction of the fluorescence by the adsorbed compound; (ii) adsorbents which have been mixed with luminous paint behave differently. In this case the zones appear as follows. The particles of the adsorbent allow the light to penetrate the column to a certain extent and fluorescence excited there is emitted. Actual adsorption of the light is stronger at places where the adsorbed compound is present, so that the fluorescing material, in this case the luminous paint particles, is not as strongly excited as in those places where the column is free: luminous paint particles thus act as small fluorescent screens.

Experimental.—That the outer layer of adsorbent allows light to penetrate is shown as follows. A test-tube, the diameter of which is such that it fits in the chromatographic tube with 3–5 mm. clearance all round, is filled with luminous paint and placed in the tube. The space between is filled with adsorbent and a colourless substance is adsorbed onto it. On illumination with ultra-violet light the zone appears surprisingly clearly. This filter action of the outer layer of the column can also play a part in the appearance of the zones when adsorbent materials containing adsorbed morin or berberine are employed. In this case each particle of adsorbent acts as a small fluorescent screen.

Whether an adsorbed colourless substance is detected through extinction of the fluorescence or through the filter effect can be decided in some cases by comparing the behaviour of an adsorbent + luminous paint with that of the adsorbent + morin or berberine. Table I gives the results of such a comparison

TABLE I

	Al_2O_3–Luminous paint	Al_2O_3–Morin
Benzaldehyde	Non-visible	Visible
o-Tolylaldehyde	,,	,,
Acetophenone	,,	,,
Anisaldehyde	,,	,,

A mercury lamp with a filter allowing the transmission of light of wavelength 360–370 mμ was used. Since the above compounds hardly absorb at 365 mμ the zones do not appear when adsorbent + luminous paint is employed. The appearance of the zones when adsorbent + morin is used must therefore be due to extinction of fluorescence. The effectiveness of the extinction is greater in the case of morin than that of berberine. Thus the phenacyl esters of aliphatic carboxylic acids, which hardly absorb at 365 mμ, are visible with light of this wavelength on Al_2O_3-morin but not on silica-berberine.

Relations Between Constitution and Adsorption Affinity.—The adsorption affinity, that is the firmness with which organic compounds are adsorbed from non-aqueous solvents *inter alia*, depends on the nature of the skeleton and of the functional groups of the adsorbed compound.

In order to find the effect of the functional groups on adsorption affinity, several compounds containing the same basic skeleton but differing only in one functional group were investigated. The first experiments were carried out with *p*-substituted stilbenes and azobenzenes. These compounds were adsorbed on Al_2O_3 from benzene or from carbon tetrachloride. Since stilbene and azobenzene under these conditions are only very weakly adsorbed, the adsorption affinity of the derivatives can be taken to be almost entirely dependent on the nature of the substituents. The functional groups in Table II are arranged in order of decreasing adsorption affinity.

Compounds separated by a horizontal dash could be separated, the others were not fully separable. The two series are in almost complete agreement with each other. Differences are only found in the three unnumbered acyl derivatives on the right-hand side: those in the azobenzene series lie higher than those in the stilbene series; the order, however, remains the

TABLE II

$R=C_6H_5.CH:CH.C_6H_4-$		$R=C_6H_5.N:N.C_6H_4-$	
R–COOH		R–COOH	
$R–CONH_2$			
R–OH		R–OH	
			R–NH–Ac
			R–O–Ac
$R–NH_2$		$R–NH_2$	
	R–NH–Ac		R–O–Bz
	R–O–Ac		
$R–COOCH_3$		$R–COOCH_3$	
$R–N(CH_3)_2$	R–O–Bz	$R–N(CH_3)_2$	
$R–NO_2$		$R–NO_2$	
$R–OCH_3$		$R–OCH_3$	
R–H		R–H	

Ac = CH_3CO- Bz = $C_6H_5.CO-$

same. This difference is probably due to the fact that the azobenzene derivatives were adsorbed from benzene and the stilbene derivatives from carbon tetrachloride. The solubility of the azobenzene derivatives in carbon tetrachloride was unfortunately too small for adsorption experiments to be carried out using this solvent.

Dependence of Adsorption Affinity on the Adsorbent.—In the adsorption of organic compounds from non-aqueous solvents only, certain points on the adsorbent particle (the so-called active points) play a part. These are mainly at positions where the crystal lattice is faulty. The adsorption activity of an adsorbent therefore does not only depend on its chemical nature, but also on the nature of its crystal lattice. α–Al_2O_3 does not adsorb whereas γ–Al_2O_3 and Böhmite are very effective as adsorbents. $CaSO_4$, like the " soluble " anhydrous salt, possesses the same crystal lattice as the hemihydrate; it shows good adsorption properties, and is, in the light of our experience, very efficient in the separation of hydroxy-anthraquinones. As the lattice of the soluble anhydrite is changed into that of the insoluble anhydrite by heating to 250°, the capacity for adsorption falls very considerably.

We have previously shown that γ–Al_2O_3 can be obtained in five different grades (by treatment with water vapour or by regulated heating) which can differentiate by adsorption of azo dyes under standardized conditions. The employment of Al_2O_3 with different and reproducible activities has proved so useful that we have attempted to prepare graded specimens of other adsorbents. By varying its water content, bentonite was obtained in five grades of activity, silica gel and precipitated silica in three, $CaSO_4$ and MgO also in three, and $CaCO_3$ in two different grades. The standardization of these adsorbents was carried out using the same dyes as in the case of alumina. In the course of these experiments we have tried to find out whether, with the same solvents, our test dyes were adsorbed in the same order as on Al_2O_3. The following experiments show this not to be the case.

Adsorption Sequence of Azo Dyestuffs in Columns of Different Adsorbents.

DYESTUFFS: *p*-hydroxyazobenzene, *p*-amino-azobenzene, Sudan Red (o-CH_3).C_6H_4.N:N.C_6H_4(o-CH_3).N:N.$C_{10}H_6$.OH, Sudan Yellow C_6H_5.N:N.$C_{10}H_6$.OH, *p*-methoxyazobenzene.

SOLVENT: Benzene–Petrol ether, 1/4.

The most strongly adsorbed dyestuff is listed at the top of each column, the least adsorbed at the bottom.

Al_2O_3	SiO_2	MgO
Hydroxyazobenzene	Sudan Red	Hydroxyazobenzene
Amino-azobenzene	Oxyazobenzene	Sudan Yellow, Sudan Red
Sudan Red	Amino-azobenzene	Amino-azobenzene
Sudan Yellow	Sudan Yellow	Methoxyazobenzene
Methoxyazobenzene	Methoxyazobenzene	

$CaSO_4$	$CuSO_4$
Hydroxyazobenzene, Sudan Yellow	Amino-azobenzene
Amino-azobenzene	Sudan Red
Sudan Red	Hydroxyazobenzene
Methoxyazobenzene	Sudan Yellow
	Methoxyazobenzene

Effect of the Acid or Alkaline Character of the Adsorbent on the Adsorption Sequence.—The adsorption sequence of acidic or basic dyes is influenced by the acidity or alkalinity of the adsorbent as the following observations show.

SOLVENT: Benzene–Petrol ether, 1/4.

Al_2O_3 alkaline	Al_2O_3 treated with HCl
Hydroxyazobenzene	Hydroxyazobenzene
Amino-azobenzene	Sudan Red
Sudan Red	Amino-azobenzene
Sudan Yellow	Sudan Yellow
Methoxyazobenzene	Methoxyazobenzene

SiO_2 treated with NaOH	SiO_2 treated with HCl
Sudan Red	Sudan Red
Amino-azobenzene	Hydroxyazobenzene
Hydroxyazobenzene	Amino-azobenzene
Sudan Yellow	Sudan Yellow
Methoxyazobenzene	Methoxyazobenzene

Effect of Different Solvents on the Adsorption Sequence.—The firmness with which a compound is adsorbed from a non-aqueous solvent depends on the nature of the solvent in two ways: (i) the solvent tends to occupy the active points of the adsorbent; (ii) the solvent reacts with the dissolved substance (solvation). Both these effects are negligible with non-polar solvents such as petrol ether, cyclohexane, carbon tetrachloride and benzene, but considerable with polar solvents. The effect of various solvents on the adsorption sequence of dyestuffs is shown in the following results.

ADSORBENT : Al_2O_3

Benzene–Petrol ether, 1/4
Carbon tetrachloride

Hydroxyazobenzene
Amino-azobenzene
Sudan Red
Sudan Yellow
Methoxyazobenzene

Ether

Sudan Yellow, Hydroxyazobenzene
Sudan Red
Amino-azobenzene
Methoxyazobenzene

Chloroform

Hydroxyazobenzene
Amino-azobenzene
Sudan Yellow
Sudan Red
Methoxyazobenzene

Tetrahydrofuran, Dioxan, Cyclohexanone

Hydroxyazobenzene
Sudan Red, Sudan Yellow
Amino-azobenzene
Methoxyazobenzene

ADSORBENT : $CaSO_4$

Benzene–Petrol ether, 1/4

Sudan Yellow, hydroxyazobenzene
Amino-azobenzene
Sudan Red
Methoxyazobenzene

Ether

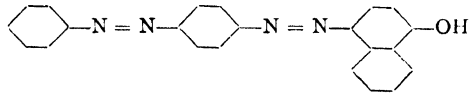
Sudan Yellow, hydroxyazobenzene
Amino-azobenzene
Sudan Red
Methoxyazobenzene

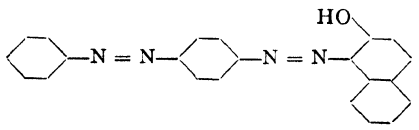
Chloroform

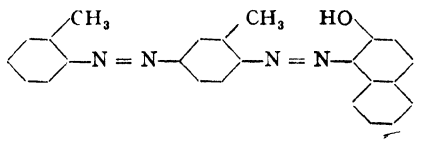
Sudan Yellow, hydroxyazobenzene
Amino-azobenzene
Sudan Red
Methoxyazobenzene

There does not seem to be a relationship between the adsorption sequence and the solubility of the test dyestuffs.

It is remarkable that Sudan Yellow and hydroxyazobenzene are adsorbed equally firmly from ether. A similar effect with ether as solvent was observed in the case of the three following Sudan dyes.

(i) 

(ii) 

(iii) 

These compounds are adsorbed on Al_2O_3 from benzene–light petroleum 1/4 in the order (i), (ii), (iii), but from ether in the order (ii), (iii), (i).

Even though no satisfactory explanation can at the moment be given of the effect of the solvent on the adsorption sequence, our observations lead to the following procedure. In the case of a complex mixture which cannot be separated by the use of one adsorbent and one eluent, it has been suggested that separation may be effected by adsorption from a non-polar solvent onto a strong adsorbent, followed by elution with a series of solvents of increasing adsorption affinity. We believe that this procedure when applied to compounds such as our dyestuffs, which in the adsorption sequence are so dependent on the nature of the solvent, is not as satisfactory as a procedure, which in our experience has proved to be of value, and which involves repeated chromatography of the mixture from one non-polar solvent on several adsorbents of varying activity.

The Adsorption Sequence which is independent of both Adsorbent and Solvent.—The marked dependence of the adsorption sequence of our dyestuffs on the nature of the solvent is perhaps due to variations in the solvation of the hydroxyl and amino groups present. Furthermore, it is possible that, depending on the nature of the solvent, the dyestuffs are adsorbed partly in the azobenzene form, partly in the tautomeric quinone-imine form.

These complications are avoided if the following compounds are used. The adsorption affinity decreases as one goes down the series.

$R = C_6H_5.N{:}N.C_6H_4$
(i) $R\text{–}NH.CO.CH_3$
(ii) $R\text{–}NH.CO.C_6H_5$
(iii) $R\text{–}O\text{–}CO.CH_3$ or $O_2N\text{–}R\text{–}N(CH_3)_2$
(iv) $R\text{–}COOCH_3$
(v) $R\text{–}NO_2$
(vi) $R\text{–}OCH_3$

Instead of dyestuff (iii) which will be partly hydrolyzed on alkaline adsorbents, *p*-bromo-*p'*-dimethylamino-azobenzene was employed. This compound, as a result of polarized adsorption, forms a bluish-violet zone. These compounds were always adsorbed in the same order from the following solvents on the following adsorbents. Al_2O_3: benzene–petrol ether 1/4, ether, chloroform. From ether, separation of (i) and (ii) is incomplete. SiO_2: benzene–petrol ether 1/4, ether, chloroform. $CaSO_4$: benzene–petrol ether 1/4, benzene, ether. MgO: benzene–petrol ether 1/4. $CuSO_4$: benzene–petrol ether.

From the above results we believe that we may draw the following conclusions concerning the relations between constitution and adsorption. The adsorption affinity of a compound is practically an additive function of the adsorption affinities of the basic skeleton and of the functional groups only when there is no possibility of tautomerism and when solvation by the solvent employed does not occur. The above-mentioned series can be taken to represent the relative adsorption affinities of the functional groups. Deviations from the above sequence are to be observed only when the adsorption behaviour of the functional groups is very similar. Strain [2] has drawn similar conclusions, using carotinoids. The knowledge that adsorption affinity depends largely on the number and nature of the functional groups only when the skeleton of the molecules is not very different enables one to predict in many cases whether chromatographic separation is possible.

J. Amer. Chem. Soc., 1948, **70**, 588.

Water-soluble Salts as Adsorbents.—Anhydrous copper sulphate has shown itself to be particularly well suited to the separation of azobenzene derivatives. The following azobenzene derivatives were adsorbed from benzene–light petroleum 1/4 and were eluted with the same solvent mixture or with benzene alone : the compounds were found to be adsorbed in the following order of decreasing adsorption affinity.

$R = -C_6H_4.N{:}N.C_6H_4-$	Colour of the zones
(i) $H-R-NH_2$	Flesh-coloured
(ii) $(CH_3)_2N-R-N(CH_3)_2$	Bluish red
(iii) $H-R-N-(CH_3)_2$	Purple
(iv) $Br-R-N(CH_3)_2$	Blue
(v) $H-R-NH.CO.CH_3$	Reddish brown
(vi) $H-R-NH.CO.C_6H_5$	Pale red

The following azobenzene derivatives, which cannot be separated on alumina are easily separated on anhydrous copper sulphate.

(1) $R-COOCH_3$, $R-N(CH_3)_2$. $R-N(CH_3)_2$ is well adsorbed from benzene onto copper sulphate, $R-COOCH_3$ on the other hand forms an easily eluted yellow zone.

(2) $R-NH_2$, $R-NH.CO.C_6H_5$. $R-NH_2$ is firmly adsorbed onto $CuSO_4$ from benzene as a flesh-coloured zone from which $R-NH.CO.C_6H_5$ is easily separated by elution.

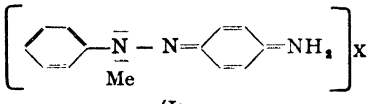
(3) $R-NH_2$, $R-O-CO.CH_3$. $R-NH_2$ is adsorbed from benzene much more firmly than $R-O-CO.CH_3$.

(4) $R-NO_2$, $R-N(CH_3)_2$. The purple zone of $R-N(CH_3)_2$ is much more firmly adsorbed than the orange zone of the nitro compound.

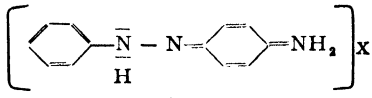
Anhydrous zinc, manganese, aluminium and magnesium sulphates can also be used for the separation of azobenzene derivatives.

We believe that it is probable that the addition compounds of the type (I) analogous with salts of *p*-amino-azobenzene (II) are formed on the surface of the adsorbent.

The above-mentioned salts, particularly aluminium sulphate, can be used for the separation of other compounds such as hydroxy-anthraquinones. Compounds that are very firmly adsorbed onto these salts can be isolated simply by dissolving the inorganic material in water and extracting the organic compounds with a suitable solvent.



(I)



(II)

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THE FUNCTION OF ADSORBENT ACTIVITY IN THE CHROMATOGRAPHIC SEPARATION OF CERTAIN ANTHRAQUINONE COMPOUNDS

By A. Stewart

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The activity of adsorbents used in chromatography with organic solvents is easily controlled by preliminary incorporation of water in the adsorbent The effect on the band movement of simple anthraquinone compounds in toluene on activated alumina and unactivated magnesium carbonate is described. The conditions for preparing activated alumina by heating between 250° and 500° C have considerably less effect on the behaviour than has the deactivation treatment. The band movement measurements were made under satisfactory comparative conditions but are affected by several errors; in particular the greater solvent movement in mid-column than at the periphery renders values obtained at the periphery 11 % low.

The distribution of the anthraquinone compounds between solvent and partially deactivated alumina is independent of concentration The length of the initial chromatograph band and the mean rate of band movement are in satisfactory accord with theory. The considerable band widening in passage through a column is independent of the adsorption, little affected by the rate of solvent flow and may be caused by non-uniformity of flow through the interstitial capillary spaces; its effect on the separation of a mixture causes the various bands to have approximately equal width at the same position in the column.

The relation between constitution and chromatographic behaviour of simple anthraquinone compounds, largely influenced by the conditions used, is briefly described

The successful application of adsorption chromatography ultimately depends on the empirical selection of operating conditions, if for no other reason than that the composition of the mixtures examined is rarely fully known until the separation has been achieved. Various methods have been described for the partial deactivation of adsorbents [1-5] and it is the purpose of this paper to draw attention to the simplicity of this operation and the wide range of control exercised on the band movement, enabling this variable to be systematically applied in selecting the operating conditions. To illustrate the behaviour, band movement measurements have been made using the highly coloured simple anthraquinone compounds in toluene solution; these conditions do not necessarily represent the optimum for such separations, for which their fairly low solubility is often a controlling factor. The effect on activated alumina and magnesium carbonate adsorbents is described; it has been applied to other adsorbents, but with the wide range of control obtained on deactivation these adsorbents cover most of the activity range required when the separation is carried out from organic solvent solution. The effect of variation in the conditions for the activation of alumina is shown to have much less effect on the behaviour than has the subsequent deactivation.

Partial deactivation of activated alumina gives a distribution between adsorbent and solvent which is substantially independent of the concen-

[1] Zechmeister and Cholnoky, trans Bacharach and Robinson *Principles and Practice of Chromatography*. (Chapman & Hall, London, 1941, p. 48)
[2] Brockmann and Schodder, *Ber.*, 1941, **74**, 73
[3] Muller, *Verb. Ver Schweiz Physiol.*, 1942, **21**, 29; *Helv. chim. Acta*, 1943, **26**, 1945; 1944, **27**, 404.
[4] Schroeder, *Ann. N Y. Acad. Sci*, 1948, **49**, 204.
[5] Stewart and I C I. Ltd., *Brit. Pat.* 565,405.

tration and under these conditions the mean band movement and the length of initial band are shown to be in agreement with the theory described by LeRosen [6] and others.[7-10] Conditions affecting the widening of bands, often a limiting factor in the chromatographic separation, are considered.

Theoretical

The symbols used have the following significance:

i	Weight of interstitial solvent per unit length of the column.
m	Weight of adsorbent per unit length of the column.
K	Distribution constant = the weight of solute adsorbed per gram of adsorbent/equilibrium concentration of solution in gram per gram, this value being substantially constant under the conditions employed.
K'	Distribution constant prevailing in unit length of the column, $K' = Km/i$.
c	Concentration of solution.
w	Weight of solution.
x	Distance of any point in the column measured from the beginning (top) of the column.
D	Distance the developing solvent has moved in the column.
c_o	Initial concentration of solution added to column.
x_o	Length of the initial band formed by adding w_o to column. x_o^α, x_o^β referring to initial lengths of components α and β.
D_o	Length of column occupied by interstitial solvent of weight w_o.
$D_i^{\alpha\beta}$ ..	Distance the developing solvent requires to move to separate two bands starting from x_o^α and x_o^β.
R	Displacement of zone on column/displacement of solvent in column $R = dx/dD$. R_L for the leading edge of a band; R_F for the following edge; R_M for the mean of R_L and R_F.
% R_M ..	R_M expressed as a percentage of the solvent movement.
% H ..	Extent of water deactivation; ml. water addition per 100 g. adsorbent, the latter including any moisture initially present.
R_L/R_M ..	A measure of the widening of a band edge in passage through the column.

As LeRosen [6] and others [7-10] have shown, where the distribution between adsorbent and solvent is independent of concentration and is rapidly established, the conditions for the formation and separation of the bands can be evaluated.

If a solution of concentration c_o of a pure compound is added to a column in amount w_o equal to length D_o of interstitial solvent, the ratio of interstitial solvent to adsorbent in unit length of the column being i/m, then the solute is adsorbed until it reaches equilibrium with the initial concentration of the solution and a band of length x_o is formed such that

$$x_o c_o + K' x_o c_o = D_o c_o \text{ or } x_o = D_o/(1 + K') = D_o/(1 + Km/i) \quad . \quad (1)$$

If pure solvent is now allowed to flow through the column then the band moves down at a fraction of the rate of linear movement of the solvent equal to the fraction of the total substance present in the interstitial solvent. The movement of the band relative to the linear movement of solvent through the column R is given by

$$R = x_o/D_o = 1/(1 + K') = 1/(1 + Km/i), \quad . \quad . \quad (2)$$

or

$$K' = (1 - R)/R \text{ and } K = [(1 - R)/R]\, i/m \quad . \quad . \quad (3)$$

[6] LeRosen, *J. Amer. Chem. Soc.*, 1947, **69**, 87.
[7] De Vault, *J. Amer. Chem. Soc.*, 1943, **65**, 532
[8] Weiss, *J. Chem Soc.*, 1943, 297.
[9] Glueckauf, *J Chem Soc.*, 1947, 1302
[10] Weil-Malherbe, *J. Chem. Soc.*, 1943, 303

The movement of the chromatograph bands through a column is conveniently expressed[11] as the relative movement R, which is given here as a percentage of the solvent movement. The value depends on the ratio of solvent to adsorbent i/m for the particular column, so that the results obtained from different columns require to be calculated on the basis of eqn. (2) and (3) to correspond with a particular value of i/m.

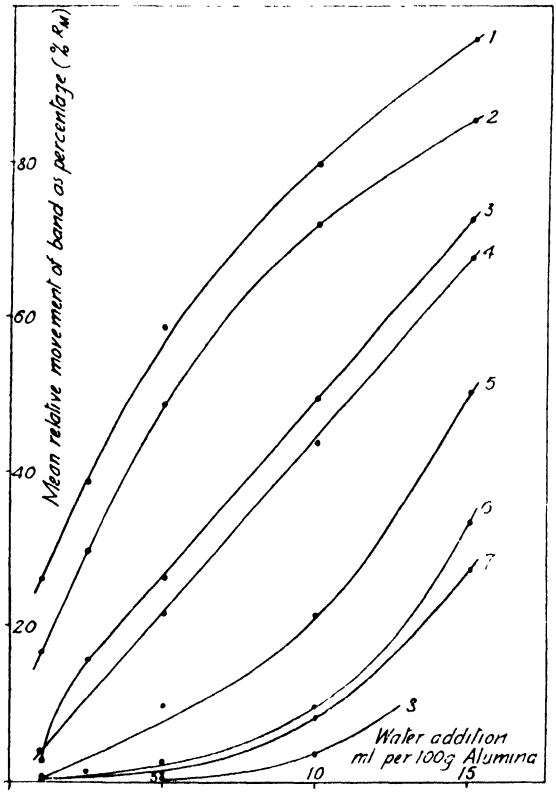

FIG. 1.—Effect of water deactivation treatment applied to activated alumina on the band movements of anthraquinone compounds in toluene.

Curve 1. 1-Chloroanthraquinone.
" 2. 1-Methylaminoanthraquinone
" 3. 1-Aminoanthraquinone.
" 4 1-Dimethylaminoanthraquinone.
" 5. 1-Amino-4-methylaminoanthraquinone.
" 6. 2-Aminoanthraquinone.
" 7. 1 : 4-Diaminoanthraquinone.
" 8. 1 . 4 : 5-Triaminoanthraquinone.

Under the above conditions the band should move down the column without changing its length, the small region in front and rear where adsorption and desorption occur remaining constant. The position of the following and leading edges of a band is given by

$$\text{Following edge}: x = x_0 D/D_0 \quad . \quad . \quad . \quad (4)$$

$$\text{Leading edge}: x = x_0 + x_0 D/D_0 \quad . \quad . \quad . \quad (5)$$

[11] LeRosen, *J. Amer. Chem. Soc.*, 1942, **64**, 1905.

If a second component is present in the initial solution and there is no mutual interference in the distribution, the bands will draw apart and commence to separate when the following edge of the faster reaches the leading edge of the slower

$$x_o^\beta D_s/D_o = x_o^\alpha + x_o^\alpha D_s/D_o \text{ or } D_s^{\alpha\beta}/D_o = x_o^\alpha/(x_o^\beta - x_o^\alpha), \quad . \quad (6)$$

the actual position on the column being given by $R^\beta D_s$.

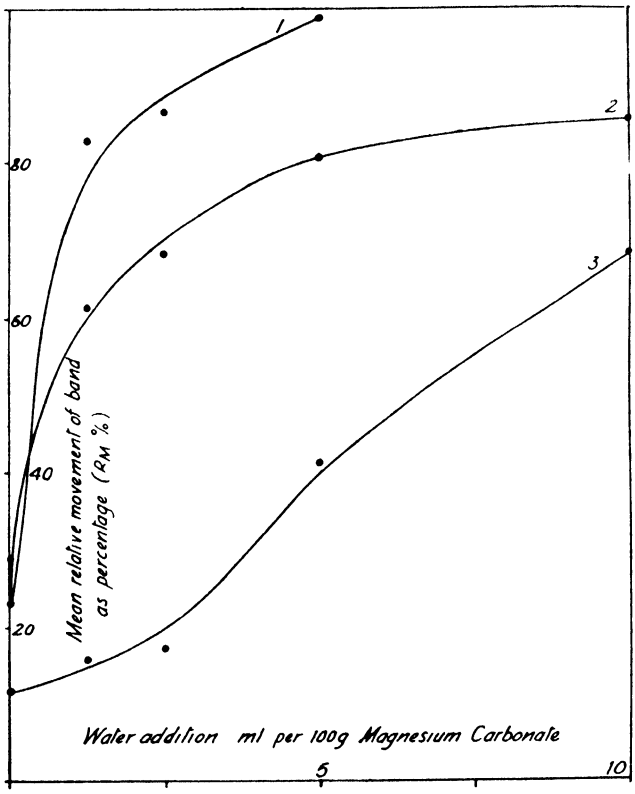

FIG. 2.—Effect of water deactivation treatment applied to unactivated magnesium carbonate on the band movement of anthraquinone compounds in toluene

Curve 1. 1-Hydroxy-4-*p*-toluidinoanthraquinone.
" 2. 1 : 4 : 5-Triaminoanthraquinone.
" 3. 1 : 4 : 5 : 8-Tetraminoanthraquinone.

The solvent flow required to produce the initial separation should depend on the ratio of the lower R value to the difference in R values, while the position at which this separation occurs and the actual rate at which the bands subsequently move apart depend on the value of R.

Certain complications limit the simple application of the above relations. The leading edge of a band moves considerably (20 %–30 %) faster than the following edge and the diminishing concentration at the band edges increases the difficulty of measuring the band movement and determining the position of separation. The bands move at a different rate in mid-column than at the periphery, probably 16 % faster in the columns considered below.

Experimental

The Control of Adsorbent Activity by Partial Deactivation.—The preferred method of deactivation consists in adding liquid water to the adsorbent, shaking to distribute the soft wet portion initially formed and mixing for 2 hr., conveniently by rotating the container. The addition necessary causes little or no change in the handling properties of the adsorbents; the method is easy to carry out and gives a reproducible behaviour. The deactivation is expressed as ml. water addition per 100 g. adsorbent, abbreviated to % H, the weight of adsorbent including any moisture initially present.

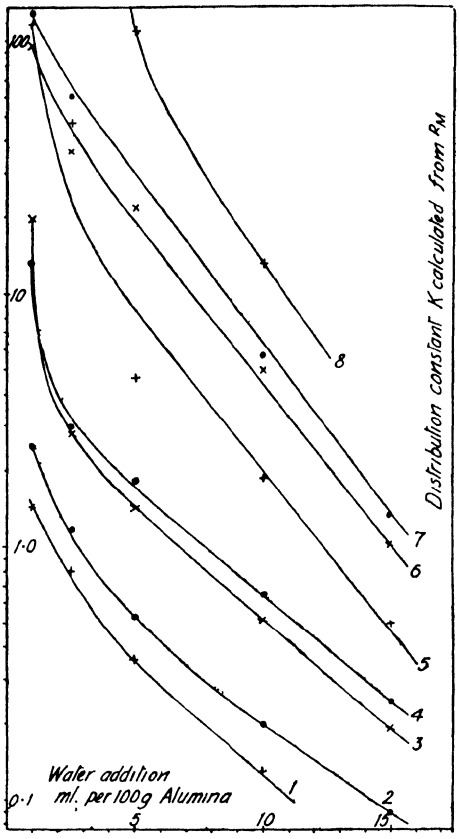

FIG. 3.—The effect of water deactivation treatment applied to activated alumina on the distribution constant of anthraquinone compounds between toluene and adsorbent. The curves are numbered as in Fig. 1.

The effect of partial deactivation on the band movement of simple anthraquinone compounds in toluene on activated alumina (Type O; P. Spence & Sons Ltd.) and on unactivated magnesium carbonate (Ponderous. B.P. quality; Cuxson, Gerrard and Co. Ltd.) is shown in Fig. 1 and 2, the band movement being given as the mean of the leading and following edge movement % R_M, determined and corrected for variation in column packing as described below.

This reproducible behaviour enables the rate of movement to be controlled over a wide range so that in conjunction with a suitable choice of organic solvent and a sufficiently high initial adsorbent activity most separations can be obtained at a convenient rate. In practice the two adsorbents mentioned, which are of satisfactory grain size for chromatographic use, meet most requirements. Inter-

mediate values of the deactivation tend to give most effective separations as the maximum increase of movement with deactivation generally occurs at low % H values for weakly adsorbed compounds and at higher values for those more strongly adsorbed. Occasionally the order of the band movement changes with the deactivation, generally at low values of % H (Fig. 3). Partial deactivation of activated alumina gives a distribution between adsorbent and solvent substantially independent of concentration and bands do not form long trails at one or other edge when this applies.

When the solvent used has affinity for water, equilibrium appears to be established with the deactivated adsorbent and any initial water present in the solvent affects the chromatographic behaviour to some extent. For separations from an aqueous medium this deactivation treatment is ineffective.

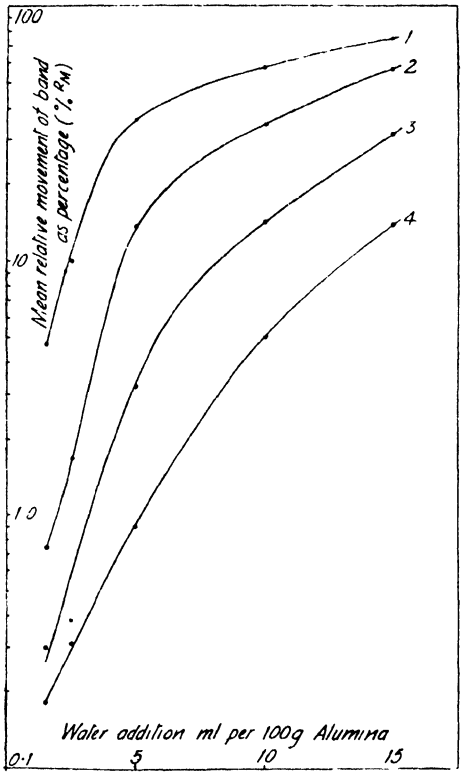

FIG. 4.—Band behaviour of the test mixture in toluene on alumina activated at 330° C for 4 hr.

Curve 1. Red band. Curve 3. Purple band.
,, 2. Blue band. ,, 4. Violet band.

Fig. 3 shows the effect of partial deactivation on the distribution constant, calculated from the % R_M values shown in Fig. 1 by means of eqn. (3). There is a very considerable change in the distribution with practically a linear relation between log K and % H when the deactivation exceeds 2·5 % H.

The Effect of Conditions of Activation of Alumina on the Deactivation Behaviour.—Activated alumina may be prepared [18] by heating aluminium trihydroxide at atmospheric pressure, the product, γ alumina, retaining some water to an extent varying with the activation temperature; it may also contain

[18] Holmes, Lava, Delfs and Cassidy, *J. Biol. Chem.*, 1933, **99**, 417.

alkaline impurities[18] which can if necessary be removed, but these rarely upset the chromatographic behaviour. Satisfactory commercial products are available which obviate the rather troublesome activation. To find the effect of activation conditions on the behaviour after water deactivation, dried hydrate of alumina (British Aluminium Co. Ltd.) was heated in an open pan at temperatures and for periods on temperature given below, together with the moisture content of the products determined by ignition to about 800° C.

Temperature ..	°C	250	330	330	330–360	400	500–550
Time	hr.	4	4	8	24	4	4
Moisture content	%	11·3	8·8	7·9	5·3	3·9	1·7

Each sample was deactivated with from 1·5 % to 15 % water addition (% *H*) and

FIG. 5.—Effect of conditions of activation on the relative movement of the violet band

Curve 1.	Activated at	250° C	4	hr.
,, 2.	,,	,, 330° C	4	,,
,, ⊙	,,	,, 330° C	8	,,
,, ×	,,	,, 330°–360° C	24	,,
,, 3.	,,	,, 400° C	4	,,
,, 4.	,,	,, 500°–550° C	4	,,

band movements measured using a toluene solution of four components which gave distinctive highly coloured bands covering a convenient range of adsorption affinity. The 10 ml. solution added to each column contained

0·77 mg. 1-Methylaminoanthraquinone (red, least adsorbed band),
0·34 mg. 1 : 4-Dimethylaminoanthraquinone (blue band),
1·28 mg. 1-Amino-4-methylaminoanthraquinone (purple band),
0·58 mg. 1 : 4-Diaminoanthraquinone (violet, most adsorbed band),

[18] Siewert and Jungnickel, *A.C.S. Abstr.*, 1943, **37**, 5898.

the actual concentration of the components being unimportant provided sufficient is present to give a highly coloured band. Fig. 4 shows the band behaviour of these components on one sample of adsorbent while Fig. 5 shows the effect of conditions of activation on the behaviour of the most strongly adsorbed component, the results for the others being similarly distributed and displaced to an extent indicated in Fig. 4. The curves in Fig. 5 show the effect of activation temperature, the effect of the heating period between 4 and 24 hr. at 330° C being within the experimental error.

The activation conditions employed have considerably less effect on the behaviour than has the subsequent deactivation treatment. There is no direct relation between the initial moisture content and the water added on deactivation, e.g., samples prepared at 250° and 530° C differ by 9·6 % in initial moisture but

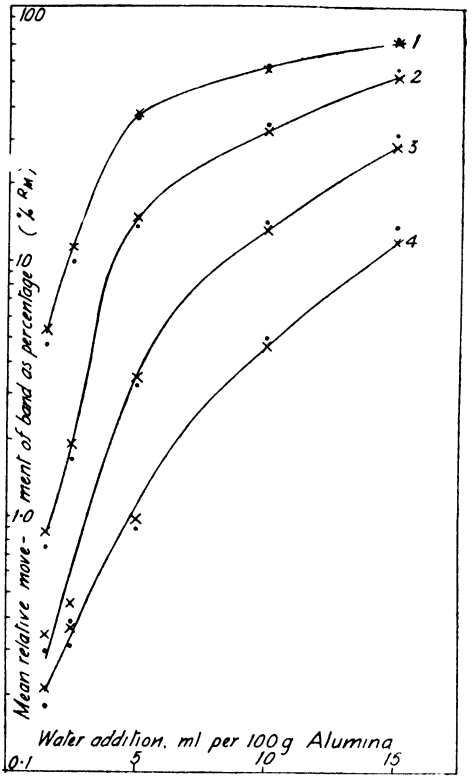

FIG 6.—Effect of the correction for variation in the ratio of solvent to adsorbent in alumina columns (×) and curves show the uncorrected values; (•) show the corrected values corresponding to the curves shown in Fig. 4. The i/m values were: 1·5 % H 0·580; 2·5 % H 0·583; 5·0 % H 0·542; 10 % H 0·462, 15 % H 0·425.

give practically the same relative band movement when a further 10 % water is added to both. For low values of % H the band movement increases with activation temperature, but the extent to which the band movement increases on further addition of water decreases with activation temperature. Qualitative tests show that activation temperatures above 500°–550° C give a progressive decrease in activity. The quality of activated alumina probably depends to some extent on the nature of the starting material and other factors, but the general behaviour on deactivation is similar

The Measurement of Band Movement.—The tubes used were 2 cm. diam. and 55 cm. long having a compact cotton wool retaining plug. Well-packed columns free from air bubbles were obtained from 150 g. alumina or

70 g. magnesium carbonate which were added as a slurry in toluene and settled, with assistance of intermittent tapping, to a constant column length before use; the interstitial solvent was determined by weighing the column before and after adding the adsorbent. The temperature remained close to 20° C throughout and no temperature correction was applied. The solvent used was high-grade technical toluene which had been passed through a column of the same adsorbent so as to minimize any possible effect on the activity. The solution under test was added (10 ml. of 0·1 % conc.) when the solvent level had dropped to the adsorption surface and as soon as this entered the column it was washed in with several small (0·5–1 ml.) lots of solvent before raising the solvent level to about 10 cm. above the adsorbent where it was maintained throughout the measurement by means of a constant level arrangement. Care was taken at all stages not to disturb the adsorbent or allow air to enter the column. Gravity flow was employed and was measured by weight. The position of the band edges and the solvent flow gave satisfactory linear relations against time from which the R_L and R_F values were obtained by means of the previously determined length of column occupied by 1 g. of interstitial solvent, and these are given throughout this paper as a percentage of the solvent movement.

A typical column was of 3·303 cm.^{2} cross-sectional area and 43·9 cm. length L, containing 150·3 g. ($m \times L$) activated alumina of 5 % H and 81·29 g. ($i \times L$) interstitial solvent; the latter occupies 0·540 cm. per g. solvent and for the observed flow of 54·34 g./hr. the linear movement of solvent was 29·29 cm./hr. The solvent movement varied between 23 and 45, average 28·8 cm /hr. for the various columns. The value of i/m was fairly constant for a single sample of adsorbent but varied from 0·6 to 0·4 as the % H increased from 1·5 to 15 %, the mean value being close to 0·5. Values of R corresponding to this mean value of i/m were obtained from the measurements on alumina columns from

$$R_{\text{corrected}} = \frac{1}{1 + \frac{1}{0{\cdot}5} \times \frac{i}{m} \frac{(1 - R)}{R}}.$$

The magnesium carbonate columns gave a mean value for i/m of 1·17, which was used in place of 0·5 for their correction.

The effect of the correction for i/m is shown in Fig. 6 where the uncorrected values corresponding to Fig. 4 are shown by (×) with curves drawn through these points and the corrected values—corresponding to the curves shown in Fig. 4—are indicated by (•). The correction is relatively small and without significant effect on the general relation between band movement and adsorbent activity.

The reproducibility of the band movement measurement was tested in a series of columns using a band having R_M 51 %; the standard deviation for the R_L and R_F values was 3·3 and 2·7 and for R_M 2·0. $R_L/R_M = 1{\cdot}15$, with standard deviation 0·06. The behaviour of these columns was found substantially unchanged after use in a series of measurements.

The reproducibility is reasonably satisfactory for comparative purposes involving fairly large differences, but apart from errors due to the difficulty of observing the exact position of the band edge and the occasional inclination of the bands there is a further error arising from the bands and presumably the solvent moving faster in mid-column than at the periphery. Since a uniform solvent movement over the cross-sectional area of the column has been assumed the R_M values are too low, results given below indicating this error to be about 11 % but no correction has been applied for this factor. Provided the comparisons are carried out in similar columns, as in the present case, this source of error should remain reasonably constant.

The Relation between Adsorption and Band Movement.—Preliminary adsorption measurements were made allowing a solvent solution to reach equilibrium with an alumina adsorbent and determining the equilibrium concentration, but this procedure was limited to low values of the latter by the low solubility of the compounds used and the photometric method employed. The results fitted the Freundlich adsorption equation,

$$\log_{10} (\text{specific adsorption}) = \log_{10} K + \alpha \log_{10} (\text{equilibrium concentration}),$$

α being about 0·75 for activated alumina but increasing to 1·0 on partial deactivation with water and becoming slightly greater than 1 for weak adsorption. Within a rather large experimental error there was agreement between the observed R_M value and that calculated from K by means of eqn. (2).

To obtain measurements at higher concentrations a 2-cm. column packed with 50 g. partially deactivated Type O alumina and of known interstitial volume was used, the solution being fed through the column until the issuing solution was at the initial concentration and the amount adsorbed determined

TABLE I

THE RELATION BETWEEN THE BAND MOVEMENT CALCULATED FROM ADSORPTION MEASUREMENT AND THE OBSERVED VALUE

Adsorbent: Partially deactivated Type O Activated Alumina.
% H = ml. water added per 100 g. adsorbent.
(w) measurement for band washing out.
(p) ,, from peripheral observation.

Adsorbate	Conditions	% R calc. from adsorption	% R obs.		
			R_L	R_F	R_M
1 : 4-Di-*p*-toluidinoanthraquinone	Toluene : 5 % H Conc. 46 mg. %	73·2, 72·7 } 73·0	(w) 81·7 (p) 74·8	55·8 51·4	68·7 63·1
	,, 18·1 ,,	76·0, 68·0 } 72·0	(p) 75·0	51·6	63·3
1-Methylaminoanthraquinone	Toluene : 5 % H Conc. 115 mg. %	55·0, 55·1 } 55·0	(p) 57·0	38·0	47·5
	,, 62·5 ,,	55·9, 54·1 } 55·0	(p) 57·3	37·0	47·2
	,, 33·2 ,,	53·5, 54·6 } 54·0	(p) 56·1	36·6	46·4
1-Amino-2-methylanthraquinone	Toluene : 5 % H Conc 46·2 mg %	37·0, 37·2 } 37·1	(w) 54·2 (p) 34·0	26·8 29·2	40·1 31·6
	,, 16·3 ,,	36·2, 36·4 } 36·3	(w) 50·3 (p) 33·0	27·4 28·2	38·8 30·6
	,, 4·4 ,,	34·6, 36·7 } 35·6	(w) 47·3 (p) 31·2	27·9 29·0	37·6 30·1
1-Amino-4-methylaminoanthraquinone	Toluene : 5 % H Conc. 23·1 mg. %	9·1, 9·5 } 9·3	(w) 13·3 9·8	7·2 7·5	10·3 8·6
	Benzene Conc. 4·0 mg. %				
	5 % H	17·7	(p) 22·4	14·1	18·2
	10 % H	46·2	(p) 51·0	35·3	43·1
	15 % H	80·1	(p) 106	60·7	83·5
1 : 4-Diaminoanthraquinone	Toluene : 5 % H Conc. 7·5 mg. %	8·8, 9·2 } 9·0	(p) 7·2	7·0	7·1

by difference and also by washing the band from the column, allowance being made for the solution held by the cotton wool retaining plug. The relative band movement was determined in the same column by observing the peripheral movement and in some cases by observing when the band started and finished washing from the column. The results are summarized in Table I.

There is reasonable agreement between the band movement calculated from adsorption measurements and the observed R_M values, the values obtained

by washing from the column being 5·1 % high and those obtained from peripheral observations 11·1 % low; as indicated above the latter are likely to be too low due to non-uniformity of solvent flow across the cross-sectional area of the column. The results confirm that band movement on the partially deactivated alumina adsorbent used is independent of concentration. A considerable difference is shown between the band movement of the leading and following edges which in the case of the peripheral results is a measure of the band widening further considered below: the somewhat greater difference obtained when the band is washed out represents the widening between the leading edge in mid-column and the following edge at the periphery.

The Widening of Bands.—(i) The leading and following edges of a band move down at a uniform rate and the widening of each edge is given by R_L/R_M. The widening is not uniform over the whole band but is greater towards the edges than for the main deeply coloured portion, the movement of 1-methylaminoanthraquinone in toluene on Type O 5 % *H* alumina being:

	R_F	R_L	R_M	R_L/R_M
Edges of band	43·2	59·2	51·2	1·15
Main portion of band ..	45·1	55·7	50·4	1·10

(ii) The R_L/R_M value is substantially the same for the bands of all components present in a column, independent of the R_M value and is little affected by the extent of deactivation Table II gives results from a set of measurements on the activation of alumina using the four component mixture as described above, which are typical of the six samples examined in this connection.

TABLE II

R_L/R_M FOR THE FOUR COMPONENT MIXTURE ON FIVE 2-CM. COLUMNS: ALUMINA ACTIVATED AT 330° C FOR 8 HR. AND PARTIALLY DEACTIVATED

Column – Deactivation (% *H*)	*i*/*m*	Violet Band	Purple Band	Blue Band	Red Band	Average
		R_L/R_M				
1·5	·565	1·14	1·04	1·05	1·12	1·09
2·5	·559	1·12	1·06	1·05	1·06	1·07
5	·526	1·29	1·23	1·17	1·11	1·20
10	·439	1·08	1·09	1·04	1·09	1·07
15	·403	1·09	1·07	1·04	1·07	1·07
Average	—	1·14	1·10	1·07	1·09	1·10
		R_M %				
1·5	—	·2	·4	1·2	7·9	2·4
2·5	—	·4	·6	2·3	14·1	4·3
5	—	1·6	5·0	19·3	41·2	16·8
10	—	5·6	15·4	38·1	61·1	30·0
15	—	18·7	39·3	64·5	80·6	50·8
Average	—	5·3	12·1	25·1	41·0	20·9

(iii) Similar band widening occurs in the absence of adsorption in passage through a column of 2 cm. diam. packed with non-adsorbent particles of similar grain size. 1 : 4-Di-*p*-toluidinoanthraquinone is not adsorbed from toluene solution by slightly moist and unactivated dried hydrate of alumina, fairly similar in particle size to the activated product used in Table II. Tests gave $R_M = 95·1$ % within the anticipated peripheral error of 100 %. The R_L/R_M value was 1·15. The concentration of the solution leaving the column was also measured photometrically, the results confirming the band widening as shown in Fig. 7.

An increase in the viscosity of the solvent by addition of liquid paraffin reduced the flow rate and band movement to a quarter but did not change the R_L/R_M value.

(iv) The above results indicate that the band widening is not dependent on adsorption but is directly proportional to the distance of travel along the column, since R_L/R_M is substantially the same for all the bands in a column, ΔD is constant and $R = \Delta x/\Delta D$.

(v) The bands from a solution containing several components move through a column in such a way that they have the same width at any given position in the column. Fig. 8 shows the observed band width plotted against the mean band position for the four component mixture referred to above, using a column of 2·4 cm. diam. There is appreciable observational error indicated

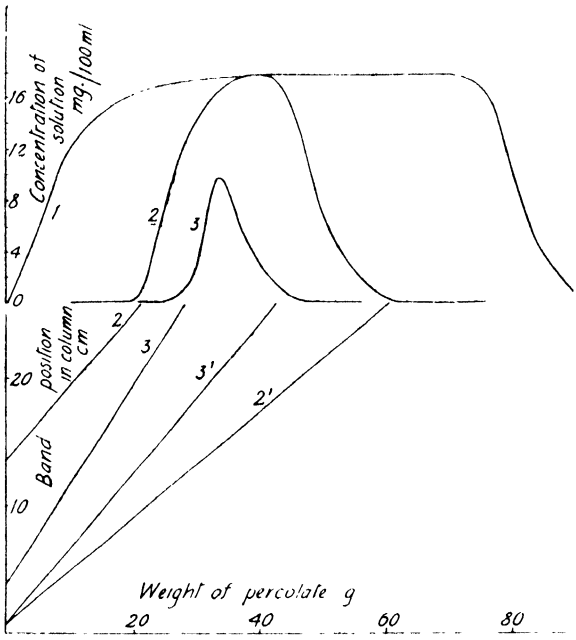

FIG 7.—Behaviour on a non-adsorbent column. Band position and concentration leaving column for 1 : 4-di-*p*-toluidinoanthraquinone in toluene on a 2-cm. column of unactivated alumina.

Curve 1 40 ml. (34·6 g.) initial solution conc. 17·8 mg per 100 ml.
,, 2 20 ,, (17·3 g) ,, ,, ,, ,, ,,
,, 3 5 ,, (4·3 g.) ,, ,, ,, ,, ,,

by vertical lines on which the mean value is marked. In the upper part of the column the widening is proportional to the distance travelled but beyond 25 cm. the rate of widening decreases, perhaps due to the band edges becoming too weak to be observed. The width of the four bands practically overlaps, although the leading band passed the 15 cm. position 48 hr. before the last band. A possible explanation of the effect is that the major part of the band width is due to the widening effect, which is similar for all the bands, and small differences arising from the length of the initial bands are concealed by the observational error.

(vi) EFFECT OF SOLVENT FLOW ON BAND WIDENING.—A moderate increase in the solvent movement D from 0·6 to 3·0 cm./min. had little effect in a 2-cm. column on the R_L/R_M value at 1·13. Increasing the flow to 15 cm./min. caused an appreciable initial widening of the band after which the rate of widening decreased to give an average value of 1·23.

(vii) EFFECT OF COLUMN DIAMETER.—The band widening appears to decrease as the column diameter increases, columns of 2, 5 and 7·5 cm. diam. giving

R_L/R_M values of 1·13, 1·06 and 1·04, the behaviour of the columns being otherwise identical. Equally small values have on occasion been obtained in 2-cm. diam. columns and the result may only indicate that the most effective packing is more easily obtained in columns of larger diameter.

(viii) A possible cause of band widening may be that the interstitial capillary spaces are not of uniform size and flow through some outdistancing flow through others leads to dilution and consequent widening of the band.

Although the band movement is faster in mid-column the rate of diffusion in a column is considered to be too small for diffusion from the mid-column to periphery to account for the widening. In addition the diffusion would be affected by the strength of adsorption, the viscosity of solvent and the rate of solvent flow.

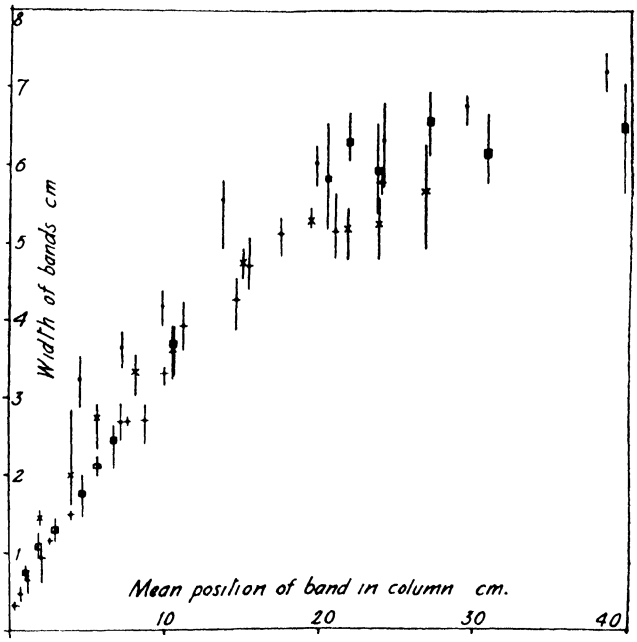

FIG. 8.—Variation in band width with mean position in column.
Solvent: Toluene; Adsorbent: Alumina activated 300° C 24 hr 5 % *H*.
Column: 2·4 cm diam

● Red band. ⊡ Purple band.
× Blue band. -●- Violet band.

Some diffusion is to be expected when a solution moves along a capillary space between two solvent layers since the movement is a maximum in mid-capillary and zero at the capillary surface. But if this was the full explanation band widening might be expected to be more sensitive to the viscosity of the solvent and rate of solvent movement, and to increase in a more closely packed column.

The Length of the Initial Band.—From eqn. (2) the length of band obtained on adding a solution of a pure compound to a column x_0 should equal RD_0, the product of the relative band movement and the length of column occupied by a volume of interstitial solvent equal to the volume of solution added. Fig. 9 shows the observed x_0 plotted against R_LD_0 and R_FD_0 from the results of 50 measurements covering 20 pure compounds, in which 10 or 20 ml. of solution was used, D_0 being approximately 5 or 10 cm.

There is fairly satisfactory agreement between x_0 and R_LD_0, whereas the value of R_FD_0 is only about 70 % of x_0. For strongly adsorbed compounds the initial band is very small and difficult to measure and the x_0 value tends to be too

large in these cases. The average values for the 40 measurements in which the band exceeded 1 cm. length were :

x_0 4·59 cm. ;	R_LD_0 4·66 cm.	R_FD_0 3·40 cm.
	R_LD_0/x_0 1·00, std. dev. 0·17	R_FD_0/x_0 0·71, std. dev. 0·14

The agreement between x_0 and R_LD_0 is to be expected since the initial band is itself a leading edge and its length will be correspondingly greater than the theoretical R_MD_0 and equal R_LD_0.

The Relation between Chemical Constitution and Chromatographic Behaviour of Anthraquinone Compounds.—Strain [14] has shown that the relation between constitution and chromatographic behaviour frequently depends on the solvent and adsorbent conditions used and this is found to apply to

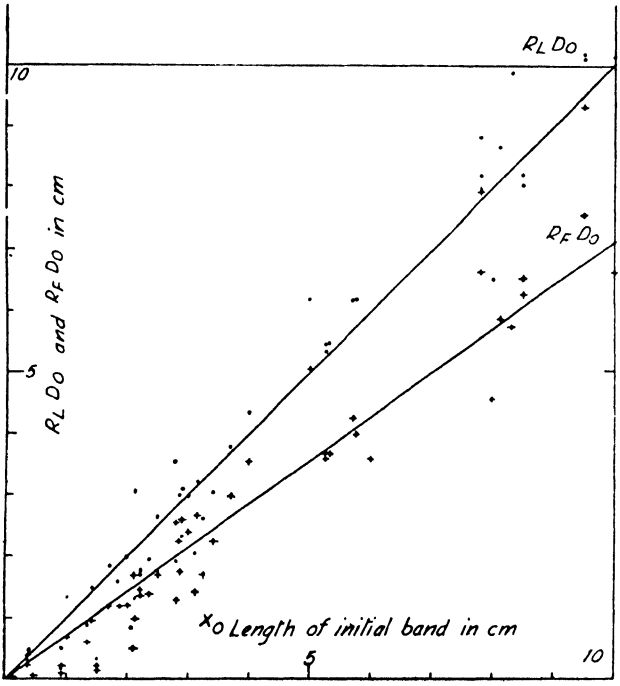

FIG. 9.—Relation between x_0 and R_LD_0 (●) or R_FD_0 (+).

anthraquinone compounds, in some cases a change in the activity of the adsorbent by water addition being sufficient to alter the order in which bands move. Within this qualification certain general rules serve as a guide to the behaviour and appear to be governed by two factors: (i) the specific action of certain groups such as hydroxy and amino in increasing the adsorption, probably as Meunier and Vinet [15] suggest due to hydrogen bonding between the group and the adsorbent; (ii) the avidity of the various compounds for the solvent employed acting against a fairly general adsorptive power.

The order of increasing adsorption for mono-substitution is generally halogeno, nitro, arylamino, alkylamino, amino, acylamido, hydroxy group in side chain, and hydroxy group attached to nucleus. There does not appear to be any systematic relation between mono-substitution in the 1 and 2 positions.

Adsorption generally increases with increasing number of substituent groups of the same composition, to an extent which varies with the position occupied

[14] Strain, *Ind. Eng. Chem. (Anal.)*, 1946, **18**, 605.
[15] Meunier and Vinet, *Chromatographie et Mesomerie* (Masson & Cie, Paris).

and the nature of the substituent, but decreased adsorption from aqueous medium occurs in the case of the sulphonate group. The introduction of further groups of different composition may either increase or decrease the adsorption, thus methyl, halogeno and arylamino groups tend to decrease and amino, methoxy and hydroxy to increase the adsorption.

Discussion

The simple method described for the deactivation of adsorbents gives remarkable control over the chromatographic behaviour and is easily applied as a further variable to the choice of solvent and adsorbent when selecting the conditions for any particular separation. It enables the separations to be obtained at a convenient rate and in this connection is of considerable assistance when a flowing chromatogram is combined with photometric measurement for the quantitative determination of the components.[16 17]

Appreciable heat of wetting is generated when a solvent is added to an adsorbent, even after partial deactivation and as Müller [3] has shown, the heat generated gives a measure of the activity. It would appear that the adsorption of a solute depends on competition between the solute, water and solvent for the available active surface. In the case of activated alumina a small degree of deactivation renders the distribution of a solute between solvent and adsorbent independent of the concentration, a factor which facilitates the chromatographic separation ; under these conditions fairly satisfactory agreement has been obtained between the mean relative movement of the bands, the length of the initial band and the values predicted from theory.

The solvent flow is greater in mid-column than at the periphery where the almost spherical adsorbent particles are in contact with a surface of much smaller curvature, and the packing would be expected to be less perfect. Although the chromatograph columns of alumina show dilatency the particles are not in closest packing—the interstitial volume is too high for this—and it seems likely that they are in a flocculated state similar to that described by Kruyt and Selms [18] for suspensions of silica particles in organic solvent, in which case the glass surface of the tube may be linked to the adsorbent particles by forces similar to those responsible for the flocculation. The troublesome band widening appears to be related to the manner of flow through the interstitial spaces.

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[16] Cropper and Strafford, *J. Soc. Chem. Ind.*, 1944, **63**, 268.
[17] Cropper, *Analyst*, 1946, **71**, 263.
[18] Kruyt and Selms, *Rec. trav. chim.*, 1943, **62**, 407.

EQUILIBRIUM AND RATE STUDIES OF CATION-EXCHANGE WITH MONOFUNCTIONAL RESINS

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The preparation of sulphonated cross-linked polystyrene and of cross-linked polymethacrylic acid is described. The sodium-hydrogen exchange equilibria for both materials have been examined. The former resin is shown to behave as a monofunctional strong acid and the latter as a monofunctional weak acid. The application of the law of mass action to the exchange equilibria is discussed.

A study of the rate of sodium-hydrogen exchange with sulphonated cross-linked polystyrene suggests that at low concentrations of sodium ions in solution the rate-determining mechanism is diffusion of ions through a thin film of liquid surrounding the resin particle. The influence of hydroxyl ion concentration on the rate of exchange of sodium for hydrogen with both resins is described, and the conditions under which the diffusion of ions within the resin particle may become the rate-controlling process are discussed.

In the application of ion-exchange resins to chromatographic techniques, more information is required on the fundamental molecular and ionic processes involved. Correlation of such information with the basic chemical and macromolecular structure may be expected to lead to the development of improved materials. In equilibrium and rate studies, monofunctional resins obtained by addition polymerization, e.g., sulphonated cross-linked polystyrene and cross-linked polymethacrylic acid, offer many advantages. In contrast to earlier materials obtained by polycondensation, e.g., sulphonated phenol-formaldehyde resins, these materials possess a fairly well-defined structure which may be systematically varied. They may also be examined over a wide range of pH without complicating factors arising due to the presence of different types of ionizable group. Moreover, the technique of suspension polymerization enables the resins to be prepared in the form of spherical beads which are especially suitable for rate studies.

In this paper the preliminary results of an investigation into the equilibrium and rate processes with sulphonated cross-linked polystyrene and cross-linked polymethacrylic acid are presented.

Experimental

Preparation of Cation-exchange Resins.—SULPHONATED CROSS-LINKED POLYSTYRENE. This material was prepared by the sulphonation of a cross-linked polystyrene bead polymer as described by D'Alelio.[1] Styrene was co-polymerized at 80° C for 18 hr. with *ca.* 10 % divinylbenzene, 1 % benzoyl peroxide being employed as catalyst. The co-polymer was sulphonated with concentrated sulphuric acid at 100° C for 8 hr. using 1 % silver sulphate as catalyst. The maximum capacity of the product (5·25 milli-equivalents of base per g. dry hydrogen form) was independent of particle size and agreed with the value calculated for a monosulphonic acid. The material was hygroscopic and the dry hydrogen form absorbed approximately 80 % water at 20° C. On conversion from the wet hydrogen form to the wet sodium form a decrease in volume of 6 %–7 % was observed.

CROSS-LINKED POLYMETHACRYLIC ACID. The carboxylic type exchange resin was prepared from methacrylic acid and divinylbenzene. Commercial methacrylic acid redistilled *in vacuo* was polymerized with *ca.* 10 % divinylbenzene in the presence of 1 % benzoyl peroxide. Polymerization was carried out in a sealed tube at 60° C for 24 hr. The product was treated with 2 N NaOH to remove soluble material, washed and dried. The maximum capacity (9·2 milli-equivalents of base per g. dry hydrogen form) was consistent with the value calculated from the composition of the monomer mixture. The dry material absorbed approximately 130 % of water at 20° C and an increase in volume of approximately 75 % was observed on conversion from the wet hydrogen to the wet sodium form.

After preliminary cycling in a column between 2 M NaCl or 2 N NaOH and 2 N HCl, the cation-exchange resins were converted to the hydrogen form and washed with distilled water. Washing was continued until the pH of the effluent attained a value of 4·0 or higher. If necessary, fines were removed by elutriation and the ion-exchange resins were air-dried to a uniform moisture content.

[1] D'Alelio, *U.S. Pat.* 2,366,007.

Equilibrium Studies.—Samples of the cation-exchange resins in the hydrogen form were weighed out into a series of bottles and further samples were taken for moisture content determinations. The latter were dried to constant weight over P_2O_5 in a vacuum desiccator Different amounts of NaOH solution with or without NaCl solution were added to the samples of resin and the solutions made up to 50 ml. For the sulphonated cross-linked polystyrene the liquid-solid ratio was 100/1, for the cross-linked polymethacrylic acid 200/1. The solutions were allowed to stand with occasional shaking until equilibrium was attained (1–7 days). Aliquot samples of the solutions were then withdrawn and titrated with standard HCl or NaOH to determine the extent of exchange. The pH values of the solutions were determined using a Cambridge pH meter and glass electrode. For the measurement of pH values greater than 9·0, a Cambridge Alki electrode was employed.

Rate Studies.—To obtain samples of resin of approximately uniform particle diameter, the resins were sieved in the air-dry state using calibrated B.S. sieves. For the determination of rates of exchange two methods were employed.

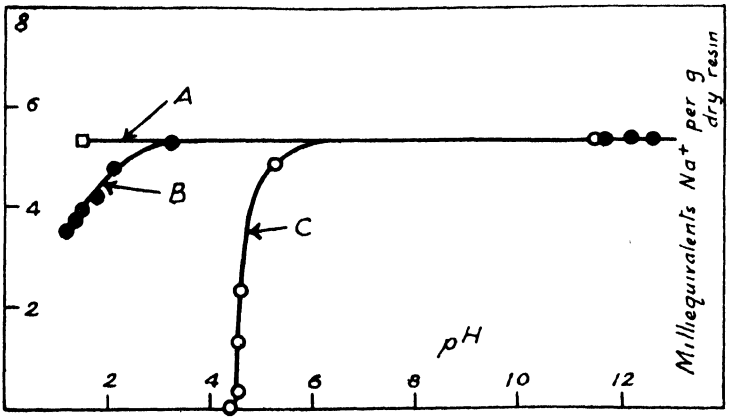

FIG 1.—Sulphonated cross-linked polystyrene. Relationship between Na^+ ion taken up by the resin and pH.

A. In presence of 5 M NaCl. B. In presence of 0·1 M NaCl. C In absence of NaCl.

(*a*) INDICATOR METHOD. This method, which is only applicable to sulphonated cross-linked polystyrene, depends on the fact that whether the solution is acid or alkaline, exchange will proceed virtually to completion if the ratio $[Na^+]/[H^+]$ in solution is sufficiently high (see below). The hydrogen form of the resin is stirred with a solution of NaCl and NaOH, the latter being less than sufficient to neutralize the hydrogen ions liberated in the exchange process. The solution, initially alkaline, becomes acid when the amount of exchange just exceeds the amount of alkali originally added An indicator preferably of the anionic type is used to show this change.

A weighed amount of resin of known moisture content was added to a known volume of water containing a few drops of bromo-cresol green indicator solution (0·04 % solution in water) in a small beaker. The mixture was stirred vigorously with a magnetic stirrer and a suitable mixture of NaCl and NaOH solutions added. The time elapsing between the addition of the alkali solution and the colour transition (blue→yellow) was measured with a stop-watch. All experiments were carried out at room temperature (18°–22° C) and with a constant volume of solution.

(*b*) SHALLOW-BED METHOD. A simple modification of the method used by Boyd, Adamson and Myers [2] was employed. The resin sample (*ca.* 0·1 g.) was supported

[2] Boyd, Adamson and Myers, *J. Amer. Chem. Soc.*, 1947, **69**, 2836.

on stainless steel gauze or a sintered-glass disc. The resin was first converted to the hydrogen form and washed free of acid. The solution containing sodium ions was then passed through the bed for an appropriate time at a known flow-rate. The bed was then immediately washed with a stream of distilled water. In the case of the sulphonated cross-linked polystyrene, the amount of residual hydrogen ion was determined by displacement, using an excess of NaCl solution and titrating the solution with standard alkali. For the cross-linked polymethacrylic acid, the amount of exchange was determined by removing the sodium ion with a measured volume of standard acid and back titration of the acid solution.

Results

Exchange Equilibria.—The amount of sodium ion taken up at equilibrium by the resins and its dependence on pH and on the ratio $[Na^+]/[H^+]$ in solution is shown in Fig. 1, 2 and 3.

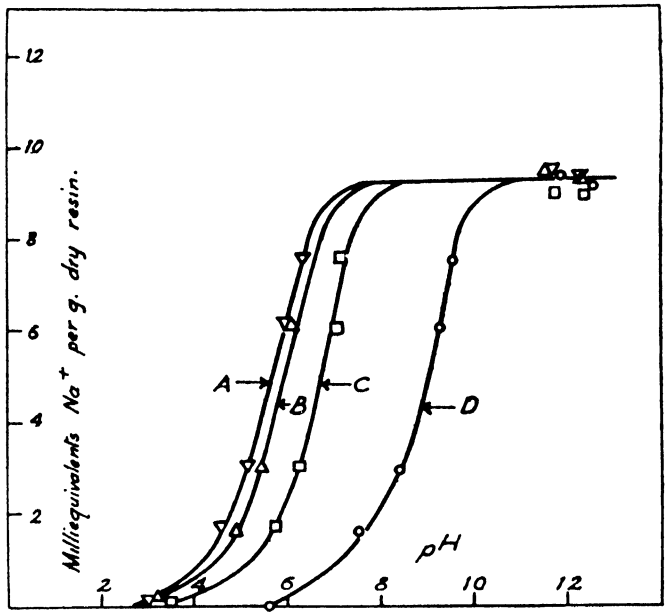

FIG. 2.—Cross-linked polymethacrylic acid. Relationship between Na^+ ion taken up by the resin and pH.

A. In presence of 2 M NaCl. B. In presence of 1 M NaCl. C. In presence of 0·1 M NaCl. D. In absence of NaCl.

It will be seen from Fig. 3 that the Na^+ ion taken up by both resins was dependent only on $[Na^+]/[H^+]$ in solution and not on $[Na^+]$ or $[H^+]$ separately. In the case of the sulphonic acid type exchanger, provided the $[Na^+]/[H^+]$ ratio in solution is greater than 100/1, virtually complete replacement of hydrogen by sodium ion is effected. For the exchange resin containing carboxylic groups, a $[Na^+]/[H^+]$ ratio of at least $10^3/1$ is necessary to effect complete conversion to the sodium form.

Exchange Kinetics.—Using the indicator method it was found that above a minimum rate of stirring the results obtained were independent of the stirring rate. This was found to apply over the whole range of sodium ion concentrations studied. Under the conditions employed, however, the degree of mixing of resin and solution may be expected to be lower than that attained with the shallow-bed method.

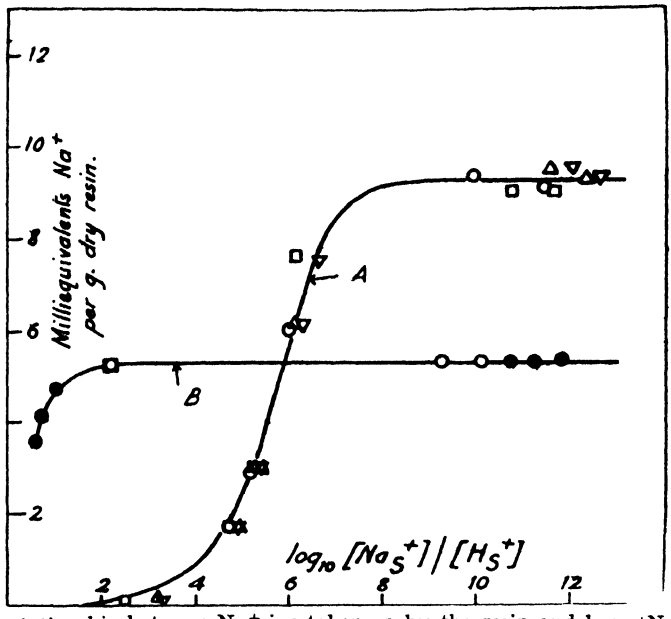

FIG. 3.—Relationship between Na^+ ion taken up by the resin and $\log_{10}[Na_s^+]/[H_s^+]$. A. Cross-linked polymethacrylic acid. B. Sulphonated cross-linked polystyrene.

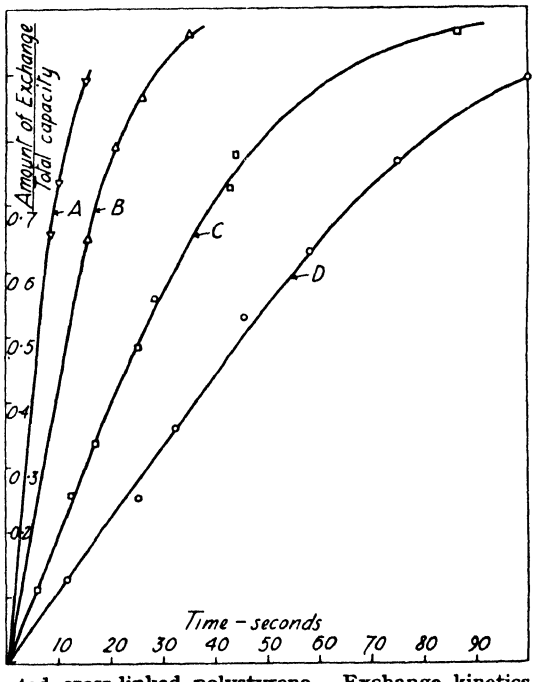

FIG. 4.—Sulphonated cross-linked polystyrene. Exchange kinetics at low Na^+ ion concentrations. (Indicator method.)

A.	Air-dry particle diameter		50–100 μ	$[Na_s^+]$	0·048–0·050 M
B.	,,	,,	,,	$[Na_s^+]$	0·026–0·029 M
C.	,,	,,	300–400 μ	$[Na_s^+]$	0·045–0·050 M
D.	,,	,,	,,	$[Na_s^+]$	0·023–0·028 M

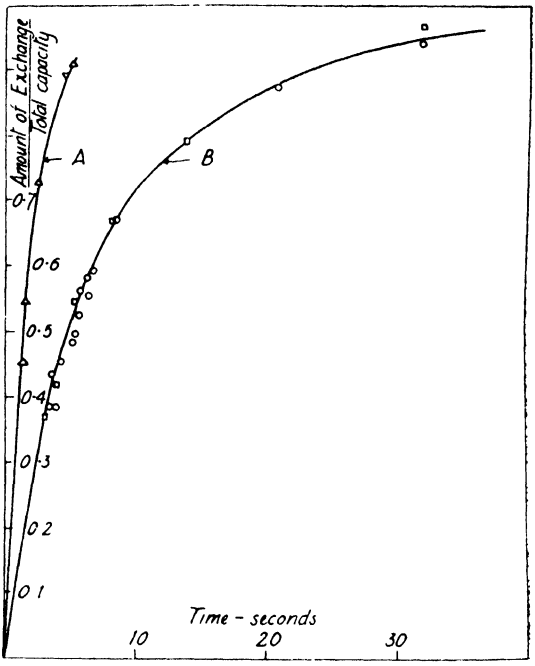

FIG. 5.—Sulphonated cross-linked polystyrene. Exchange kinetics at high Na^+ ion concentrations. (Indicator method.)

A. Air-dry particle diameter 50–100 μ — ∇ $[Na_s^+]$ 2·18 M; Δ $[Na_s^+]$ 1·09 M
B. Air-dry particle diameter 300–400 μ — □ $[Na_s^+]$ 2·18 M; ○ $[Na_s^+]$ 1·09 M

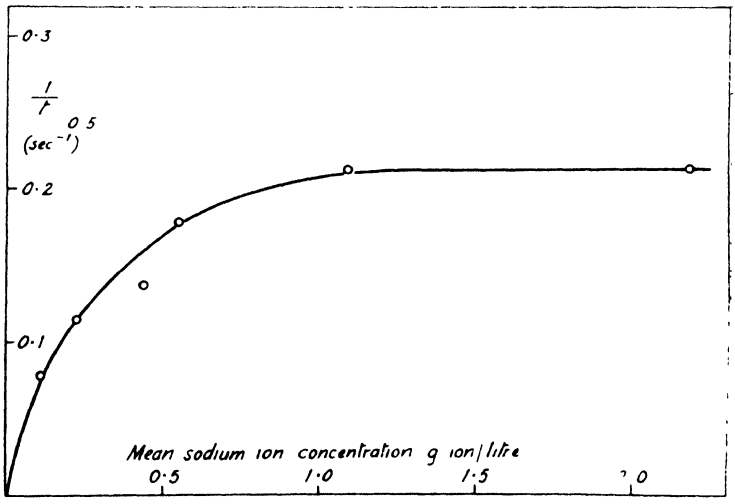

FIG. 6 —Sulphonated cross-linked polystyrene. Relationship between half-life and Na^+ ion concentration. (Indicator method.) Air-dry particle diameter 300–400 μ.

The effect of sodium ion concentration in solution and the particle size of the resin were examined using the indicator method. The results obtained are shown in Fig. 4 and 5.

At low sodium ion concentrations, as shown in Fig. 4, the exchange proceeds initially at an approximately constant rate but then slows down progressively as the exchange proceeds. At high sodium ion concentrations (Fig. 5) the form of curve obtained is similar but owing to the high rate of exchange under these conditions the initial rate of exchange cannot be determined accurately by the present method. The reciprocal of the time for half-conversion of the resin to the sodium form is plotted against the mean sodium ion concentration in solution in Fig. 6

It appears that at high sodium ion concentrations the rate of exchange is independent of the sodium ion concentration, whilst at low sodium ion concentrations the rate is proportional to the sodium ion concentration. This is shown also in Fig. 7 where the initial rate of exchange has been plotted against the mean sodium ion concentration in solution

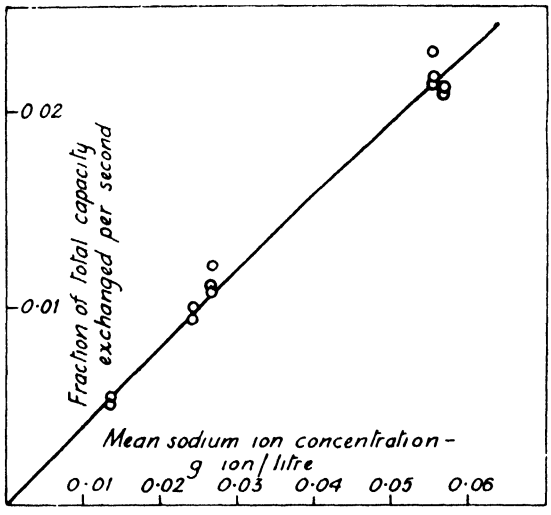

FIG. 7 —Sulphonated cross-linked polystyrene Relationship between rate of exchange and Na^+ ion concentration at low Na^+ ion concentrations. (Indicator method.) Air-dry particle diameter 300–400 μ.

At low sodium ion concentrations there is a change in the sodium ion concentration as the reaction proceeds. It has however been found that the results are in agreement with the relationship

$$d(Na)/dt = Kw[Na^+],$$

where (Na) is the total amount of exchange, w the weight of resin, $[Na^+]$ the sodium ion concentration in solution and K a constant.

It will be seen from Fig. 4 that at low sodium ion concentrations the rate of exchange with particles of diameter 50–100 μ is about four times as great as that with particles of diameter 300–400 μ. Thus, at low sodium ion concentrations the rate of exchange is approximately inversely proportional to the particle diameter. At high sodium ion concentrations no quantitative conclusions can be drawn from the present data, but it is apparent from Fig. 5 that exchange takes place more rapidly with the smaller particles.

The indicator method cannot be used for the direct investigation of the effect of hydroxyl ion concentration on the rate of exchange, and the shallow-bed method was employed for this purpose (see Fig. 8).

Results obtained by the indicator method were confirmed in that, with NaCl solutions, the exchange rate was independent of sodium ion concentration

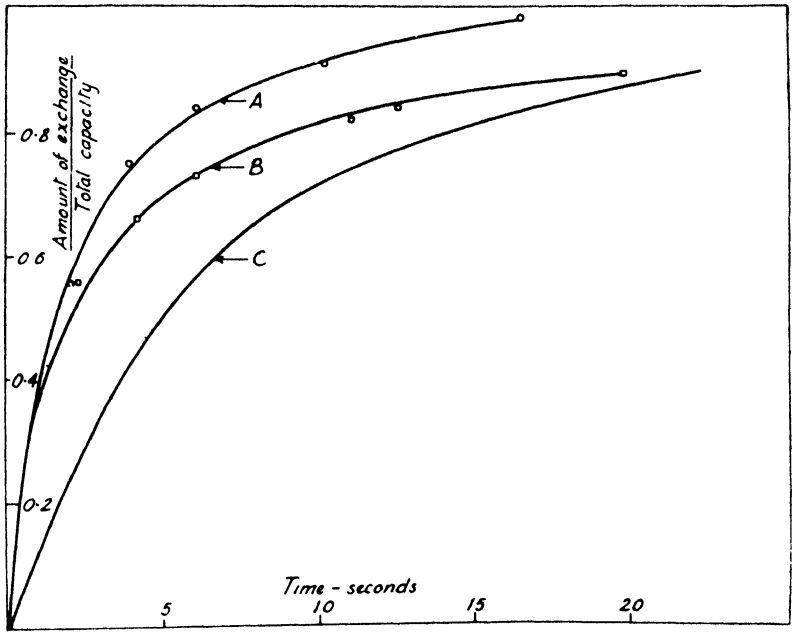

FIG. 8.—Sulphonated cross-linked polystyrene. Exchange kinetics at high Na^+ ion concentration. Air-dry particle diameter 300–400 μ.

A. Shallow-bed method. 2 N NaOH and 1 N NaOH solutions. Flow-rates 15 cm./sec. and 30 cm./sec.
B. Shallow-bed method. 2 M NaCl and 1 M NaCl solutions. Flow-rates 15 cm./sec. and 30 cm./sec.
C. Indicator method. Na^+ ion concentration 2 M and 1 M.

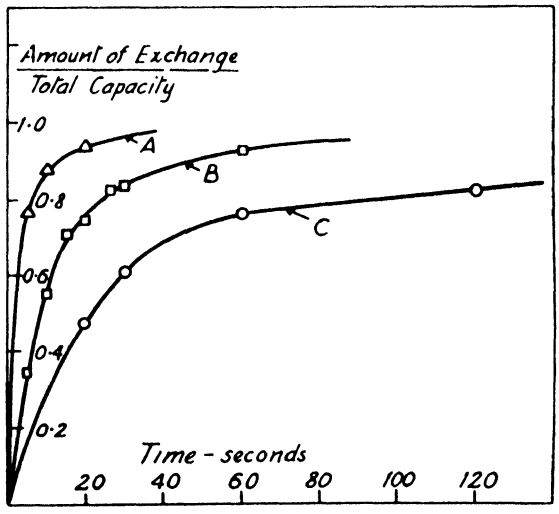

FIG. 9.—Cross-linked polymethacrylic acid. Exchange kinetics at high Na^+ concentrations. Air-dry particle diameter 250–380 μ. Flow-rate 1 cm./sec.

A. Na^+ concentration 2·2 M, OH^- concentration 0·2 M.
B. ,, ,, 2·1 M, ,, ,, 0·1 M.
C. ,, ,, 2·0 M, ,, ,, 0·025 M.

above 1 M. In addition, it was shown that the rate of exchange increased with increase in hydroxyl ion concentration and was independent of hydroxyl ion concentration above 1 M. Variations in flow-rate from 15 cm./sec. to 30 cm./sec. did not affect the rate of exchange, but the shallow-bed technique using M NaCl gave faster rates of exchange than the indicator method.

The results obtained with cross-linked polymethacrylic acid, using the shallow-bed method and keeping the flow-rate and Na^+ concentration virtually constant, are given in Fig. 9.

It will be seen that increase in hydroxyl ion concentration markedly increases the rate of exchange.

Discussion

Exchange Equilibria.—From the study of the exchange equilibria with sulphonated cross-linked polystyrene, we conclude that if the $[Na^+]/[H^+]$ ratio in solution is greater than 100 the resin is fully ionized. It is not possible to say, on the present evidence, whether the resin is fully ionized at all values of $[Na^+]/[H^+]$. However, it is clear that the resin behaves as a fairly strong acid and only one type of grouping appears to be present.

Consideration of the exchange equilibria of the polymethacrylic acid resin

$$H^+_R + Na^+_S \rightleftharpoons Na^+_R + H^+_S$$

leads to the mass-action relation

$$\frac{[Na^+_R]}{[H^+_R]} = K_1 \frac{[Na^+_S]}{[H^+_S]}, \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

activity coefficients being neglected.

$[Na^+_R]$ and $[H^+_R]$ are the concentrations of Na^+ ion and H^+ ion in the resin in g. equiv./l.

$[Na^+_S]$ and $[H^+_S]$ are the concentrations of Na^+ ion and H^+ ion in the solution in g. equiv./l. K_1 is the relative affinity constant of sodium and hydrogen *ions* for the resin. By analogy with sulphonic acid resins, we may expect K_1 to have a value of from 1 to 2.

In order to compare eqn. (1) with experimental data, we must have a relationship between $[H^+_R]$ and the total concentration of carboxylic hydrogen on the resin. We may assume that

$$\frac{[H^+_R]}{[\text{Total carboxylic hydrogen}]} = K, \quad . \quad . \quad (2)$$

where K is a constant. This leads to the equation

$$\frac{[Na^+_R]}{[\text{Total carboxylic hydrogen}]} = KK_1 \frac{[Na^+_S]}{[H^+_S]}. \quad . \quad . \quad (3)$$

It has been found that an equation of this type fits the experimental data quite well, with a value of $2{\cdot}24 \times 10^{-6}$ for KK_1.

Alternatively, the ionization of the resin may be assumed to follow the laws holding for dilute solutions. We then write

$$\frac{[H^+_R]\,[R^-]}{[HR]} = K_2, \quad . \quad . \quad . \quad . \quad (4)$$

where $[HR]$ and $[R^-]$ are the concentrations of unionized and ionized resin in g. equiv./l. K_2 may be expected to have a value of the same order (10^{-5} g. equiv./l.) as for the carboxylic group in simple compounds.

From eqn. (1) and (4) and assuming electro-neutrality of the resin phase, the following relation may be derived:

$$[Na^+_R] = \frac{-xK_1K_2}{2} + \tfrac{1}{2}\left[xK_1K_2\left\{xK_1K_2 + \frac{4c}{a\left(1 + \frac{1}{xK_1}\right)}\right\}\right]^{\frac{1}{2}}, \quad . \quad (5)$$

where $x = [Na^+_S]/[H^+_S]$.

c = total capacity of resin in g. equiv. per g. dry resin.

a = volume of wet resin in l./g. dry resin.

It is assumed that a is constant and independent of both $[Na^+_S]$ and $[H^+_S]$. While large variations in the volume of the resin are observed, the effect is small considered in relation to the enormous variations in $[Na^+_S]/[H^+_S]$.

Since $K_1 \simeq 1$ and $[Na^+_R]$ only becomes significant for values of $[Na^+_S]/[H^+_S]$ greater than 10^3, $1/K_1x$ is less than 10^{-3} and may be neglected in comparison with unity. Therefore

$$[Na^+_R] = \frac{-xK_1K_2}{2} + \tfrac{1}{2}\left[xK_1K_2\left\{xK_1K_2 + \frac{4c}{a}\right\}\right]^{\frac{1}{2}} \quad . \quad . \quad . \quad (6)$$

Expressing the amount of sodium ion on the resin in g. equiv. per g. dry resin, we have

$$y = a\,[Na^+_R] = \frac{-x(aK_1K_2)}{2} + \tfrac{1}{2}\left[x(aK_1K_2)\left\{x(aK_1K_2) + 4c\right\}\right]^{\frac{1}{2}} \quad . \quad (7)$$

From eqn. (5) we see that the amount of sodium taken up by the resin depends on the ratio $[Na^+_S]/[H^+_S]$ and not on $[Na^+_S]$ and $[H^+_S]$ separately. As constants, eqn. (7) contains only the capacity c and the parameter aK_1K_2.

This latter may be estimated. $K_1 \simeq 1$, $K_2 \simeq 10^{-5}$ g. equiv./l. and since the density of the wet resin is approximately unity, $a \simeq 10^{-3}$ l./g. Hence $aK_1K_2 \simeq 10^{-8}$ g. equiv./g. dry resin. Alternatively, a value for aK_1K_2 may be found from the experimental data. It may be shown from eqn. (7) that when $y = \frac{1}{2}c$,

$$x = \tfrac{1}{2}\frac{c}{(aK_1K_2)}$$

This leads to a value of $1{\cdot}05 \times 10^{-8}$ g. equiv./g. for aK_1K_2.

With $c = 9{\cdot}24 \times 10^{-3}$ g. equiv./l. and $aK_1K_2 = 10^{-8}$ g. equiv./l., values of y were calculated for various values of $\log_{10}[Na^+_S]/[H^+_S]$ using eqn. (7). The results are represented by the smooth curve in Fig. 3. The agreement between the calculated curve and the experimental points is good in view of the assumptions made.

It is concluded that the behaviour of cross-linked polymethacrylic acid is well-accounted for by assuming that only a small fraction of the carboxylic hydrogen is ionized. The exact relationships governing the degree of ionization is a matter for further investigation.

Exchange Kinetics.—Boyd, Adamson and Myers[2] have studied the rates of exchange of various cations on the phenolic resin, Amberlite IR-1. In their discussion they consider three mechanisms, each of which might be rate-controlling under appropriate conditions:

(i) Diffusion of ions through a thin film of liquid surrounding the particles (or through liquid in macropores inside the particles).

(ii) Diffusion of ions through the resin material itself.

(iii) The chemical process of exchange.

In the present work, if the chemical process of exchange were the sole rate-controlling process, the measured rate of exchange would be independent of the particle size. The fact that, both at high and low sodium ion concentrations, the rate varied markedly with particle size would seem to rule out the chemical process as a major rate-controlling factor.

If any type of film diffusion is a rate-controlling process, this is because, despite stirring, rapid flow or any other attempt to make the solution

homogeneous right up to the surface of the particle, there is a thin film inside which mixing is imperfect. (The film may also be due to cracks or macropores in the particle; in this case, the rate of stirring will have practically no effect on the characteristics of the film.)

If the diffusion of sodium ions across the film towards the resin is the sole rate-controlling process, the concentration of sodium ions at the inside boundary of the film will be virtually zero (owing to the diffusion of ions into the particle being very rapid in comparison). Hence the concentration gradient (and the diffusion rate) of sodium ions across the film will be proportional to the concentration of sodium ions in solution. To a first approximation, the film thickness may be assumed to be independent of particle size. The rate of exchange will then be inversely proportional to the particle diameter (provided macropores play a negligible part in film diffusion) since the surface area per particle is proportional to the square of the diameter and the capacity is proportional to the cube of the diameter.

It is probable that the diffusion rates of sodium and hydrogen ions within the resin particle are largely coupled, owing to the powerful electrostatic forces which oppose the entry of anions and also any variation of the total cation concentration in the resin. We may therefore speak of diffusion of ions within the resin being the rate-controlling process without specifying sodium or hydrogen ions separately. If diffusion of ions within the resin is the sole rate-controlling process, this means that the effect of film diffusion has been eliminated and the surface of the resin is in equilibrium with the bulk of the solution. Since in all the present work with sulphonated polystyrene, the $[Na^+]/[H^+]$ ratio in the solution was at least 10^4, the surface of the resin will be saturated with sodium ions. Hence diffusion of sodium ions into the interior of the resin particle will occur from a constant surface concentration which is independent of the sodium ion concentration in the solution. Hence the rate of exchange will be independent of the sodium ion concentration in the solution.

If diffusion of hydrogen ions through the film away from the resin is the sole rate-controlling process, the rate of exchange will be independent of sodium ion concentration but will be dependent on the hydroxyl ion concentration of the solution. Hydroxyl ions will diffuse towards the surface of the particle and will neutralize hydrogen ions in the film thus increasing the rate of removal of hydrogen ions.

The results obtained at low sodium ion concentrations ($< 0{\cdot}1$ M) show exactly the characteristics expected if film diffusion of the sodium ions is the rate-controlling process. At high sodium ion concentrations, the rate is independent of sodium ion concentration and hence the rate-controlling process might be either the diffusion of ions within the particle or the diffusion of hydrogen ions across the film or both. However, Fig. 8 shows that, using the shallow-bed technique, increase in the hydroxyl ion concentration from zero to 1 M increased the exchange rate while a further increase to 2 M had no effect. Hence we may conclude that with M or 2 M NaCl solutions the film diffusion of hydrogen ions is rate controlling, while with N or 2 N NaOH solutions diffusion of ions within the particle is the sole rate-controlling process. The diffusion coefficients of sodium, hydrogen and hydroxyl ions are of the same order. Similar concentrations (1 M) of hydroxyl or sodium ions in solution are therefore likely to be required to eliminate film diffusion of hydrogen and sodium ions as rate-controlling processes.

No satisfactory explanation can be given for the observation that with the shallow-bed method increase in the flow-rate from 15 to 30 cm./sec. failed to increase the rate of exchange with NaCl solutions. The fact that at low sodium ion concentrations the rate was inversely proportional to particle diameter appears to preclude the possibility of macropores playing

an appreciable part in film diffusion. We can only suppose that owing to the highly turbulent nature of the flow in our experiments the effective film thickness was not altered by the apparent flow-rate.

We consider now in more detail the way in which, at high Na^+ concentrations and virtually zero OH^- concentrations, the rate of diffusion of hydrogen ions through the film controls the exchange process. This rate of diffusion is initially zero but increases progressively as the concentration of hydrogen ions outside the particle surface builds up. However, long before the rate of hydrogen ion film diffusion becomes equal to the rate of diffusion of ions within the resin, the ratio $[Na^+]/[H^+]$ in the solution immediately outside the particle surface will have fallen appreciably, causing a decrease in the concentration of Na^+ ions *inside* the particle surface. Hence the rate of diffusion of ions within the particle will decrease.

When we consider the rate of Na–H exchange with the polymethacrylic acid resin, it is apparent that the film diffusion of hydrogen ions will be much more important than with sulphonated polystyrene resins, owing to the much larger $[Na^+]/[H^+]$ ratio (10^8) in the solution necessary to maintain the surface of the resin particle saturated with sodium ions. The results (Fig. 9) confirm this expectation.

In conclusion, it appears that for the diffusion of ions within the resin to be the rate-controlling process, it is necessary to have in solution high concentrations of both Na^+ and OH^- ions.

The work described above has been carried out as part of the research programme of the Chemical Research Laboratory and this paper is published by permission of the Director of the Laboratory. The authors wish to thank their colleagues on the staff of the Chemical Research Laboratory for advice and assistance.

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CATION EXCHANGE WITH A SYNTHETIC PHENOLSULPHONATE RESIN

Part V. Kinetics

BY T. R. E. KRESSMAN AND J. A. KITCHENER

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A study has been made of the kinetics of exchange between solutions of various simple inorganic and substituted quaternary ammonium salts and the ammonium form of the sulphonated phenol–formaldehyde resin for which equilibrium measurements have been reported elsewhere.

Two mechanisms are observed, where the rate is controlled by diffusion in the particles of the exchanger (*P*-mechanism) and in the bounding Nernst film (*F*-mechanism) respectively. These are distinguished by the form of the kinetics, by interruption tests and by the influence of stirring. The factors that decide which mechanism applies in a given system are discussed.

Measurements of the influence of temperature suggest that so long as the cation is small compared with the pores of the resin (e.g., Na^+ and NMe_4^+ with the present resin) the energy of activation for diffusion is *ca.* 5 kcal /mole as for free diffusion in water. Larger ions, which have difficulty in penetrating, show a higher value, e.g., 8 kcal./mole with $PhNMe_3CH_2Ph^+$.

Exchange occurs readily between a granular resin and a finely divided resin in suspension, proving that soluble anions are not needed to release cations from the resin. The high mobility of the cations is consistent with a diffuse double-layer model.

The potentialities of ion exchange separation techniques can be fully exploited only if consideration is given to both equilibrium and kinetic aspects. These are largely independent and they differ with different ion exchangers even though the exchange groups may be the same. There is no simple relationship between affinities and rates of exchange for a series of ions, although with the phenolsulphonate resin used in the present study the rate generally decreases as the affinity increases.

The possibilities of separations based on differences of *rates* of exchange have not yet been fully explored. Fig. 1 shows an example where such an effect might be employed: the concentrations of H^+, NH_4^+ and NEt_4^+ in a solution (initially equimolar with respect to NH_4^+ and NEt_4^+) are shown during the course of an exchange experiment with a H-resin (containing $-SO_3^-$ groups) in a stirred system. It is seen that the equilibrium affinity of NEt_4^+ for the resin is greater than that of NH_4^+ but the *rate* of exchange is greater for the NH_4^+ ion. If the experiment were stopped after 2 or 3 min. the solution would be relatively enriched in NEt_4^+, but at equilibrium the reverse would apply.

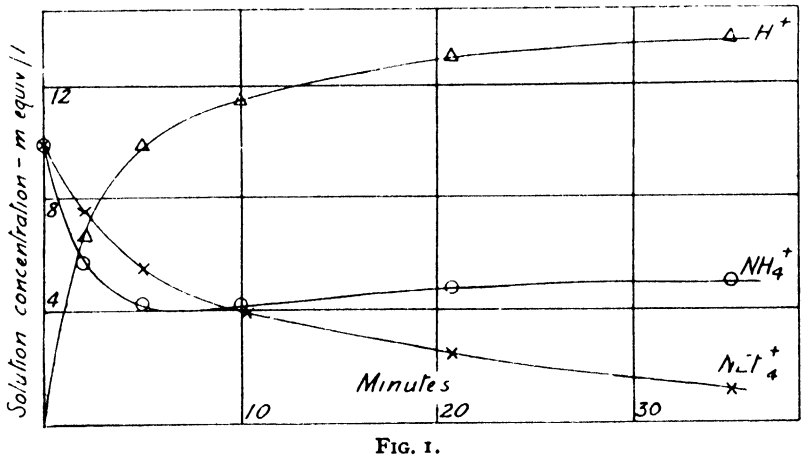


FIG. 1.

Both equilibria and kinetics for a series of ions depend very greatly on the structure of the solid exchanger. The equilibrium relationships for a phenolsulphonate resin similar to Zeo-Karb 215 have already been described elsewhere in Parts I–IV of this series of papers.[1] The present paper describes studies of the kinetics of exchange with the same resin.

Experiments and Results

Materials.—The sample of resin was taken from the same batch of H-resin as that used in earlier studies.[1] The general technique of converting the material to the salt form and of determining the equivalent weights, air drying, etc., is the same as described there. With the exception of the experiments illustrated in Fig. 1, where the H-form of the resin was used, the NH_4-form was used throughout the work and, after preparing it from the H-resin, it was air-dried and sieved $-10 + 22$ mesh.

Two samples were sieved out and all the results except those illustrated in Fig. 8 were obtained with one of these samples. Those in Fig. 8 were obtained with the second sample and, although the resin granules were also $-10 + 22$ mesh, a larger fraction of the larger mesh size was present, and the velocity of exchange is thus somewhat lower with this sample than with the first.

[1] Kressman and Kitchener, *J. Chem. Soc.*, 1949, 1190.

In the series of experiments illustrated in Fig. 1 the H-form of the resin was used and, because of its slight instability,[1] it was prepared by thoroughly washing the free acid from a sample taken from the main batch, air-drying rapidly and sieving — 10 +22 mesh ; it was then used immediately. In this way the experiments were completed before the H-resin had begun to hydrolyze.

Determination of Effective Sphere Radius of the Swollen Resin Granules.—A large number of particles—of the order of 500—were counted, allowed to swell in water, the water removed and the resin granules mopped between filter papers until the surface moisture had been removed. The swollen granules were then weighed. The density of the swollen granules was determined with the aid of a density bottle in the usual way. The effective sphere radius r of the swollen granules is calculated from

$$4/3 \cdot \pi r^3 \rho N = W,$$

where ρ is the density of the swollen resin, N the number of granules and W the weight of the N granules.

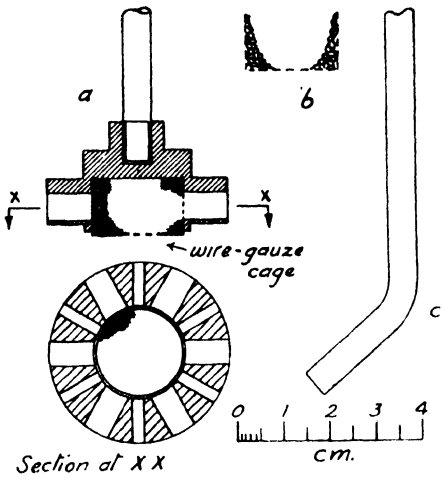


FIG. 2.

The first sample of NH_4-resin, which was used for the majority of the work (see under Materials), was found in this way to have an effective sphere radius of 0·446 mm. ; and the second sample, used for the experiments illustrated in Fig. 8, an effective sphere radius of 0·517 mm. The density of the swollen granules was 1·33 g./cm.[3]

Determination of Exchange Velocity.—The limited bath method was used throughout the work and not the column technique with infinite bath as used by Boyd *et al.*[2] The essential requirements in such heterogeneous studies of standardized vigorous agitation and the necessity of starting and stopping the reaction sharply, were achieved by using a centrifugal type of stirrer as shown in Fig. 2 *a*. The resin was placed in the wire-gauze cage and as the stirrer rotated in the solution, a very rapid stream of the solution passed over the resin, which was forced into a roughly cylindrical " wall " on the inside of the cage (see Fig. 2 *b*).

The constancy of speed of rotation of the stirrer was followed stroboscopically. By using several stroboscope discs constant speeds of rotation from 300 rev./min. at intervals to 1200 rev./min. were attained. The reaction was started by lowering the already rotating stirrer, with the resin in the cage, into the solution ; and it was stopped after the appropriate interval of time by raising it, still rotat-

[2] Boyd, Adamson and Myers, *J. Amer. Chem. Soc.*, 1947, **69**, 2836.

ing, out of the solution. An aliquot portion of the solution was then analyzed for NH_4^+ or H^+ as appropriate. Temperatures between 14° and 45° C were thermostatically controlled to within ± 0·2°; outside this range the limits were ± 0·5°.

The weight of resin taken in every run was such that it contained 2·5 m.equiv. of exchangeable cation, and 2·5 m.equiv. of the other cation were also present in the aqueous phase in the form of 125 ml. 0·02 N solution.

Fig. 3 shows that the mixing is at a maximum when the speed of rotation is between 1000 and 1100 rev./min.; higher speeds caused entrainment of air and, accordingly, an apparent decrease in velocity. The speed of rotation was, therefore, standardized at 1000 rev./min. throughout the work.

Influence of Agitation.—Fig. 3 shows how the time for half-change depends upon the speed of rotation of the stirrer for two systems, NH_4R + NaCl and NH_4R + NEt_4Br respectively. The time for half-change in the first system

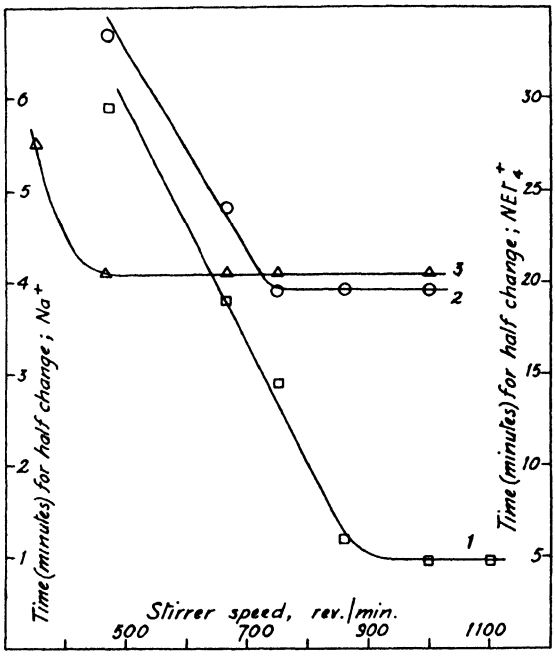


FIG. 3.—Effect of stirring on the time for half-change.
1. Centrifugal stirrer, Na^+.
2. Centrifugal stirrer, NEt_4^+.
3. Glass stirrer, NEt_4^+.

decreases as the speed of rotation is increased, approaching a minimum at 1000 rev./min. when the stationary liquid film surrounding the particles has reached a minimum. (Above about 1100 rev./min. entrainment of air occurs and erratic results are consequently obtained.) No greater velocity was obtained even when the solution was circulated over the resin (in the form of a shallow bed) extremely rapidly by means of a pump.

The time for half-change in the NEt_4^+ system is also practically at a minimum at 1000 rev./min. but, unlike the Na^+ system, it remains at a minimum at speeds down to 750 rev./min. and only then, when very little relative motion of solid and liquid is occurring, does it begin to increase.

Fig. 3 also shows a curve obtained in the NEt_4^+ system with a simple bent glass-rod stirrer (Fig. 2*c*). This stirrer is seen to be more efficient at slower speeds of rotation but at higher speeds the centrifugal stirrer results in a thinner

liquid film than does the simple stirrer. Evidently, so long as the glass stirrer keeps the particles in suspension, increased agitation has an immeasurable effect upon the thickness of the liquid film.

Preparation of NEt_4-resin in Fine Suspension.—A series of rate experiments was carried out in which no soluble anions whatever were present in the system, the exchange occurring between the ordinary NH_4-resin and a fine aqueous suspension of NEt_4-resin. This suspension was prepared by milling well-washed NEt_4-resin with water.

A sample of H-resin was washed free of acid and converted to the NEt_4-form with a solution of NEt_4Br. The rate of reaction is considerably less than with ammonium chloride and a lower rate of flow was accordingly used, and a greater volume of solution was found necessary to remove the whole of the exchangeable hydrogen ions from the resin. The resin—about 40 g.—was thoroughly washed to remove all traces of electrolytes and then ground in a laboratory porcelain ball mill for about 24 hr. with about 1 l. water. The milky suspension so obtained was allowed to settle for about 1 hr. and the liquid decanted. This consisted of a suspension of the finest particles, the largest of which were of the order of 1 μ diam. It was adjusted by dilution to contain 0·02 equiv. of exchangeable NEt_4^+ per l. of suspension, as indicated by a Kjeldahl nitrogen determination.

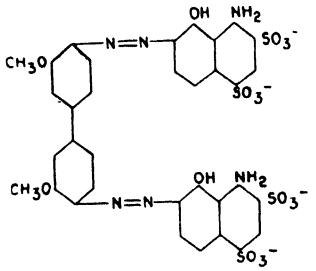


FIG. 4.—Anion of Chlorazol Sky Blue FFS.

Care was taken when using this suspension to ensure that homogeneous samples were taken from it. It was vigorously shaken just prior to, and kept gently agitated during, the removal of the sample. The NH_4-resin was washed with water before each run was started to remove any traces of electrolytes which might be contaminating it.

The velocity determination with this suspension was carried out exactly as with the aqueous salt solutions. The results obtained are shown in Fig. 5, curve 10, and Fig. 11, curve 6, and graphs of Q_t/Q_∞ against $\sqrt{t}$ for the NEt_4-resin suspension and for NEt_4Br indicate that the apparent rate constants are in the ratio of 0·74/1.

Preparation of the Salts of Chlorazol Sky Blue FFS.—The sodium salt was prepared from the crude dye by dissolving it in water and salting-out with sodium acetate. This was repeated three times. Adhering sodium acetate was finally removed by washing repeatedly with alcohol. The purified product was dried at 100° C.

The NEt_4-salt was prepared in solution from NEt_4OH and the pure dye acid. The NEt_4OH was prepared in known concentration (about 0·2 N) by adding excess moist silver oxide to a solution of NEt_4Br and filtering. The dye acid was prepared from the pure sodium salt by passing quantitatively a dilute solution (about 0·01 N) containing 4·96 g. of the salt through a 100 ml. column of Zeo-Karb 215 (The Permutit Co., Ltd.) containing exchangeable hydrogen ions. The solution was then evaporated on the water bath until its concentration was of the order of 0·03 N, cooled and "titrated" with the NEt_4OH to pH 7, using a pH meter. The quantity of NEt_4OH required was exactly that calculated from its known concentration. The solution of the NEt_4-dye salt was then diluted to 1 l.

In the runs with the dye salts, as in those with the NEt_4-resin suspension, the resin was washed with water before each run was started, to remove traces of electrolytes.

Velocity with Different Cations.—Since the equilibrium positions are very different with the various cations, and since only the kinetics are at present being considered, the extent of exchange at time t is expressed as Q_t/Q_∞, i.e., the fraction of the amount of exchange occurring at time t to that occurring at equilibrium. Fig. 5 shows the graphs of Q_t/Q_∞ against t for a number of different

cations at 25° C. The same results are plotted in Fig. 6 as $-\log_{10}(1 - Q_t/Q_\infty)$ against t and Q_t/Q_∞ against $\sqrt{t}$ for analysis of mechanism—see below.

Influence of Temperature.—The activation energies of the exchange reactions were determined from velocity measurements at several temperatures with the NH_4-resin and, respectively, Na^+, NMe_4^+ and $PhNMe_2CH_2Ph^+$.

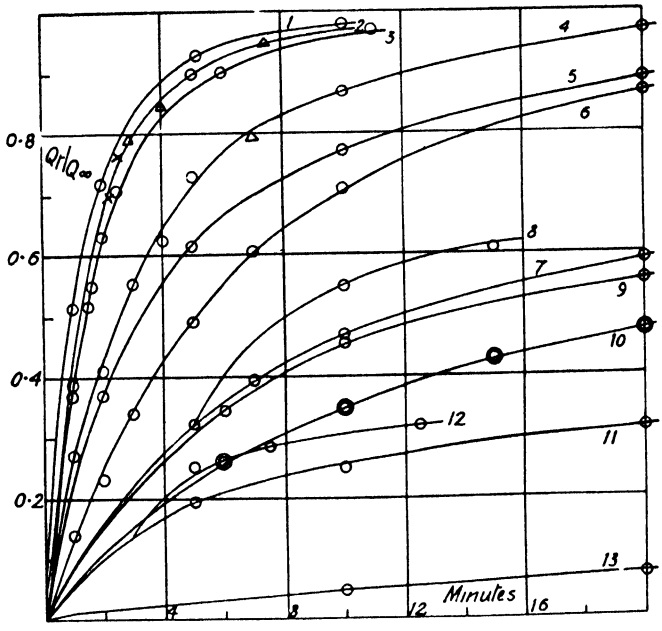


FIG. 5.—Rate of exchange between NH_4-resin and various cations at 25° C.

1. K^+ (chloride).
2. Na^+ O chloride, × interrupted, Δ Chlorazol Sky Blue salt.
3. Li^+ (chloride).
4. Mg^{++} and Ba^{++}, O chloride, Δ $MgSO_4$.
5. NMe_4^+ (bromide).
6. Al^{+++} (chloride).
7. NEt_4^+ (bromide).
8. NEt_4^+ (bromide) interrupted at 5 min. for 30 min.
9. NMe_3 *n*-Amyl$^+$ (bromide).
10. $PhNMe_2Et^+$ (bromide) and NEt_4-resin suspension.
11. Th^{++++} (nitrate).
12. Th^{++++} (nitrate) interrupted at 2·5 min. for 20 min.
13. $PhNMe_2CH_2Ph^+$ (chloride).

The results are shown in Fig. 7, 8, and 9, where they are plotted in terms of $-\log_{10}(1 - Q_t/Q_\infty)$ against t, and Q_t/Q_∞ against $\sqrt{t}$ as appropriate. The logarithms of the limiting slopes of the lines so obtained are plotted against the reciprocals of the absolute temperature in Fig. 10. The values for the activation energies given in Table I are obtained from the slopes of these straight lines.

TABLE I

Cation	Temperature range	Activation energy kcal /mole
Na^+ (P)	2°–14·8°	5·1
Na^+ (F)	25°–45·7°	5·0
NMe_4^+	1°–61°	5·1
$PhNMe_2CH_2Ph^+$	25°–50°	8·2

Discussion

The structure of the phenolsulphonate exchange resin is a three-dimensional rigid network resembling a sponge and containing five $-SO_3$ groups to every seven phenol residues (see Part I).[1] The cations are highly mobile inside the water-filled interstices, and form a Gouy diffuse double layer round each resin granule.

Consequently, cation exchange does not involve any " chemical " step, since no covalent bond has to be broken, and the rate of exchange depends simply on one or more of a series of consecutive *transport* steps—namely, forced convection of the fluid, diffusion through the unmixed boundary

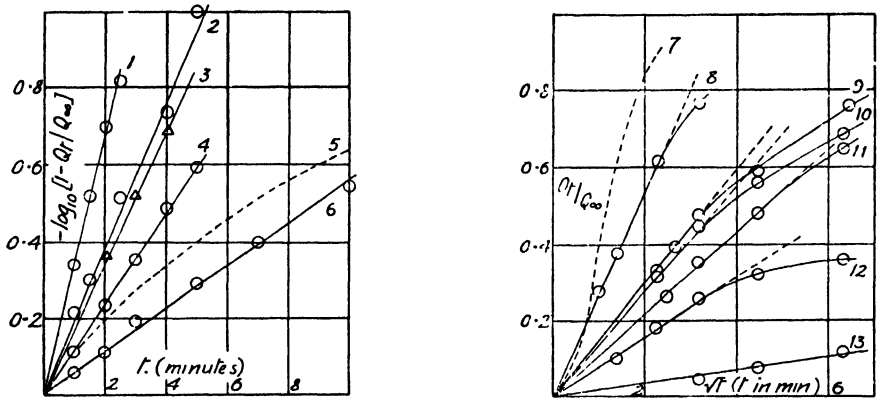


FIG. 6.—Tests of mechanism.

1. K^+.
2. Li^+.
3. Ag^+.
4. Mg^{++} and Ba^{++}.
5. NEt_4^+.
6. Al^{+++}.
7. Na^+(25°).
8. NMe_4^+.
9. NEt_4^+.
10. NMe_3,*n*-Amyl$^+$.
11. $PhNMe_2Et^+$ and NEt_4-resin suspension
12. Th^{++++}.
13. $PhNMe_2CH_2Ph^+$.

layer and diffusion inside the particles. As Boyd *et al.*[2] have shown, three possible types of kinetics may therefore be encountered in a practical, dynamic exchange process:

(i) Diffusion in the boundary liquid film is rate-determining (*F*-mechanism).

(ii) Diffusion in the solid particle is rate-determining (*P*-mechanism).

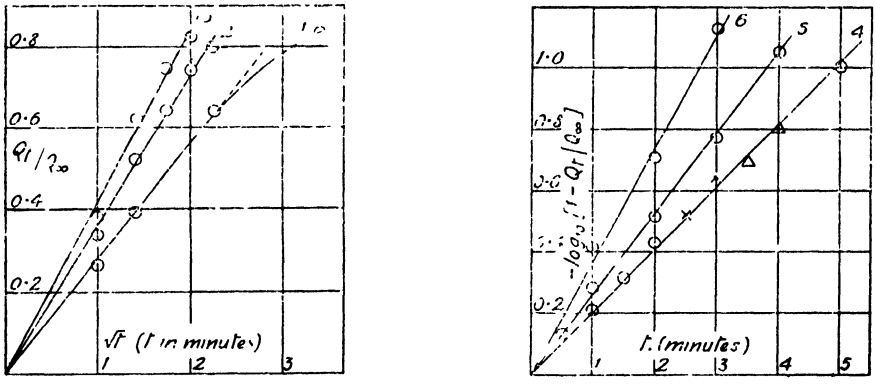
(iii) In an intermediate region the rate is influenced by diffusional resistances in *both* phases (*I*-mechanism).

The factors that may decide which mechanism applies in a given system are (1) particle size, (2) degree of agitation of the solution, (3) diffusion coefficients of the ions in the solution and inside the resin particles, (4) temperature, (5) the equilibrium distribution coefficient and (6) solution concentration. Variation of any one of these factors may produce a change of mechanism. For example, Boyd *et al.*[2] have shown that the Na^+–K^+ exchange (under their conditions of experiment) is *F* at 0·001 M ionic strength, *I* at 0·01 M and *P* at 0·1 M.

Criteria for Distinguishing Mechanisms.

(*a*) **Influence of Stirring.**—The rate increases with the degree of mixing of the liquid as long as the *F*- or *I*-mechanisms are rate-controlling, whereas with *P*-mechanism it should be independent. With increasing speed of rotation of a given stirrer, a maximum exchange rate may be reached, beyond which the rate may remain constant or even decrease (Fig. 3). Only exceptionally does such a maximum indicate the point where *I*-mechanism gives place to *P*. More usually, the rate reaches a limit for hydrodynamical reasons, the stirrer having reached its limiting efficiency for mixing, and it may still be possible to achieve faster rates of exchange by using a different type of stirrer (Fig. 3).

However, in border-line cases a change of mechanism may sometimes be detected by the influence of stirring—notably, when a maximum exchange rate is reached in the stirring range slower than the known hydrodynamical limit. The exchange with NEt_4^+ in Fig. 3 provides an instance of this.



(*a*) (*b*)

FIG. 7.—Influence of temperature, Na^+.

1. 2·0° C
2. 8·4°
3 14·8°
4. 25·0° C
5. 34·8°
6. 45·7°

O Chloride
× interrupted
Δ Chlorazol Sky Blue salt

(*b*) **Form of the Kinetics.**—(i) FILM DIFFUSION (*F*-MECHANISM).—The well-known Nernst static diffusion film theory is clearly only a crude approximation for the complex situation which exists near an irregular solid surface in a stirred liquid; in particular, the "thickness" of such a layer is a mathematical fiction which has proved of little use since this quantity must usually be deduced from the kinetics (not vice versa), and its dependence on stirring, temperature, viscosity, etc., is problematical. Only in a few idealized cases has it been possible to calculate the diffusional transport up to a body in a stirred liquid.[3] However, the Nernst layer approximation is useful for treating the kinetics of exchange under fixed conditions of stirring and temperature.[2]

In the present system, let Q_0 be the number of milli-equivalents of the pure A^+ form of the resin, which at time $t = 0$ is brought into contact with V ml. solution containing Q_0 milli-equivalents of a salt of the cation B^+. Suppose the resin consists of n particles of mean equivalent sphere radius r,

[3] Levich, *Acta Physicochim*, 1942, **17**, 257, 1944, **19**, 117, 133; *Faraday Soc. Discussions*, 1947, **1**, 37.

and that the effective Nernst diffusion layer thickness is δ. Let the amount of exchange which has occurred after time t be Q_t (milli-equivalents). The diffusion process taking place in the Nernst layer is essentially a cation exchange at constant ionic strength. The rate is therefore proportional to the gradient of concentration of A^+ (or B^+); let D_L be the Fick's law diffusion coefficient for the inter-diffusion of A^+ and B^+ under these conditions.

After time t the concentration of A^+ on the outer side of the Nernst layer is Q_t/V. The concentration of A^+ at the surface of the particle is more difficult to estimate. Boyd *et al.*[2] took this as the concentration which would be found in a solution in equilibrium with the prevailing resin composition. Under their conditions this was simply proportional to the $[A^+]$ in the resin, but under the present conditions it would be necessary to introduce the mass action equilibrium constant

$$K = \left(\frac{[A^+]}{[B^+]}\right)_{soln.} \times \left(\frac{[B^+]}{[A^+]}\right)_{resin}$$

(see Part I),[1] and the resulting kinetics would be mathematically very complicated.

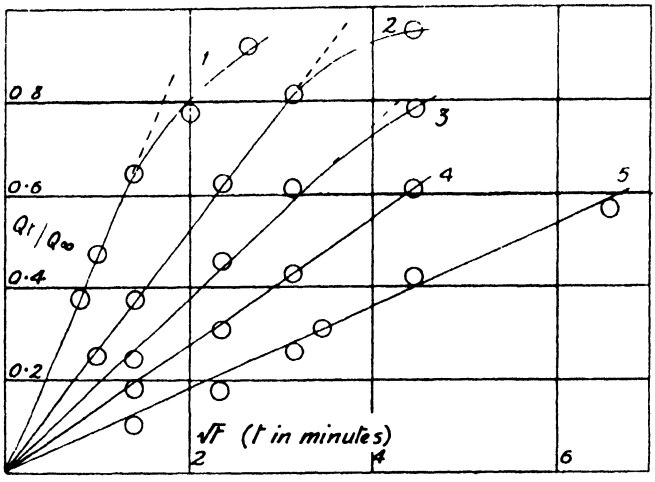


FIG. 8.—Influence of temperature, NMe_4^+.

1. 60·6° C
2. 41·3°
3. 25·0°
4. 12·6° C
5. 1·2°

However, it will be shown that the cations in the exchanger are highly mobile; there seems therefore to be no reason to distinguish between "free" and "bound" cations in the resin phase—at least for simple inorganic cations. Further, the number of cations present at the outer surface of the particles as gegenions to the $-SO_3^-$ groups far exceeds those present as soluble electrolyte from the solution phase since soluble anions are largely repelled by the opposing zeta potential.[4] Consequently, the number of A^+ ions which are free to participate in diffusion away from the surface of the sphere at any instant is simply proportional to the number present in the resin: let it be put equal to $k'(Q_0 - Q_t)$.

[4] See, for example, Verweg and Overbeek, *Theory of the Stability of Lyophobic Colloids*, (Elsevier, 1948), p. 31.

Application of Fick's law to the Nernst layer (the diffusion gradient being assumed linear) gives

$$\frac{dQ_t}{dt} = \frac{D_L}{\delta}\left[k'(Q_\infty - Q_t) - \frac{Q_t}{V}\right].$$

Integration leads finally to the equation

$$\frac{Q_\infty}{Q_0} \ln\left(1 - \frac{Q_t}{Q_\infty}\right) = kt,$$

where Q_∞ is the amount of A^+ which has passed into the solution when equilibrium has been reached, and k is a constant, equal to Dk'/δ.

This equation is of the same form as that obtained by Boyd *et al.*[2] (for an infinite bath), which was found to fit the kinetics of their Na^+–K^+ exchange in dilute solutions at least up to $Q_t/Q_\infty = 0{\cdot}4$. In the present work the equation fits the kinetics well in appropriate cases (see Fig. 6 *a* and 7 *b*). For example, the NH_4^+–Na^+ exchange at 25° C fits accurately from $Q_t/Q_\infty = 0$ up to at least $Q_t/Q_\infty = 0{\cdot}9$. Other cases which conform to these kinetics include Ag^+, K^+, Li^+, Mg^{++}, Ba^{++}, Al^{+++}.

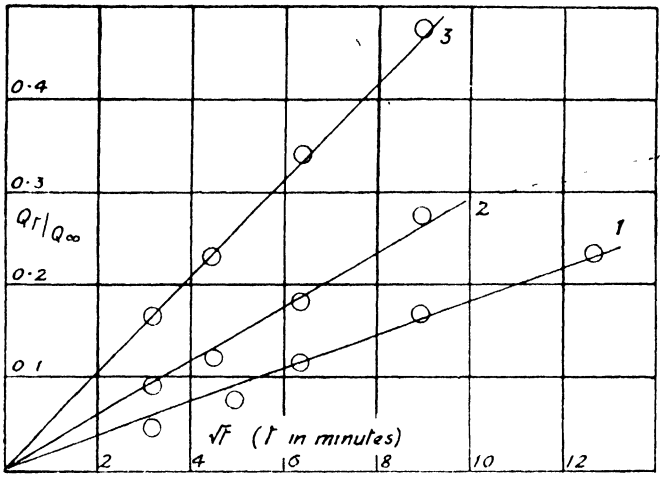


FIG. 9 —Influence of temperature, $PhNMe_2CH_2Ph^+$.
1. 25·0° C 2. 36·5° C 3 50·0° C

(ii) PARTICLE DIFFUSION (*P*-MECHANISM).—Assuming the solution effectively uniform up to the particle surface and the rate controlled by radial diffusion inwards with a constant diffusion coefficient, the kinetics of the *P*-mechanism should be formally similar to those for conduction of heat into a sphere from a well-stirred bath. Boyd *et al.*[2] have successfully applied the well-known theory for conduction of heat into a sphere from an infinite bath. The corresponding theory for a limited bath is given by Carslaw and Jaeger.[5] The form of their solution, however, is less convenient than that of an alternative solution recently given by Paterson,[6] viz.,

$$\frac{Q_t}{Q_\infty} = \frac{w+1}{w}\left\{1 - \frac{1}{\alpha-\beta}\left[\alpha e^{\alpha^2\tau}(1 + \operatorname{erf}\alpha\sqrt{\tau}) - \beta e^{\beta^2\tau}(1 + \operatorname{erf}\beta\sqrt{\tau})\right]\right\},$$

[5] Carslaw and Jaeger, *Conduction of Heat in Solids* (Oxford Univ. Press, 1947), pp. 83, 201.

[6] Paterson, *Proc. Physic. Soc.*, 1947, **59**, 50.

where $\tau = \frac{\varkappa t}{r^2}$, $\varkappa$ being the thermal diffusivity; and α and β are the roots of the equation

$$x^2 + 3wx - 3w = 0$$

and w is (heat capacity of the sphere)/(heat capacity of the bath). This solution is valid up to $\tau = 0{\cdot}1$ which, in practice, covers most of the process (e.g., up to $Q_t/Q_\infty = 0{\cdot}84$, when $w = 1$).

In attempting to apply Paterson's solution to the ion-exchange process with, e.g., NEt_4^+ and NH_4-resin, $\varkappa$ is identified with the cation-exchange diffusion coefficient inside the resin (D_P), and Q_t/Q_∞ with the ratio (NH_4^+ out in time t)/(NH_4^+ out at equilibrium), and w with the ratio (NEt_4^+ in

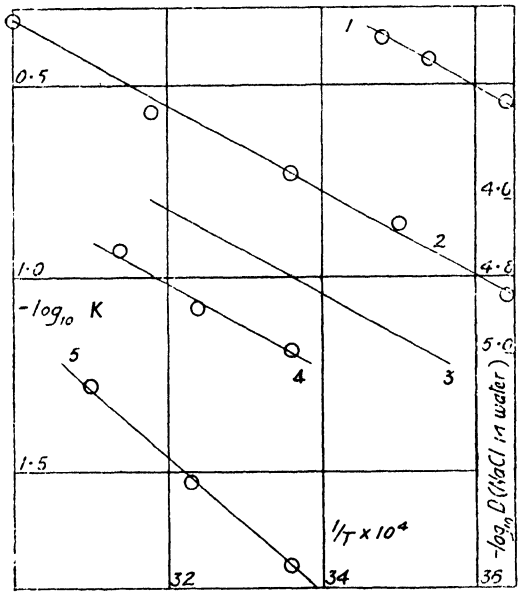


FIG. 10.—Activation energy data.

1. Na^+ (P).
2. NMe_4^+.
3. Diffusion coefficient data for NaCl in water.[7]
4. Na^+ (F) (−0·5 added to vertical scale).
5. $PhNMe_2.CH_2Ph^+$.

resin at equil.)/(NEt_4^+ in solution at equil.). From the value of w, α and β can be calculated and a graph of Q_t/Q_∞ as a function of τ can be computed. Since

$$\tau = (D_P/r^2)t,$$

the best value of (D_P/r^2) can be found to fit the theoretical curve to the experimental points (provided, of course, the theory gives the correct form of the kinetics); hence, r being determined independently, a value of D_P can be obtained.

The attempt to fit the equation in this way to the data in Fig. 11 did not lead to satisfactory agreement: when experiment and theory were fitted at $Q_t/Q_\infty = 0{\cdot}5$, the deviations were always in the sense that the equation predicted a more rapid reaction initially than in fact occurred. Barrer [8]

[7] *Int. Crit. Tables*, Vol. 5.
[8] Barrer, *Trans. Faraday Soc.*, 1949, **45**, 358.

has recently discussed possible causes for deviations in the somewhat analogous system of gas sorption by zeolites. Lack of conformity in the present system could be due to (1) slow swelling of the initially dry resin, and (2) change of diffusion coefficient with resin composition. (Curiously enough, good agreement was obtained when w was taken as (NH_4^+ in resin at equil.)/(NH_4^+ in solution at equil.); the lines in Fig. 11 were obtained in this way. The significance, if any, of this is not clear.)

Barrer [8] has shown that Paterson's solution, like the well-known one for diffusion into a sphere from an infinite bath, approximates to a $t^{\frac{1}{2}}$-relation for small values of Q_t/Q_∞, the limiting expression being

$$\frac{Q_t}{Q_\infty} = \frac{6}{r} \cdot \frac{Q_0}{Q_0 - Q_\infty} \cdot \sqrt{\frac{Dt}{\pi}}.$$

However, examination of such graphs shows that whereas the line for an infinite bath is within 2 % of linear up to about $Q_t/Q_\infty = 0{\cdot}5$, the linear range becomes progressively shorter as the bath becomes more limited. Thus, at $w = 1$ the $t^{\frac{1}{2}}$ graph is linear only within 4 % up to $Q_t/Q_\infty = 0{\cdot}25$. Consequently, in testing for P-mechanism by this method of plotting the data, a straight line is to be expected only at small values of Q_t/Q_∞. Fig. 6 *b* and 7 *a* show examples of exchange reactions which conform approximately to these expectations. Values for the nominal diffusion coefficients obtained from these graphs are given in Table II.

TABLE II

DIFFUSION COEFFICIENTS AT 25° C

Cation	D_P(cm.^{2}sec.$^{-1}$ × 10^6)
NMe_4^+	2·1
NEt_4^+	0·5
NMe_3 *n*-Amyl$^+$	0·3
$PhNMe_2Et^+$	0·1
$PhNMe_2CH_2Ph^+$	0·006

The $t^{\frac{1}{2}}$ graph is used as the first test for P-mechanism. It is fortunate that the F- and P-kinetics are sufficiently different in form to be distinguished readily by conformity to either log $(1 - Q_t/Q_\infty)$ against t or Q_t/Q_∞ against $t^{\frac{1}{2}}$ graphs respectively. Fig. 6 (curves 5 and 7) shows how data which fit one are clearly excluded from fitting the other.

(iii) INTERMEDIATE (I-MECHANISM).—The full kinetics of the I-process, transitional between F- and P-, have not yet been worked out for a limited bath. The analogous problem of heat conduction into a composite sphere has been solved for the case of a constant surface temperature.[9] However, the algebra is already very complicated, and with a finite bath would be much more so. Crank and Godson [10] have obtained approximate numerical solutions for certain cases of composite infinite cylinders in a limited bath using the method of finite differences. The same method could be used for the present problem, but it would be necessary to have more explicit knowledge of the constant k' in the F-kinetics theory than is at present available.

The only example of what appears to be I-mechanism encountered in the present work is the Na^+–NH_4^+ exchange within the range 15°–25° C, the kinetics at 25°, 35° and 46° fitting F-mechanism and those at 15°, 8° and 2° fitting P-mechanism.

(*c*) **Interruption tests** [11] provide a simple criterion for the existence of a large concentration gradient inside the particles, thus differentiating P- or I-mechanism from F. In the present work such tests have, in every case where applied, confirmed the mechanisms already suggested from the

[9] Carslaw and Jaeger, ref. 5, p. 288.
[10] Crank and Godson, *Phil. Mag.*, 1947, **38**, 794.
[11] Kunin and Myers, *J. Physic. Chem.*, 1947, **51**, 1111.

form of the kinetics. Thus, the Na^+–NH_4^+ exchange at 25° gives *F*-kinetics and shows no discontinuity on interruption (Fig. 7, curve 4) whereas Na^+–NH_4^+ at 2°, and NMe_4^+ and NEt_4^+–NH_4^+ at 25° give *P*-kinetics and show a large discontinuity in the expected direction. Likewise Th^{++++}–NH_4^+ gives *P*-kinetics and shows a similar discontinuity on interruption (see Fig. 5, curves 8 and 12).

(*d*) **Temperature coefficient** might be expected to afford a criterion of mechanism, since the temperature coefficient for diffusion through the solid might reasonably be supposed to be distinctly higher than for free diffusion in solution. This is certainly the case with large molecules such as dyes penetrating into fibres, where the activation energy is about

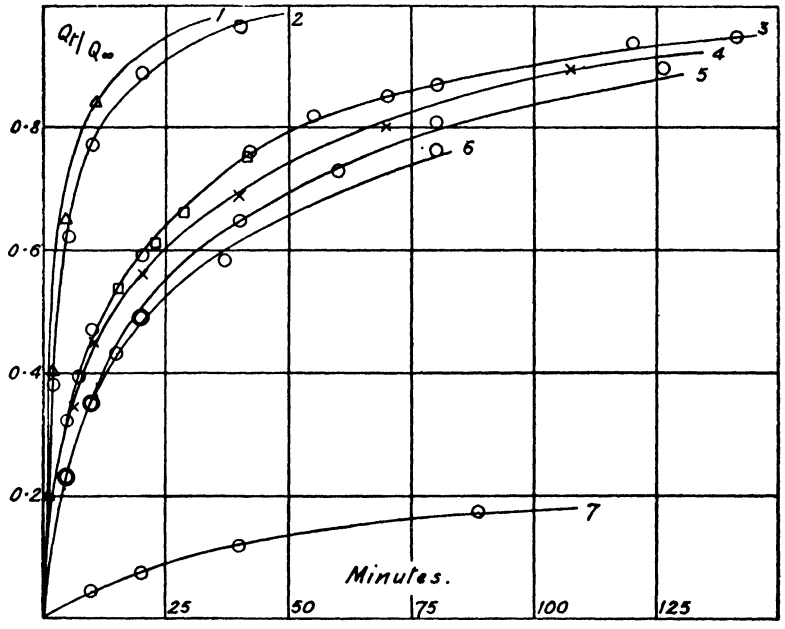


FIG. 11.—Systems showing particle diffusion.

1. Na^+ 2° C
2. NMe_4^+ 25° C
3. NEt_4^+ 25° C. ○ bromide □ Chlorazol Sky Blue salt
4. NMe_3.*n*-amyl$^+$ 25° C
5. $PhNMe_2Et^+$ 25° C
6. NEt_4-resin suspension 25° C
7. $PhNMe_2CH_2Ph^+$ 25° C

10–30 kcal./mole,[12] whereas that for diffusion of most salts in water is about 5 kcal./mole.[7] Similarly, Boyd *et al.*[2] found 4 kcal./mole for Na^+ (*F*-mechanism) and 8 kcal./mole for *P*-mechanism with Amberlite IR-1.

Table I shows values of the energy of activation for several exchange reactions studied in the present work (see also Fig. 10). The exchange between NH_4-resin and Na^+ at 25°–46° (*F*-) shows the expected value of about 5, but it is remarkable that both Na^+ at 2°–15° (*P*-) and NMe_4^+ (*P*-) have the same low value. A high value of 8·2 kcal./mole is found for $PhNMe_2CH_2Ph^+$. This is the largest ion yet studied which is capable of

[12] See, e.g., Speakman and Smith, *J. Soc. Dyers Col.*, 1936, **52**, 121. Garvie, Griffiths and Neale, *Trans. Faraday Soc.*, 1934, **30**, 271.

penetrating and reaching all the exchanging sites in this resin and it seems likely that the higher activation energy is a consequence of the steric difficulty which it experiences in moving through the molecular pores of the resin. On the other hand, the smaller ions showing *P*-mechanism evidently do not experience any such " wall-effect " and move simply through the water which fills the pores.

It is not invariably true that diffusion in solids requires large energies of activation, and results in agreement with the present picture have been obtained by other workers. The high value with fibres may reflect distortion of a flexible macromolecular structure by the dye molecule. With rigid porous solids, the activation energy for the diffusion of molecules *smaller than the pores* may be almost normal for an aqueous medium. For example, Tiselius [18] found 5·4 kcal./mole for the diffusion of water normal to the (201) face in the zeolite, heulandite. On the other hand, diffusion normal to the (001) face required 9·1 kcal/mole ; presumably the interstitial holes in this direction are much smaller, and of about the same size as the water molecule. Diffusion of salts in 2 % agar gels shows the same temperature coefficient as for free diffusion in aqueous solution.[7]

Role of the Anion.—To preserve electro-neutrality, the ions leaving the resin must be replaced by others of the same total charge. If the ions were bound to specific sites the situation might arise where the rate of dissociation from sites was the factor limiting the rate of exchange, and it might then be necessary to provide extra (soluble) anions by diffusion of salt from the ambient solution into the interstices of the resin before rapid cation exchange could take place.

This possibility was studied by the experiments (recorded above) in which the anion was provided by the dye Chlorazol Sky Blue FFS, which is found to diffuse extremely slowly and to an extremely small extent into the resin. With both Na^+ and NEt_4^+ the rate of exchange was the same as with the simple halide salts (see Fig. 5, curve 2 ; Fig. 7, curve 4 ; Fig. 11, curve 3), indicating that it is not necessary to have soluble anions within the pores for exchange to occur rapidly.

The high mobility of the cations alone is proved conclusively by the experiment with finely divided NEt_4-resin *suspension* in place of NEt_4Br solution. This suspension exchanges cations with normal NH_4-resin grains almost as rapidly as does the homogeneous salt solution, the apparent rate constants being in the ratio of 0·74/1 (cf. Fig. 5, curves 7 and 10), proving that soluble anions play no significant part at all in the kinetics of cation exchange, at least with the resinous exchanger studied here.

In this experiment the process starts with the interpenetration of the Gouy diffuse double layers of the large grains with those of the small suspended particles. At the very low ionic strengths prevailing, the double layers spread so far from the microscopic particles that the cation distribution in the liquid is similar to that in a true salt solution. Consequently, the outside part of the double layer from the large granules can mix rapidly with the cations of the suspension, leaving diffusion of cations within the pores of the resin as the rate-controlling process, as it is with the ordinary salt solution. A similar process no doubt occurs in normal exchanges with soluble salts—the soluble cations exchange first with the resin cations in the outer diffuse double layer, thus setting up a concentration gradient within the pores which is eliminated by ordinary diffusion, this latter process requiring no soluble anions.

[18] Tiselius, *Z. physik. Chem. A*, 1934, **169**, 425 ; 1935, **174**, 401.

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ION EXCHANGE STUDIES

II. The Determination of Thermodynamic Equilibrium Constants

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A critical examination of the postulates used by different authors to evaluate the activities of ions in an exchanger is given and an experimental test is made using the barium–hydrogen and lanthanum–ammonium exchange systems. It is shown that the assumption that the activities in the exchanger are proportional to the molar concentrations (in arbitrary units) gives an approximately constant value for the mass product, but even on this postulate the mass product varies by about 50 %, rising to a maximum at an equivalent fraction of about ¼ for the multivalent ion in the exchanger. It is suggested that the activities in the exchanger have been wrongly evaluated because of the influence of some secondary process, such as adsorption, solution of ions in the exchanger or swelling of the resin.

In this paper the following symbols have been used :

a, thermodynamic activity.
K_a, thermodynamic equilibrium constant.
c, molar concentration
x, molar fraction.
C, equivalent concentration.
X, equivalent fraction, defined as the ratio of the number of equivalents of a given cation to the total number of equivalents of all cations in the same phase
n, number of moles.

Suffixes have been used to indicate the ion and the particular phase to which reference is made, thus

x_{Ba_R} is the molar fraction of barium ions in the resinous exchanger.
a_{H_S} is the thermodynamic activity of hydrogen ions in solution.

The thermodynamic equilibrium constant of an ion-exchange reaction of the type

$$Ba^{2+}_S + 2H_R \rightleftharpoons Ba_R + 2H^+_S \quad . \quad . \quad . \quad (1)$$

is written

$$K_a = \frac{a_{Ba_R}}{a^2_{H_R}} \cdot \frac{a^2_{H_S}}{a_{Ba_S}} \quad . \quad . \quad . \quad . \quad . \quad (2)$$

Whilst the activities a_{Ba_S}, a_{H_S} in solution may be readily evaluated in favourable cases from known activity data, some assumption must be made for the activities of the two ions in the solid phase. Hitherto, at least three different assumptions have been made for a_{Ba_R} and a_{H_R} as follows :

(i) Boyd, Schubert and Adamson [1] have assumed that the ions in the solid phase exist in ideal solution, and have put the activities in the solid phase equal to the molar fractions of the two species. If the x terms are molar fractions and the n terms are the number of moles of Ba_R and H_R, then

$$K_a = \frac{x_{Ba_R}}{x^2_{H_R}} \cdot \frac{a^2_{H_S}}{a_{Ba_S}} = \frac{(n_{Ba_R} + n_{H_R})\, n_{Ba_R}}{n^2_{H_R}} \cdot \frac{a^2_{H_S}}{a_{Ba_S}} \quad . \quad . \quad . \quad (3)$$

By plotting log $(x_{Ba_R}/x^2_{Na_R})$ against log $(a_{Ba_S}/a^2_{Na_S})$ for the sodium–barium system a straight line was obtained by Boyd, Schubert and Adamson, which seemed to indicate that the concept of ideal solution in the solid phase was a reliable hypothesis.

(ii) Bauman and Eichhorn [2] on the other hand have written the equilibrium constant of a reaction of the above type in a form corresponding to

$$K_a = \frac{c_{Ba_R}}{c^2_{H_R}} \cdot \frac{a^2_{H_S}}{a_{Ba_S}}, \quad . \quad . \quad . \quad . \quad . \quad (4)$$

where the c terms are molar concentrations, and have derived an equation of the form

$$\frac{x_{Ba_R}}{(1 - 2x_{Ba_R})^2} = K_a \cdot \frac{c_R}{c_S} \cdot \frac{x_{Ba_S}}{(1 - 2x_{Ba_S})^2}, \quad . \quad . \quad (5)$$

where c_R is the total molar exchange capacity of the exchanger and c_S the total molar concentration of the solution. It is difficult to see how this equation can be derived from eqn. (4) unless some assumption has been made which has not been specifically mentioned in their paper. Nevertheless, these authors make a test of their equation which appears to support their postulates.

(iii) Kressman and Kitchener [3] have assumed that the activities of the ions in the exchanger are proportional to the equivalent fractions defined as

$$a_{Ba_R} = \frac{C_{Ba_R}}{C_R} = X_{Ba_R}, \text{ and } a_{H_R} = \frac{C_{H_R}}{C_R} = X_{H_R} = 1 - X_{Ba_R},$$

where the C terms are the number of equivalents of the ions in the exchanger, C_R is the total capacity, and the X terms are the equivalent fractions in the exchanger. Hence

$$K_a = \frac{X_{Ba_R}}{(1 - X_{Ba_R})^2} \cdot \frac{a^2_{H_S}}{a_{Ba_S}} \quad . \quad . \quad . \quad . \quad (6)$$

In this treatment the activity coefficients of the ions in solution have also been neglected, and the equation written

$$K_a = \frac{X_{Ba_R}}{(1 - X_{Ba_R})^2} \cdot \frac{C^2_{H_S}}{C_{Ba_S}} \quad . \quad . \quad . \quad . \quad (7)$$

The mass-law product estimated from an equation of this form was found to be constant over a range of $0{\cdot}76 < X_{Ba_R} < 0{\cdot}88$ for the barium–ammonium system (and over similar ranges for the other systems), and it was concluded that the changes occurring in $\gamma_{Ba_S}/\gamma^2_{H_S}$ must be accompanied by proportionate changes in $\gamma_{Ba_R}/\gamma^2_{H_R}$.

It appears surprising that three different assumptions for the activities of the ions in the exchanger can lead to equations which are apparently

[1] Boyd, Schubert and Adamson, *J. Amer. Chem. Soc.*, 1947, **69**, 2818.
[2] Bauman and Eichhorn, *ibid.*, 2830.
[3] Kressman and Kitchener, *J. Chem. Soc.*, 1949, 1201.

equally successful in describing the results. All three approaches when applied to exchanging ions of the same valency lead to the same equations, which would therefore be expected to give a constant mass-law product for homovalent exchange. We have previously shown [4] that the mass-law product for the sodium–hydrogen system is not constant but rises to a maximum for a value of 0·12 for the molar fraction of sodium ions in the exchanger. This suggests that the simple assumptions made for the activities of ions of the same valency in the exchanger is not valid, and, *a priori*, one would expect these assumptions to be in greater error for heterovalent exchange, for which the system is likely to be less ideal.

The situation is further confused by the apparent contradictory nature of eqn. (3) and (4). If the capacity of the exchanger is constant, the postulates of Kressman and Kitchener and of Bauman and Eichhorn are such that a constant mass-law product can be obtained from the same set of results by use of both eqn. (4) and (7) (provided the ratio of the activity coefficients of the ions in solution does not vary greatly), for

$$\frac{X_{Ba_R}}{(1 - X_{Ba_R})^2} = \frac{C_{Ba_R} \cdot C_R}{C^2_{H_R}} = \frac{2c_{Ba_R} \cdot C_R}{c^2_{H_R}} \quad . \quad . \quad . \quad (8)$$

The postulates of Boyd, Schubert and Adamson and those of the other two sets of authors are, however, mutually exclusive, for if eqn. (4) is written in terms of the number of moles of the two ions in a given weight of exchanger we arrive at

$$K_a = \frac{n_{Ba_R}}{n^2_{H_R}} \cdot \frac{a^2_{H_S}}{a_{Ba_S}} \quad . \quad . \quad . \quad . \quad . \quad (9)$$

This is different from eqn. (3) which has a term $(n_{Ba_R} + n_{H_R})$ equal to the total number of moles of the two ions in the exchanger, which must vary according to the position of equilibrium. Thus it is impossible to obtain a constant mass-law product using eqn. (3) from results which give a constant with eqn. (4) and (6).

In view of this confusion, a thorough test of the mass-action concept has been made by estimating the mass-law product from the same results using eqn. (3), and also by assuming the activities in the exchanger to be equal to the molar concentrations (i.e., neglecting the activity coefficients of the ions in the exchanger). In order to simplify the presentation of the results, eqn. (4) obtained on the latter assumption has been used in the form given by substitution of the equivalent fractions for the concentration terms. Thus

$$\begin{aligned} K_a &= \frac{c_{Ba_R}}{c^2_{H_R}} \cdot \frac{a^2_{H_S}}{a_{Ba_S}} \\ &= \frac{C_{Ba_R}}{2C^2_{H_R}} \cdot \frac{c^2_{H_S}}{c_{Ba_S}} \cdot \frac{\gamma^2_{H_S}}{\gamma_{Ba_S}} \\ &= \frac{C_{Ba_R}}{2C^2_{H_R}} \cdot \frac{2C^2_{H_S}}{C_{Ba_S}} \cdot \frac{\gamma^2_{H_S}}{\gamma_{Ba_S}} \\ &= \frac{X_{Ba_R}}{X^2_{H_R}} \cdot \frac{X^2_{H_S}}{X_{Ba_S}} \cdot \frac{\gamma^2_{H_S}}{\gamma_{Ba_S}} \cdot \frac{C_S}{C_R} \end{aligned}$$

Hence

$$K_a = \frac{X_{Ba_R}}{(1 - X_{Ba_R})^2} \cdot \frac{(1 - X_{Ba_S})^2}{X_{Ba_S}} \cdot \frac{\gamma^2_{H_S}}{\gamma_{Ba_S}} \cdot \frac{C_S}{C_R}, \quad . \quad . \quad (10)$$

[4] Duncan and Lister, *ibid.* (in press).

where C_s is the total equivalent concentration in solution, and the X terms are equivalent fractions of the respective ions in the exchanger and in solution. The ratio of the K_a values given by eqn. (10) and (6) is equal to the capacity of the exchanger.

For the barium–hydrogen system, plots of K_a against x_{Ba_R} and against X_{Ba_R} were made to test eqn. (3) and (10) respectively. In both cases the mass-law product was found to vary, but the curve obtained using eqn. (10) was more nearly constant and had a form similar to that obtained for the sodium–hydrogen system. The same was true of the exchange of lanthanum and ammonium ions, the value of K_a being evaluated according to the equation,

$$K_a = \frac{X_{La_R}}{(1 - X_{La_R})^3} \cdot \frac{(1 - X_{La_S})^3}{X_{La_S}} \cdot \frac{\gamma_{H_S}^3}{\gamma_{La_S}} \cdot \frac{C_S^2}{C_R^2} \quad . \quad . \quad . \quad (11)$$

Experimental

The position of equilibrium between exchanger and solution has been determined (i) by batch equilibration methods and (ii) by determining the breakthrough volume necessary for a column of exchanger in the hydrogen form to be saturated by a given mixture of metal ions and hydrogen ions. The apparatus was in principle the same as that used for investigating the sodium–hydrogen system.[4]

Radiochemical tracer methods were used to study both the barium and the lanthanum systems, the two tracers being ^{139}Ba (half-life 86 min., β-energy 2·3 MeV, γ-energy 0·6 MeV) and ^{140}La (half-life 40 hr., β-energy 1·45 MeV, γ-energies 0·87, 0·49 and 0·33 MeV) respectively. In the lanthanum–ammonium batch equilibration method, volumes of radioactive lanthanum nitrate solution from 100 ml. to 5 l. were made up with ammonium nitrate to a total concentration of 0·1 N, the lanthanum concentration being varied from 10^{-2} to 10^{-6} N. To these solutions, known weights of Dowex 50 (0·15 to 0·007 g.) were added, the exchanger (in the ammonium form) having been previously dried in an oven at 110° C to constant weight, and the capacity having been determined by the column method using lanthanum. A similar batch equilibration method was also used for the barium–hydrogen system.

In the breakthrough volume method a mixture of barium chloride and hydrochloric acid at a total concentration of 0·2 N, or of lanthanum nitrate and ammonium nitrate at a total concentration of 0·1 N, was passed down the column saturated with the univalent ion. Since the multivalent ion is held more strongly, a sharp boundary was obtained and was easily observed by measuring the radioactivity of the liquid leaving the column by means of a Geiger-Muller counter of the liquid flow type.[4] After correcting for the paralysis time of the counting assembly and the decay of the radio-tracer used, the concentration of the barium or lanthanum entering the column was equated to the activity observed after the column was saturated. By measuring the shaded area shown in Fig. 1 the amount of the multivalent ion taken up by the column for a given concentration in solution may be estimated and hence the mass-law product determined.

In order to estimate the mass-law product for values of X_{Ba_R} and X_{La_R} below about 0·2 it is necessary to reduce the concentration of the respective ions in solution to 10^{-5} M or less. With such low concentrations very large breakthrough volumes are necessary unless the capacity of the column is kept low. In one experiment a column containing about 10 mg Dowex 50 of capacity 0·0446 m. equiv. required 1000 ml. of a solution containing $1{\cdot}8 \times 10^{-5}$ g./l. of lanthanum nitrate to saturate the column at a value of $X_{La_R} = 0{\cdot}417$. To study the equilibrium at very low concentrations of the multivalent ion it was necessary to use batch methods, since the flow velocity becomes too fast for reasonably sharp boundaries to be obtained if the breakthrough volume is to be reached within a time during which the radio-tracer is still active.

The values of X_{Ba_R}, X_{Ba_S}, C_R and C_S obtained experimentally were substi-

tuted in eqn. (10). Corrections for the activity coefficients $\gamma^{+}_{H_S}$ and $\gamma^{2+}_{Ba_S}$ for mixtures of these two ions are necessary and may be estimated from the activities of the electrolytes in mixtures of barium chloride and hydrochloric acid, for

$$\frac{\gamma^{2}_{H_S}{}^{+}}{\gamma_{Ba_S}^{2+}} = \frac{\gamma^{4}_{\pm HCl\,(BaCl_2)}}{\gamma^{3}_{\pm BaCl_2\,(HCl)}}, \quad . \quad . \quad . \quad . \quad (12)$$

where $\gamma_{\pm HCl\,(BaCl_2)}$ and $\gamma_{\pm BaCl_2(HCl)}$ are the activity coefficients of the electrolytes in mixtures of the two. Now it is possible to estimate $\gamma_{\pm HCl\,(BaCl_2)}$ from the data of Randall and Breckenridge [5] by use of the equation

$$\log \gamma_{\pm HCl\,(BaCl_2)} = \log \gamma^{\circ}_{\pm HCl} + \alpha_{12} c_{BaCl_2 S},$$

where γ°_{HCl} is the activity coefficient of hydrochloric acid in solutions of the same ionic strength. From these data plots were made of $\log \gamma_{\pm HCl\,(BaCl_2)}$ against $c_{BaCl_2 S}$ for solutions of constant ionic strengths, from which α_{12} was estimated and used to determine $\gamma_{\pm HCl\,(BaCl_2)}$ in the solutions.

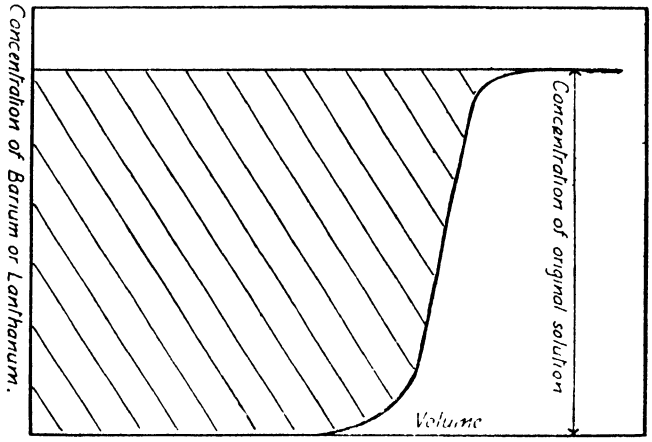

FIG 1.—Diagrammatic representation of concentration volume plot for breakthrough experiment.

Unfortunately data were not available to enable $\gamma_{\pm BaCl_2\,(HCl)}$ to be estimated, but since $\gamma_{\pm HCl\,(BaCl_2)}$ was found to differ from $\gamma^{\circ}_{\pm\,Cl}$ by less than 1 % it was considered reliable to use $\gamma^{\circ}_{\pm BaCl_2}$ as $\gamma_{\pm BaCl_2\,(HCl)}$ in solutions of the same ionic strength.

The expression corresponding to eqn. (12) for the lanthanum–ammonium system is

$$\frac{\gamma^{3}_{NH_4{}^{+}S}}{\gamma_{La^{3+}S}} = \frac{\gamma^{6}_{\pm NH_4NO_3\,(La(NO_3)_3)}}{\gamma^{4}_{\pm La(NO_3)_3\,(NH_4NO_3)}} \quad . \quad . \quad . \quad . \quad (13)$$

In this case the activity data even for pure lanthanum nitrate are not available and no correction could be made The results given below therefore do not represent the true value of the mass-law product, but the general variation will be qualitatively correct. The concentration of ammonium nitrate did not vary by more than 0·09 to 0·10 N for $X_{La_R} < 0{\cdot}9$ whilst the lanthanum nitrate concentration for the same range was always less than 0·01 N. For values of X_{La_R} below 0·8 the ammonium ion concentration was 0·099–0·1 N whilst the lanthanum ion concentration was less than 0·001 N. Although the shape of the mass-law product plot may be slightly in error for $X_{La_R} > 0{\cdot}8$, the activity correction is almost certainly constant within about 1 % for lower values of X_{La_R}.

[5] Randall and Breckenridge, *J. Amer. Chem. Soc.*, 1927, **49**, 1435.

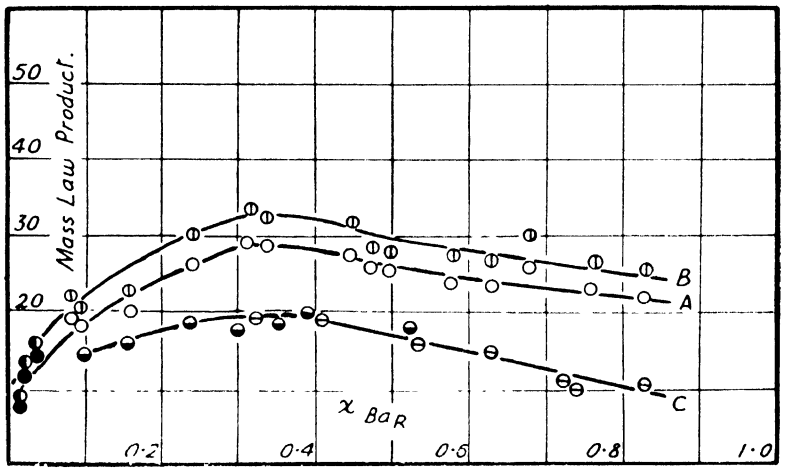

FIG. 2.—Mass-law product plot for barium–hydrogen system according to eqn. (10). A.—Room temperature (with no correction for activity coefficients in solution). B.—Room temperature (with activity coefficient correction). C.—87° C (with no correction for activity coefficients in solution).

○ ◑ ⊖ Breakthrough experiments.
● ◐ ◒ Batch equilibration experiments.

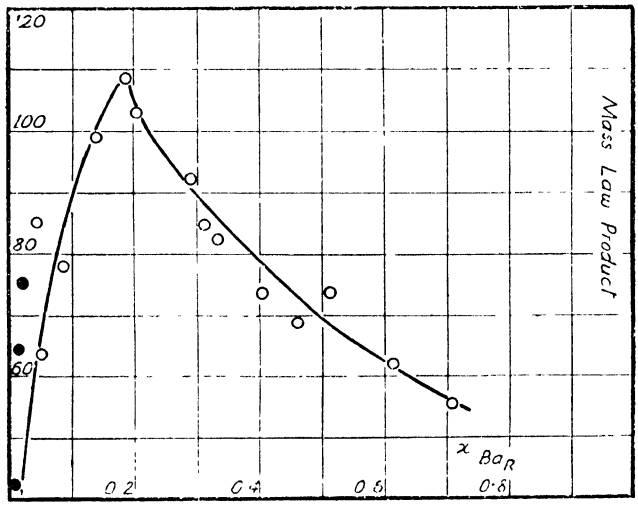

FIG. 3.—Mass-law product plot for barium–hydrogen system at room temperature according to eqn. (3) (with no correction for activity coefficients in solution).

○ Breakthrough experiments.
● Batch equilibration experiments.

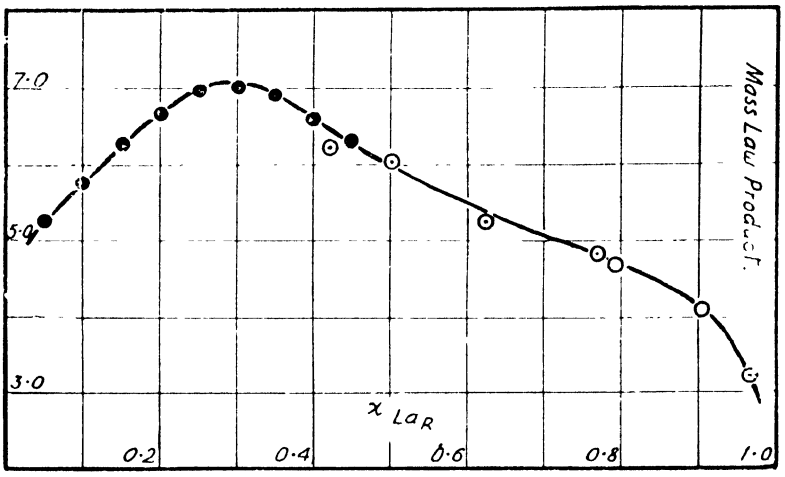

FIG. 4.—Mass-law product plot for lanthanum–ammonium system, according to eqn. (11) (with no correction for activity coefficients in solution).

⊘ Breakthrough experiments.

● Points interpolated from X_{La_R}–X_{La_S} curve constructed from batch equilibration data.

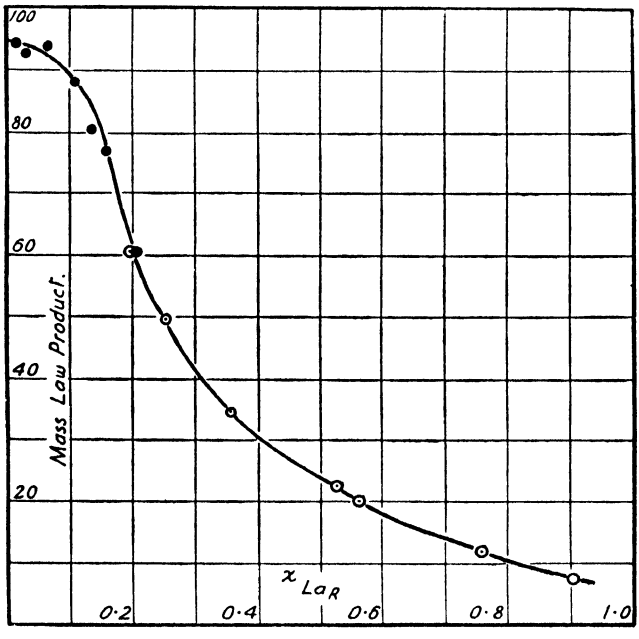

FIG. 5.—Mass-law product plot for lanthanum–ammonium system according to solid solution concept (corresponding to eqn. (3), with no correction for activity coefficients in solution).

⊘ Breakthrough experiments.

● Points interpolated from X_{La_R}–X_{La_S} curve constructed from batch equilibration data.

Results and Discussion

The variation in the mass-law product calculated according to eqn. (10) from the equilibrium data given in Table I and II for the barium chloride–hydrochloric acid system is shown in Fig. 2. It will be seen that at room temperature the mass-law product varies by about 50 %, from high values of X_{Ba_R} to a maximum at a value of $X_{Ba_R}=0{\cdot}31$. By comparison Fig. 3 shows that the mass-law product derived from the same experimental results by use of eqn. (3) varies by a factor of 2 or more over the same range, the maximum being obtained at $x_{Ba_R}=0{\cdot}17$ corresponding to $X_{Ba_R}=0{\cdot}31$. From this it was concluded that neither approach was strictly valid, although the activities of the ions in the exchanger were more nearly approximated by the use of molar concentrations in the exchanger expressed in arbitrary units. Fig. 2 may be used if desired to estimate the variation of the activity coefficients of the ions in the exchanger, although the values obtained depend on the value of K_a which is assumed to be valid and on the units of concentration.

For the lanthanum–ammonium system, the equilibrium data are given in Table III, and the curve obtained by use of eqn. (11) without making corrections for the activities of the ions in solution is shown in Fig. 4. The curve calculated assuming the validity of the solid solution concept (corresponding to eqn. (3)) is shown in Fig. 5. Much greater variations of K_a are obtained, thus supporting the conclusion that eqn. (11) (which assumes

TABLE I

EXPERIMENTAL DATA FOR Ba^{2+}–H^+ SYSTEM (TOTAL CONCENTRATION 0·2 N)

Total Equivalent Concentration (C_S)	Equivalent Concentration of Barium (C_{Ba_S})	Capacity of Exchanger (m. equiv.)	No. of m. equiv. of Barium in Exchanger	$\frac{\gamma^2_{\pm HCl}}{\gamma_{\pm BaCl_2}}$
Breakthrough Experiments				
0·220	0·01254	0·500	0·414	1·15
0·210	0·0055	0·500	0·380	1·15
0·210	0·00254	0·500	0·340	1·15
0·207	0·00191	0·500	0·315	1·15
0·209	0·00135	0·500	0·289	1·15
0·209	0·000767	0·500	0·248	1·15
0·208	0·000658	0·500	0·237	1·15
0·211	0·000542	0·500	0·224	1·15
0·210	0·000272	0·500	0·168	1·15
0·210	0·000231	0·500	0·156	1·15
0·210	0·000163	0·500	0·121	1·15
0·195	0·0001025	0·130	0·0211	1·14
0·204	0·000063	0·500	0·0481	1·14
0·195	0·0000436	0·130	0·0106	1·14
Batch Equilibration Experiments				
0·200	0·0000263	0·549	0·0201	1·14
0·200	0·0000225	1·360	0·0239	1·14
0·200	0·0000211	0·995	0·0253	1·14

TABLE II

EXPERIMENTAL DATA FOR Ba^{2+}–H^+ SYSTEMS AT 87° C (TOTAL CONCENTRATION 0·2 N)

Total Equivalent Concentration (C_S)	Equivalent Concentration of Barium (C_{Ba_S})	Capacity of Exchanger (m. equiv.)	No. of m. equiv. of Barium in Exchanger
Breakthrough Experiments			
0·206	0·0201	0·796	0·6573
0·218	0·0105	0·796	0·5890
0·212	0·0080	0·796	0·5760
0·213	0·00320	0·796	0·5029
0·212	0·00159	0·796	0·4260
0·210	0·000629	0·796	0·3258
0·208	0·000365	0·796	0·2560
Batch Equilibration Experiments			
0·206	0·00121	0·432	0·2258
0·206	0·000498	0·427	0·1657
0·206	0·000443	1·719	0·6077
0·206	0·000334	0·832	0·2476
0·206	0·000217	1·292	0·3060
0·206	0·000135	0·859	0·1333
0·206	0·0000812	1·718	0·1672

TABLE III

EXPERIMENTAL DATA FOR La^{3+}–NH_4^+ SYSTEM (TOTAL CONCENTRATION 0·1 N)

Total Equivalent Concentration (C_S)	Equivalent Concentration of Lanthanum (C_{La_S})	Capacity of Exchanger (m. equiv.)	No. of m. equiv. of La^{3+} in Exchanger
Breakthrough Experiments			
0·1000	0·500	3·107	3·000
0·1002	0·0102	3·107	2·810
0·1000	0·00100	0·410	0·3250
0·0998	0·000676	0·0446	0·03424
0·1000	0·000119	0·0446	0·02778
0·1000	0·0000364	0·0639	0·03208
0·1000	0·0000186	0·0446	0·01860
Batch Equilibration Experiments (Typical results from a series of 70 experiments)			
0·1000	0·0000797	0·151	0·08800
0·1000	0·0000339	0·0709	0·03470
0·1000	0·0000203	0·0321	0·01400
0·0999	0·00000936	0·0426	0·01485
0·1000	0·00000767	0·0353	0·01110
0·1000	0·00000625	0·0606	0·01800
0·1000	0·00000575	0·0697	0·01950
0·1000	0·00000121	0·0378	0·00375

proportionality between activities and molar concentrations) is more nearly valid.

The fact that variations in the mass-law product are obtained here for systems shown by other authors to give constants is evidently attributable to the fact that most of the earlier mass-law product determinations were made over quite small ranges. For example, Kressman and Kitchener worked within the ranges $0{\cdot}76 < X_{Ba_R} < 0{\cdot}88$ and $0{\cdot}71 < X_{Al_R} < 0{\cdot}88$ over which the mass-law product is seen not to vary by more than about 10 %, and Boyd, Schubert and Adamson similarly used restricted ranges (estimated for the barium–sodium system as $0{\cdot}64 < X_{Ba_R} < 0{\cdot}91$, and for the lanthanum–sodium system as $0{\cdot}87 < X_{La_R} < 0{\cdot}92$).

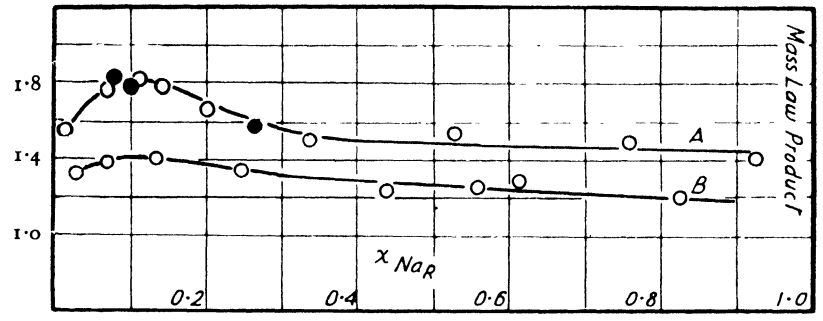

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FIG 6.—Mass-law product for the sodium–hydrogen system (C_s = 0·2 N)
A.—Room temperature. B.—87° C (correction for activity coefficient in solution).

⊘ Breakthrough experiments.
● Batch equilibration experiments.

The use of eqn. (10) and (11) is seen to give results for the exchange of uni-bi and uni-tervalent ions which are very similar to those obtained for uni-univalent exchange. For comparison, the mass-law product of the sodium–hydrogen system [4] at room temperature is shown in Fig. 6. Since the concepts of Kressman and Kitchener and of Bauman and Eichhorn must lead to mass-law products which vary in the same way as those shown in Fig. 2, 4 and 6, no method yet proposed is satisfactory in evaluating the activities of the ions in the exchanger, other than to a first approximation. Whilst it is possible that some other approach may be satisfactory, it seems to us that a fundamental feature may have been omitted in all the methods listed. For instance, whilst it is undoubtedly true that to a first approximation the total capacity of an exchanger is constant, and it is probably correct to assume the *exchange* capacity to be constant, it is possible that the resin takes up non-exchangeable material by adsorption on the surface, by solution in the exchanger or by other processes. In this respect, there is a little experimental evidence; the height of the maximum obtained in the mass-law product plots for the sodium–hydrogen and the barium–hydrogen systems decreases as the temperature increases (see Fig. 2 and 6) as would be expected for the contribution of a non-exchange process (e.g., an adsorption phenomenon) which usually decreases with increasing temperature.

Differences in mass-law product may also be caused by swelling phenomena as has been suggested by Gregor [6] on thermodynamic grounds. It is already

[6] Gregor, *ibid.*, 1948, 70, 1293.

known that ion exchange material swells and contracts in solutions of different electrolytes or in solutions of the same electrolyte in different concentrations and it is possible that swelling may also be different according to the ratio of the ions in the exchanger. Further experimental work is required to ascertain whether such secondary processes, hitherto unconsidered, have an effect on the exchange equilibrium.

We should like to acknowledge the assistance of Mr. P. E. Brown with the experimental work, and Mr. M. A. Hewitt with the computing. We are indebted to Dr. E. Glueckauf for his interest in this investigation and we should like to thank the Director of the Atomic Energy Research Establishment for permission to publish this paper.

ADDENDUM.—Some confusion seems to have arisen concerning the method of evaluating K_a by use of eqn. (10) from the experimental results. The figures shown in column 3 of Table I–III were not used as C_R, as this would lead to a value of K_a which depends on the amount of exchanger used. In order that K_a should refer to the same quantity of exchanger, C_R was taken as the capacity of 1 g. of exchanger (4·3 m. equiv./g.)[4].

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EXCHANGE EQUILIBRIA IN ANION-EXCHANGE RESINS: POROUS EXCHANGERS

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The exchange equilibria of strong-base and weak-base anion-exchange resins, prepared with various degrees of cross-linking, were examined to determine the effect of this quantity upon the behaviour of the products. By suitable improvements in " porosity " the exchange capacity for large molecules, such as penicillin or coloured bodies in beet molasses, can be increased. The swelling and hydraulic characteristics are also affected by changes in cross-linking The results are quite analogous to the molecular-sieve effect noted for the adsorption of gases by zeolites and may therefore be considered an " ionic-sieve " effect.

Chromatographic techniques that have employed ion-exchange substances as adsorbents have aided considerably in the analysis of rare earth mixtures,[1] solutions of amino acids,[2] purine bases, nucleic acids[3] and other systems. The principles involved in these separations are similar to those involved in the chromatographic analysis of non-ionic constituents; however, superimposed on these principles are several added ones deriving from the ion-exchange process. Notably successful separations have been achieved through the application of variations in elution agents, degree of column loading, flow-rates during development and particle size of adsorbent. Differences in exchange equilibria have been called into service through searches for adsorbents with specific adsorptive properties, or investigations

[1] Boyd, Schubert and Adamson, *J. Amer. Chem. Soc.*, 1948, **69**, 2818.
[2] Winters and Kunin, *Ind. Eng. Chem.*, 1949, **41**, 460.
[3] Cohn, *Science*, 1949, **109**, 377.

into the effects of complexing agents and of pH on elution. Further extension of the application of exchange equilibria principles in chromatographic analysis has been made through the use of the newer synthetic resin exchange adsorbents that contain various functional groups and possess various degrees of selectivity based upon differences in ionic size.

Equilibria in anion exchangers have been studied extensively by Wiklander,[4] Myers, Eastes and Urquhart,[5] Bhatnagar and co-workers,[6] Griessbach [7] and Kunin and Myers.[8] Most of these studies were conducted on weak-base type anion exchangers, that is, the synthetic resins employed were based upon alkylene polyamines or aromatic diamines. Kunin and McGarvey [9] examined the equilibria relationships in a strong-base or quaternary-type anion exchanger.

Titration curves of anion exchangers with strong acids clearly indicate the strength of the basic group. The order of decreasing exchange ability differs for the weakly basic resins as compared with the strong base chiefly with respect to the position of the hydroxyl ion. In the case of the strongly basic resin the order was found by Kunin and McGarvey [9] to be as follows for the strong-base exchanger, Amberlite IRA-400: citrate > sulphate ≥ oxalate > iodide > nitrate > chromate > bromide > thiocyanate > chloride > formate > hydroxyl > fluoride > acetate. The order of replacing ability is determined by means of "symmetry" studies, in which the percentage exchange effected is plotted against the symmetry value, or milli-equivalents of ion added per milli-equivalent of dry resin. Exchange equilibria relationships such as the Freundlich, Langmuir or Rothmund-Kornfeld equations hold only over certain ranges of concentration, and are best applied when ions of similar size and charge are involved. In general, symmetry plots are readily obtained and are more meaningful in discussions of practical problems in chromatography.

The availability of both weakly and strongly basic anion exchangers offers a range of operations to the laboratory investigator. Such an operating range is applicable mainly in cases of relatively small ions. When larger molecules such as complex colour bodies, penicillin and ascorbic acid are under consideration it is desirable to employ modified exchangers that exhibit greater capacities for large ions. Recent advances in synethtic resin technology have led to the development of ion-exchange resins with different degrees of "porosity" such that larger ions may be adsorbed and eluted effectively. Since the successful application of chromatographic techniques requires operation at or near equilibrium conditions, the porosity of the exchanger becomes a vital factor since rates of diffusion throughout the resin particle vary with both the porosity of the resin and size of the ion.

Samuelson [10] and Bauman [11] have studied the effects of swelling and porosity upon the equilibria of cation exchange in sulphonic acid cation-exchange resins. It is an object of this paper to present some preliminary data on the performance of laboratory preparations of porous anion exchangers of the weak-base and strong-base type.

Experimental

The resins selected for this study were modifications of the Amberlite IRA-400 (Series A) studied by Kunin and McGarvey [9] in which the density was altered

[4] Wiklander, *Ann. Roy. Agric. Coll., Sweden*, 1946, **14**, 1.
[5] Myers, Eastes and Urquhart, *Ind. Eng. Chem.*, 1941, **33**, 1270
[6] Bhatnagar *et al.*, *J. Indian Chem. Soc.*, 1941, **18**, 447.
[7] Griessbach, *Z. Ver. Deutsch. Chenuker. Beih.*, 1939, **31**, 1.
[8] Kunin and Myers, *J. Amer. Chem. Soc.*, 1947, **69**, 2874.
[9] Kunin and McGarvey, *Ind. Eng. Chem.*, 1949, **41**, 1265.
[10] Samuelson, *Diss.* (Valhallavagen, Sweden, 1944).
[11] Bauman, *Abstr. Div. Coll. Chem.* (*Amer. Chem. Soc.*), 1949, 14.

TABLE I

PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE ANION-EXCHANGE RESINS OF SERIES A AND B

SERIES A

Relative Degree of Cross-linking	Moisture Capacity %	Apparent Density g. (dry)/ml	Decrease in Resin Volume			Swelling dry→wet %	Total Exch. Capacity m.-equiv./g.	Penicillin Capacity m.-equiv./g.	Equil. Exch. S = I[a]		
			$\mu = 1.0$ %	$\mu = 0.1$ %	$\mu = 0.01$ %				I^- %	Cl^- %	F^- %
1	93	0.15	64	40	26	540	3.2	3.2	66	51	44
2	79	0.19	23	5	1	225	3.1	2.6	76	67	52
3	69	0.26	10	2	0	180	3.1	2.3	80	73	56
4	63	0.32	5	0	0	160	2.9	1.8	81	74	59
8	50	—	4	0	0	125	2.6	0.1	83	76	66

SERIES B

								Beet Sugar Decolor. Cap. g sugar/g.			
1	80	0.14				525	7.7	13.0			
2	69	0.20				220	7.2	8.5			
3	61	0.26				175	6.8	6.8			
4	55	0.32				150	6.4	5.0			
8	45	—				110	5.1	0.5			

[a] % exchange on equilibrating the hydroxyl form of the resin with an equivalent amount of the sodium salt of the respective halogen.

by varying the degree of cross-linking, and Series B, a similar set of resins in which the basic groups were of the weak-base type.

The total exchange capacities and exchange equilibria were determined in the manner described by Kunin and Myers [8] and by Kunin and McGarvey.[9] The total capacities of the strong-base resin were also determined by the extent of splitting of sodium chloride. The percentage swelling was determined by a measurement of the difference in volume between two equal weights of resin, one of which was put in the chloride form, the other in the hydroxyl form.

The apparent densities and moisture contents were determined as described previously.[8,9] The capacity for large ions and decolorizing capacity were determined in column experiments. The capacity for decolorizing beet molasses was determined by passing a 15 Brix sugar solution over a 20 ml. bed of resin at a rate of 0·067 ml. per ml /min. The end-point was taken as 20 % colour leakage. The capacity for penicillin was determined by passing dilute solutions (usually about 0·01 N) of the compound over a 2 ml. bed in a 2 ml. pipette at a slow rate, with contact times from three to four minutes. Appropriate chemical tests were employed to detect breakthrough.

The moisture capacity was determined by equilibrating the salt (chloride) form of the resin in water and determining the moisture content of the equilibrated resin.

Results

Physical Characteristics.—The physical characteristics of both resin series are described in Table I in which the variation of moisture capacity, apparent density and swelling characteristics are reported as a function of the degree of resin cross-linking. It is quite obvious that the ability to imbibe moisture and swell decreases markedly upon increasing the degree of cross-linking. Similarly, the degree of resin de-swelling upon electrolyte addition decreases as the degree of cross-linking increases.

Chemical Characteristics.—Exchange Capacities.—The total capacities of the two resin series are shown in Table I. An increase in the degree of cross-linking is accompanied by a decrease in total exchange capacity for both resin series. However, it is interesting to note that the weak-base resins decrease more rapidly than the corresponding strong bases.

The capacities for penicillin *G* as determined by a column procedure are shown as a function of degree of cross-linking in Table I. The adsorption of penicillin was studied using 0·01 N solutions of pure penicillin *G*. Nessler's reagent was used to detect leakage and breakthrough capacity. The much greater capacity for penicillin, in the porous exchangers, is shown clearly by the data. As the degree of cross-linking increases the fraction of the total resin capacity that is available to penicillin decreases markedly. In fact, at high degrees of cross-linking practically no adsorption of the large penicillin anion can be detected Similar results were obtained with the Series B resins for the adsorption of the weakly acidic coloured bodies in beet molasses.

Exchange Equilibria.—The effect of the degree of cross-linking upon anion-exchange equilibria was determined solely with the Series A, strong-base type resins The extent of exchange of various halogen anions with the hydroxyl ions of the resin A series is described in Table I. It is quite obvious that the degree of cross-linking has a marked effect upon the exchange equilibria. The results appear to indicate that as the degree of cross-linking increases the equilibrium constant approaches unity.

Discussion

The results indicate another parameter of importance in chromatography with ionic adsorbents, namely, the degree of porosity of the resin structure in addition to those of pH and basicity or acidity of the adsorbent is to be considered in applications of ion exchangers. By suitable modifications of presently available ion-exchange resins, the exchange capacity for large ions can be increased. Such " porous " exchangers are modified also with respect to swelling and hydraulic characteristics, but certain sacrifices can

be made in these matters to achieve appreciably improved capacities for large anions. Finite limitations on the size and shape of large ions that may be adsorbed are to be expected and will be determined by the structure characteristics of the resin particle. As the degree of cross-linking is increased, the ability to imbibe water and swell is decreased thereby limiting the penetration and exchange capacity towards large ions. These results are quite analogous to the molecular-sieve effect noted by Barrer [12] for the adsorption of gases by the zeolites and may therefore be considered an " ionic-sieve " effect.

The lack of ionic selectivity as the internal pore space increased due to a low degree of cross-linking has been noted by Samuelson [10] and Walton.[13] Walton attributes this effect to the fact that the environment inside of the resin particle tends to approach that of the external solution thereby decreasing the difference between the adsorption of the various ions.

Conclusions.—" Porous " modifications of conventional anion-exchange resins have been prepared and examined for their exchange and physical characteristics. It has been found that as the degree of cross-linking decreases the ability to adsorb large anions increases. It has also been noted that decreasing the degree of cross-linking markedly affects the exchange equilibria, decreasing ion selectivity.

The resins examined were synthesized by Dr. Charles McBurney and Dr. Fred Boettner in our Philadelphia laboratories. Miss Ruth Barry and Mr. Frank McGarvey assisted in the determination of exchange capacities.

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[12] Barrer, *Trans. Faraday Soc.*, 1944, **40**, 206
[13] Walton, *J. Chem. Educ.*, 1946, **23**, 545.

FULLY SWOLLEN ALGINATE GELS AS PERMUTITES: KINETICS OF CALCIUM-SODIUM ION EXCHANGE

BY J. L. MONGAR AND A. WASSERMANN

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On addition of sodium chloride solution to fully swollen cylindrical calcium alginate gels, a stationary sodium chloride concentration in the gel phase is established before appreciable conversion of the calcium alginate into the sodium salt has taken place. These observations, together with the results of other experiments, make it possible to estimate approximate initial values of third-order velocity coefficients characterizing a homogeneous ionic replacement reaction, not a diffusion step

The overall rate of previously investigated ion-exchange reactions appears to depend on the rate of a diffusion stage.[1] Some authors [2] interpreted the results of their kinetic measurements as indicating that the ion exchange itself is the slowest step; but, so far as we are aware, these experiments

[1] See, e.g., Boyd, Adamson and Meyers, *J. Amer. Chem. Soc.*, 1947, **69**, 2836.
[2] For references, see Duncan and Lister, *Quart. Rev.*, 1948, **2**, 307.

were done with adsorbents of non-uniform shape, and no attempt was made to find out how the velocity coefficients, computed from the analytical data, are influenced by the surface/volume ratio of the exchange material.

The work now to be described deals with the following reversible metathesis:

$$\text{calcium alginate} + \text{sodium chloride} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{mixed sodium-calcium alginate} + \text{calcium chloride.} \quad . \quad . \quad . \quad . \quad (1)$$

The chloride ions do not participate stoichiometrically and, therefore, the cation exchange proper can simply be represented by

$$Ca^{\cdot\cdot}{}_g + 2\,Na^{\cdot} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} 2\,Na^{\cdot}{}_g + Ca^{\cdot\cdot} \quad . \quad . \quad . \quad (1a)$$

where the species with the subscript g are confined to the gel phase, viz., they are " non-permeant," and k_1 and k_2 are third order velocity coefficients relating respectively to the forward and to the reverse process.

The results of the experiments are compatible with the assumption that the initial rate of the forward reaction relates to the homogeneous ion exchange itself, not to a diffusion step, and approximate numerical values of the velocity coefficient k_1 are estimated. The measurements were done with cylindrical alginate gels of high water-content, the surface-volume ratio of the test pieces being varied in the range 34–140 cm.$^{-1}$.

Results

The alginate gels, containing 92 % of water, were prepared, surface-dried and analyzed by methods described in a paper which we hope to publish in the *J. Chem. Soc.* (referred to below as Part I). Table I shows the numerical values of diffusion-coefficients D of some simple electrolytes dissolved in these gels.* These measurements were made by using a method similar to that outlined by Eggleton, Eggleton and Hill,[3] the D value in the last line being calculated with the help of an equation taken from Barrer.[4]

TABLE I

DIFFUSION COEFFICIENTS OF SIMPLE ELECTROLYTES IN ALGINATE GELS

Temperature ° C	Gel	Surface/volume Ratio of Gel (cm.$^{-1}$)	Permeant Electrolyte	Initial conc. of Permeant Electrolyte in Gel (g.-mole per l Gel)	Diffusion coef. × 10^6 (cm.^{2} × sec.$^{-1}$)
20	Sodium alginate	2	NaOH	2·30	18 ± 2
20	Sodium alginate	2	Na_2CO_3	1·00	8 ± 2
18	Calcium alginate	5	$CaCl_2$	0·0500 to 0·500	8·0 ± 0·8*
0	Calcium alginate	5	$CaCl_2$	0·0500 to 0·500	6·5 ± 0·7
18	Calcium alginate	60	$CaCl_2$	0·0500 to 0·500	7 ± 1†

* This value was calculated from observations in which only the earliest stage of the diffusion of calcium chloride from the gel to the solution was taken into account.

† This value relates to the latest stage of the diffusion.

[3] *Proc. Roy. Soc. B*, 1928, **103**, 620

[4] *Diffusion in and Through Solids* (Cambridge, 1941); cf. also Hill, *Proc. Roy. Soc. B*, 1928, **104**, 39.

* Sodium alginate is soluble in water, but if the sol is 2 N with respect to sodium hydroxide or carbonate a gradual transition into a gel occurs.

A comparison with the figures listed in *Landolt-Boernstein's Tables* shows that the D values of NaOH, Na_2CO_3 and $CaCl_2$ in water are similar to those listed in the last column of Table I; this is of interest because the diffusion coefficients of other simple electrolytes dissolved in a less highly swollen cation exchange material are 5–10 times smaller.[1 5]

In carrying out the kinetic measurements, fully swollen calcium alginate fibres of diameter varying between 0·24 and 1·2 mm. were hung over a hook and rinsed first with water and then with sodium chloride solution of the same temperature, thereby obtaining an effluent e_1. After known time intervals the flow of the sodium chloride solution was interrupted, the fibre was quickly surface-dried, weighed and rinsed with water until permeant electrolytes were removed from the gel, the effluent thus obtained being designated e_2. The two effluents were analyzed for calcium, chloride determinations in e_2 being

TABLE II

RESULTS OF EXPERIMENTS, WITH FULLY SWOLLEN CALCIUM ALGINATE FIBRES OF 0·48 MM. DIAM. AT 20°, SHOWING THE RAPID ATTAINMENT OF A STATIONARY SODIUM CHLORIDE CONCENTRATION IN THE GEL

Time of Treatment of Calcium Alginate Fibres with N NaCl Soln. (sec.)	Rate of Flow of NaCl Soln. (cm.³/min.)	Weight of Surface-dry Fibre (mg)	g. equiv. × 10⁴		Stationary NaCl concn. in Gel (g.-mole/l. Gel)
			Chloride Released from Fibre*	Calcium Released from Fibre†	
5	40	178	1·88	0·03	1·0 ± 0·1
5	18	179	1·4	0·03	0·8 ± 0·1
10‡	40	186	1·8	0·07	1·0 ± 0·1
10	40	180	1·8	0·07	1·0 ± 0·1
10	18	188	1·6	0·07	0·9 ± 0·1
20	40	186	2·1	0·14	1·1 ± 0·1
30	40	192	2·0	0·20	1·0 ± 0·1
60	40	159	2·0	0·35	1·1 ± 0·1
90	40	192	2·3	0·42	1·1 ± 0·1

* Into effluent e_2, during the final rinsing with water.

† Into effluent e_1, during the treatment with the NaCl solution; 100 % ion exchange corresponds to a release of $0·72 \times 10^{-4}$ g.-equiv. calcium.

‡ Using the appropriate equation for cylindrical diffusion (see ref.[4] a diffusion coefficient of 12×10^{-6} cm.² sec $^{-1}$ and assuming that the NaCl diffused into the gel is not used up, it can easily be shown that after 10 sec. the NaCl concentration should be about 0·9 g.-mole/l. gel.

TABLE III

KINETICS OF CALCIUM-SODIUM ION EXCHANGE: TYPICAL RESULTS

Temperature 37 ± 1°; Initial diam. of fully swollen calcium alginate fibre 1·15 mm.; initial concentration of non-permeant calcium in gel 0·2 g.-mole/l fully swollen gel; rate of flow of sodium chloride solution 5 cm.³/min.; stationary sodium chloride concentration in gel 0·0800 g.-mole/l. fully swollen gel.

Time of treating of fibre with sodium chloride solution (sec.)	180	300	600	840	1020
% Non-permeant calcium converted into $CaCl_2$ (which was analyzed in effluent e_1)	12	18	27	32	35
$10^4 \times k_1 \times a^2$ (sec.$^{-1}$)	6·9	6·6	5·2	4·6	4·2

[5] Bauman and Eichhorn, *J. Amer. Chem. Soc.*, 1947, **69**, 2832.

also carried out; it was possible, therefore, to estimate the sodium chloride concentration in the alginate gel and to establish that the quantity of calcium chloride in e_2 was negligibly small. The results of typical measurements are in Table II and III. The figures in the last line of Table III were obtained by integration of the following rate equation,

$$dx/dt = k_1 a^2(b - x), \quad . \quad . \quad . \quad . \quad (2)$$

where a is the stationary sodium chloride concentration in the gel, b and $b - x$ are the concentration values of the non-permeant calcium respectively at zero time and time t. The latter concentration could be estimated by taking into account the figures in the second line of Table III and the results of control tests (Part I) which showed that no significant storage of $CaCl_2$ in the fibre occurs, that there is no membrane hydrolysis, and that the ratio

$$\frac{\text{No. of g.-equiv. non-permeant calcium removed from gel as result of ion exchange}}{\text{No. of g.-equiv. non-permeant sodium incorporated into gel}}$$

is unity.

TABLE IV

KINETICS OF CALCIUM-SODIUM ION EXCHANGE: SUMMARY OF RESULTS

Run No.	Temp. ° C	Initial Diam. Fibre* (mm)	Stationary NaCl conc in Gel† (g-mole/l. Gel)	Rate of Flow of NaCl Soln (cm³/min)	pH of Soln	k_1^0 (l² g-mole⁻² sec.⁻¹)
1		0·28	1·10	2		0·020
2		0·43	1·00	2		0·014
3		0·55	1·00	2		0·016
4		0·55	1·00	10		0·014
5		0·55	1·00	20		0·014
6		0·96	0·800	2		0·016
7		0·28	0·800	2		0·016
8	20	0·40	0·400	2		0·030
9		0·28	0·130	2		0·14
10		0·45	0·100	2	5–6	0·16
11		0·45	0·100	10–20		0·16
12		0·50	0·100	40–45		0·12
13		1·1	0·08	5		0·14
14		1·1	0·08	30		0·18
15		0·28	0·07	2		0·16
16	5	0·55	0·10	40		0·16
17	5	1·1	0·08	5		0·12
18	30	1·0	0·08	5		0·16
19	37	1·1	0·08	5		0·13
20‡	20	0·45	0·10	45	10	0·12
21‡	20	1·1	0·08	5	10	0·12

* The diameter $2r$ of all fibres was much smaller than their length, the surface/volume ratio is therefore $2/r$

† Each figure listed in this column was obtained from the result of tests similar to those indicated by the data in Table II.

‡ In these experiments the calcium alginate was rinsed with a sodium chloride solution containing carbonate-free sodium hydroxide

Graphical extrapolation of $k_1 a^2$ to small conversion ratios leads to an initial value of $8{\cdot}5 \pm 0{\cdot}5 \times 10^{-4}$ sec.$^{-1}$, corresponding to an initial velocity coefficient of $k_1^0 = 0{\cdot}13 \pm 0{\cdot}04$ l.² g.-mole^{-2} sec.$^{-1}$ Similar experiments were carried out under conditions indicated by the figures in column 2–6 of Table IV, the relevant initial velocity coefficients being listed in the last column.

The accuracy of these k_1^0 values is $\pm$ 30 % in most runs, but in a few experiments, e.g., No. 15, it is $\pm$ 45 %. Such errors are considerably larger than

those of most published rate measurements relating to one-phase systems or to heterogeneous reactions involving less highly swollen adsorbents. It should also be noted that the fibre diameter could only be varied within a rather narrow range.

Discussion

The kinetic significance of velocity coefficients deduced from eqn. (2) is based on the validity of the following assumptions: (i) the diffusion of NaCl from the outside solution into the gel is fast compared to the rate of formation of $CaCl_2$; (ii) the volume of the alginate gel remains constant throughout the course of observation; (iii) the diffusion of $CaCl_2$ from the gel to the outside solution is fast compared to the rate of the reverse reaction (1). The figures in Table II and the results of similar tests show that a stationary NaCl concentration in the gel is rapidly attained before an appreciable conversion of calcium alginate has occurred; and it appears, therefore, that as a first approximation (i) is valid. It is known,[6] on the other hand, that the conversion of calcium alginate into the mixed salt is accompanied by considerable swelling, so that the second assumption is not compatible with the actual facts. The swelling of the alginate gels must give rise to a decrease of $b - x$ in eqn. (2), which in turn should bring about an increase of k_1a^2. The figures in the last line of Table III,

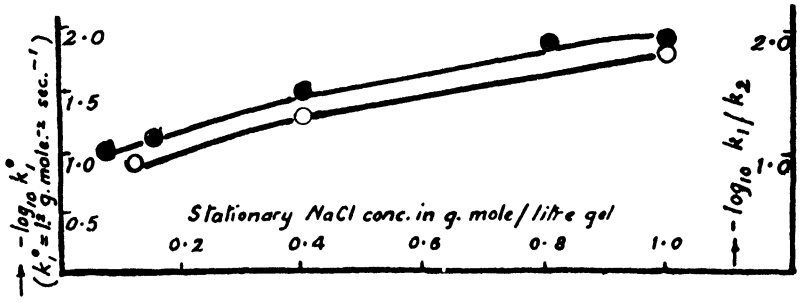

FIG. 1.—Concentration dependence of velocity and equilibrium coefficients. Temperature 20°; (A) black circles left ordinate; (B) open circles right ordinate.

and the results of other experiments, show, however, that the k_1a^2 values decrease with increasing conversion, and we believe that under these conditions the influence of the back reaction over-compensates the effect due to the swelling. The instantaneous rate of the reverse reaction (1), which is probably proportional to the square of the concentration of the non-permeant sodium ions, must decrease rapidly with decreasing conversion into the mixed alginate, and it is reasonable, therefore, to postulate the validity of (iii) for the initial stage of the interaction between calcium alginate and sodium chloride. Such a hypothesis is in accordance with experiments showing that the k_1° values are not detectably dependent on the rate of flow of the NaCl solution or on the fibre diameter (cf. Runs Nos. 1–7 and 12–16 of Table IV). If it should be possible in future to carry out more accurate measurements it may be found that the initial velocity coefficients increase somewhat with increasing surface/volume ratio of the test piece. Observations of this kind would not invalidate any essential conclusions to be derived from this work; but the correct initial velocity coefficients would have to be estimated by plotting the fibre diameter, r, against $k_1^{\circ(r)}$ and by extrapolating to $r = 0$.

[6] Mongar and Wassermann, *J. Physiol.*, 1947, **106**, 32P. MacArthur, Mongar and Wassermann, *Nature*, 1949, **164**, 110.

The k_1^o values in Table IV increase with decreasing NaCl concentration, as shown by curve A in the Figure. The trend can be taken to be a solvent effect, the dielectric constant and other properties of a medium being dependent on the salt concentration. Curve B represents the concentration dependence of the equilibrium coefficient k_1/k_2, the later values having been determined by measurements done in a static system (Part I). These graphs provide confirmatory evidence for the correct interpretation of the kinetic measurements, it being well known that in many cases an alteration of chemical conditions gives rise to similar changes of rate and equilibrium coefficients.[7]

The association of certain simple electrolytes involves covalent forces [8] and it is not impossible that similar effects play a role, to a certain extent, in the salts of alginic acid. Moreover, the solvation of permeant sodium or calcium ions is probably not identical with that of these cations if they are stoichiometrically combined with the alginate residues, thereby being held in proximity of a highly charged poly-anion. The ion exchange may be accompanied, therefore, by an alteration of both covalent and solvation forces and this should give rise to an appreciable activation energy or to a small probability of reaction. The observed temperature dependence of the velocity coefficient k_1^o (see Runs Nos. 16–19 in Table IV) is not compatible with an overall activation energy exceeding a few kcal. It has to be taken into account, however, that the calcium-sodium ion exchange, like other homogeneous ionic processes [9] of the stoichiometric type 3 reactants→product, can take place as a result of consecutive bimolecular processes. These can be formulated in the present case as follows:

$$\left.\begin{aligned} \mathrm{Ca^{\cdot\cdot}}_g + \mathrm{Na^{\cdot}} &\underset{k'_{-1}}{\overset{k'_1}{\rightleftharpoons}} \{\mathrm{Ca^{\cdot\cdot}}_g\} + \mathrm{Na^{\cdot}}_g \\ \{\mathrm{Ca^{\cdot\cdot}}_g\} + \mathrm{Na^{\cdot}} &\xrightarrow{k''} \mathrm{Ca^{\cdot\cdot}} + \mathrm{Na^{\cdot}}_g \end{aligned}\right\}, \quad . \quad . \quad . \quad (3)$$

where $\{\mathrm{Ca^{\cdot\cdot}}_g\}$ is the symbol for an intermediate in which the non-permeant calcium is combined with only one carboxylate grouping of an alginate residue; k'_1, k'_{-1} and k'' are bimolecular velocity coefficients and the significance of the other symbols is the same as in (1*a*). It can be shown, with the help of the stationary state approximation, that k_1^o is proportional to $k''k'_1/k'_{-1}$, provided $k'_{-1}[\mathrm{Na^{\cdot}}_g] \gg k''[\mathrm{Na^{\cdot}}]$. The overall activation energy E of the forward reaction (1) would be given, therefore, by $E = E'' + E'_1 - E'_{-1}$, where the various terms on the right-hand side relate to the corresponding velocity coefficient in (3). Thus a small overall activation energy does not necessarily imply a small activation energy of elementary bimolecular replacement steps.

The work described in this paper forms part of an investigation into the influence of ion exchange on the molecular shape of chain-like poly-electrolytes. A grant from the Department of Scientific and Industrial Research is gratefully acknowledged.

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London.

[7] For a theoretical treatment of such correlations see, e g, Evans and Polanyi, *Trans Faraday Soc.*, 1935, **31**, 492.

[8] Bell and Prue, *J. Chem. Soc*, 1949, 362, where references to previous work will be found.
E g, Weiss, *J. Chem. Soc*, 1944, 309.

SOME METHODS FOR EXTENDING THE SCOPE OF PARTITION CHROMATOGRAPHY

By Alfred A. Levi

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Three methods are described for extending partition chromatography to substances with limited solubilities in water.

Although in a large majority of cases adsorption chromatography is a very convenient and efficient process, partition chromatography offers certain advantages, especially for colourless substances and for quantitative work. Thus:

(i) Partition chromatography as described by Martin and Synge [1] offers scope for the separation of acids, since few adsorbents have been found which will give satisfactory chromatograms with acidic substances.

(ii) Substances left as bands on the column after a partition chromatogram are always easily eluted by simple extraction with more of the stationary phase. The quantitative elution of adsorbed material is sometimes difficult, or impossible.

(iii) Conditions on a partition chromatogram are generally easily reproducible. This is not always the case with adsorption, since it is difficult to make successive batches of adsorbents with reproducible properties.

(iv) Distribution * isotherms for partition are conveniently measured in tap funnels, or on columns. Hence the data required for calculations based on chromatographic theory are easily obtained.

(v) Experience has shown that with silica gel as support little or no "coning" occurs in carefully prepared columns, and the fronts of the bands are substantially horizontal even in quite large columns.

On the other hand, the original method of partition chromatography is applicable only to the comparatively few substances which have at least a moderate solubility in water. The present paper reviews some methods by which this limitation can be to some extent overcome.

In the first method water is replaced by another solvent immiscible or only partially miscible with the flowing phase. This method has given some excellent separations, but is again severely limited by the relatively small number of solvent pairs which can be used. Examples are: (i) For the separation of isomeric hexachlorocyclohexanes [2]: stationary phase, nitromethane; flowing phase, *n*-hexane. (ii) For the separation of fatty acids [3]: stationary phase, methanol; flowing phase, low-boiling liquid paraffins.

In the few examples so far published, isotherms for substances separated appear to be linear or nearly so. This method should therefore be worth

[1] Martin and Synge, *Biochem J.*, 1941, **35**, 1358.
[2] Ramsay and Patterson, *J. Ass Off. Agric. Chem*, 1946, **29**, 337.
[3] Ramsay and Patterson, *J Ass Off Agric Chem*, 1948, **31**, 139, 164

* It is suggested that the terms "distribution ratio, distribution isotherm," etc., be used to cover all cases where a substance distributes itself between two phases, whether by adsorption, partition, ion exchange, or other mechanism.

considering whenever a mixture with suitable physical properties is encountered.

In the second method, water as stationary phase is replaced by a solution of some substance which reacts reversibly with some or all of the constituents of a mixture. The most generally useful system consists of a solution of a suitable buffer as stationary phase. An immiscible solvent forms the flowing phase. By this means mixtures of acids or bases can be separated. This technique, which requires no unusual practical procedure, offers the following advantages:

(i) By the choice of a suitable solute in the stationary phase a partition chromatographic technique becomes available for substances which have a relatively low solubility in water.

(ii) Another variable for the control of the rate of movement of the bands becomes available in the case of acids and bases.

(iii) Extremes of acidity and alkalinity can be avoided when handling sensitive material.

(iv) By the use of concentrated solutions as stationary phase relatively large quantities of material can be separated.

(v) Impurities in the support, such as traces of alkali or foreign metals in silica gel, become of less importance in the presence of the buffer.

This technique was successful in the separation of the different penicillins,[4] and has also given some useful results in the analysis of alkaloid mixtures.[5] It suffers from two disadvantages:

(i) With colourless substances, indicators on the column can no longer be used, as in the Martin and Synge technique. This means that observations must be largely confined to the effluent solution. There are now a number of procedures available for this and in view of the flexibility of the system, this is no great disadvantage.

(ii) In general the distribution of substances between buffer and solvent seems to follow isotherms of the Freundlich type, viz., $Q = aC^n$. The bands obtained are unsymmetrical and with large loads much overlapping of bands may occur. It has been shown that the position and shape of a band of phenylacetic acid on such a column with ether as solvent can be predicted from chromatographic theory, and from the isotherms determined in tap funnels.[5]

The difficulty of "tailing" was serious with the penicillins, and in all cases chromatographic column fractionation has had to be supplemented by crystallization to obtain homogeneous material.

It is worth pointing out that curved isotherms are by no means confined to buffered solutions. Many instances are known where substances are distributed between solvent and water in conformity with Freundlich distribution isotherms and in some cases the value of the exponent is quite low (*ca.* 0·5). This difficulty can be turned to advantage by making use of the technique of displacement development,[6] since for this procedure to be successful, distribution ratio must vary with concentration, i.e., the isotherms must be curved. The study of displacement development on partition columns in these laboratories has brought to light some points of interest not previously discussed, and these are described below.

For partition chromatography isotherms can be determined by three methods:

(i) By simple partition experiments in tap funnels.

(ii) By passing sufficient of a solution of known concentration C_0 through

[4] Levi *et al.*, *Biochem. J.*, 1948, **43**, 257, 262.
[5] Evans and Partridge, *Quart J. Pharm. Pharmacol.*, 1948, **21**, 126; **31**, 441.
[6] Tiselius, *Arkiv. Kemi, Min. Geol. A*, 1943, **16**, 1.

a column to give an incompletely developed band. From measurements of the volumes of effluent and of the concentration variation of the tail, the isotherm can be calculated. For the Freundlich isotherm it is only necessary to find the retention volume V of the front of the constant concentration zone, and the volume v at which the constant concentration zone just finishes. If Q_0 is the concentration in the stationary phase, V_0 the initial volume S the pore volume of the column, and P the weight of stationary phase, then :

$$Q_0/C_0 = aC_0^{n-1} = (V - S)/P ,$$
$$dQ_0/dC_0 = anC_0^{n-1} = (v - V_0 - S)/P ,$$
$$n = (v - V_0 - S)/(V - S) .$$

In practice v is liable to be somewhat too low, with a corresponding effect on n, but the procedure is convenient, and sufficiently accurate for such purposes as choice of developer for a displacement chromatogram.

(iii) By determination of specific retention volumes at a series of known initial concentrations. This is the most accurate method and for partition is convenient, if some device is available for detecting the issuing zones.

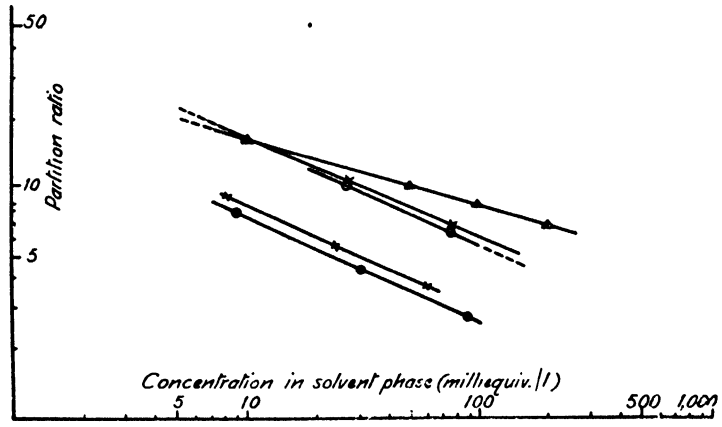

FIG. 1.—Series of distribution isotherms determined on partition chromatograms by method (iii).

From the family of isotherms so obtained a developer at suitable concentration is chosen, and the characteristic step concentrations are read off from the graphs (see Fig. 1), or are calculated. Thus for the Freundlich isotherm if C_D is the developer concentration, and C_1, C_2, ... the final step concentrations of the components,

$$Q_1/C_1 = Q_2/C_2 = \ldots\ldots Q_D/C_D ,$$
$$\text{or } \log a_1 + (n_1 - 1) \log C_1 = \log a_2 + (n_2 - 1) \log C_2 = \ldots\ldots \log a_D + (n_D - 1) \log C_D .$$

The following interesting deduction can be made from this equation. If we assume for simplicity that n is the same for all the substances, we have

$$\log C_2 - \log C_1 = (\log a_1 - \log a_2)/(n - 1).$$

Since n usually has values lying between 0·5 and 1·0 it is seen that concentration differences between steps may be quite large for very small differences in the values of a. In fact, the only limit to the resolving power of this technique is the sensitivity to small changes in concentration of the device used to follow the issuing zones.

If an approximate knowledge of the composition of the mixture to be analyzed is available the length of column occupied by the completed displacement chromatogram can be calculated. This is useful as a guide to the amount of charge which can be put on a given column. Experience has shown that the column should be some three to four times the above calculated length. If the front of the developer moves a distance x_D when unit volume passes through the column, and there are m_1, m_2 ..... milli-equivalents of the components present in the initial charge, then the length of column occupied by the completed chromatogram will be

$$x_D \left[\frac{m_1}{C_1} + \frac{m_2}{C_2} + \ldots\ldots \right].$$

If from a previous frontal analysis, or by other means, x_o, the distance travelled per unit volume by the front of the original mixed zone before development commences is known, then the volume of developer required to complete the displacement chromatogram can be calculated.

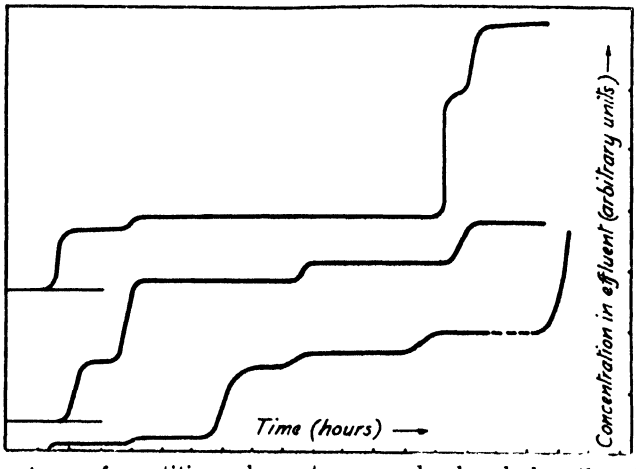

FIG. 2.—Tracings of partition chromatograms developed by the displacement technique.

Thus, assume that until displacement is complete the front of the whole system continues to move at a rate x_o per unit volume. When displacement is completed the front of the new zones will have moved the same distance.

Hence $x_o (V_o + V_D) = x_D V_D + x_D [m_1/C_1 + m_2/C_2 + \ldots.]$,

where V_o is the volume of the original solution.

Therefore $$V_D = \frac{x_D (m_1/C_1 + m_2/C_2 + \ldots.) - V_o x_o}{x_o - x_D}.$$

Since in most cases the front of the undisplaced zones will move a distance less than $x_o V_D$ this expression will give a maximum value for V_D.

From what has been said above, it can be seen that this technique offers very great scope for the quantitative analysis of complex mixtures of closely related materials (e.g., homologous series, position isomerides, and stereoisomerides). By working on a small scale with narrow columns the technique of coupled filters, or "front straighteners" [7] has not been found necessary in partition chromatograms (see Fig. 2). These would doubtless

[7] Claesson, *Arkiv. Kemi, Min. Geol. A*, 1947, **24**, 1.

be advantageous on a preparative scale, but this possibility has not yet been adequately explored.

The great sensitivity of the above method of displacement development to small differences in partition characteristics involves some restriction on the range of substances which can be separated under any given set of conditions. Hence for a very crude mixture some preliminary separation is desirable. For this purpose partition chromatography offers another device in the use of a stationary phase consisting of a solution or suspension of some substance which reacts irreversibly with the components of a mixture.[8] The chromatogram which results from passing a mixture through such a column resembles a frontal analysis in that a number of zones are formed equal to the number of substances present. Each new zone from the bottom to the top of the column contains a new component (in addition to those already present) in the order of increasing affinity for the stationary phase. The picture differs from frontal analysis in that the total molecular concentration in each step is the same (except in so far as it may be modified by physical partition between solvent and water alone). Separation cannot be complete in such a system, and the method is of little quantitative value. The products, being in solution, are readily recovered from the silica or other support by simple extraction with water. This procedure was valuable in the case of crude penicillin, especially for the earlier samples before the fermentation process was under adequate control. Large quantities of material could be considerably improved in quality without serious loss by treatment in ether solution on silica gel columns impregnated with caustic soda solution. A partial separation of bases (e.g., a crude extract of alkaloids) could obviously be similarly effected.

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[8] Catch, Cook and Heilbron, *Nature*, 1942, **150**, 163.

PARTITION CHROMATOGRAPHY ON PAPER WITH SPECIAL REFERENCE TO QUANTITATIVE SEPARATIONS

By A. H. Gordon

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Recent improvements in the method of partition chromatography on paper are reviewed. By modification of the solvents used new groups of substances can be separated. In some cases (e.g., esters of the fatty acids) reversed phase chromatograms are most convenient. Many attempts have also been made to employ the paper chromatogram for quantitative estimations; considerable success has been achieved in the case of the amino acids which, after elution from the paper, can be converted into Cu salts suitable for colorimetric determination. Some of the factors making for low recoveries, such as the impurities present in the filter paper, are also discussed.

The method of partition chromatography on paper is at present being developed in two different directions. New groups of substances are being brought within the scope of the method by the introduction of new solvent mixtures and modified techniques for the detection of the spots on the

chromatogram. In addition, efforts are being made to obtain quantitative recoveries of those materials whose separation is already well established. No attempt will be made here to list all the groups of substances to which the method has already been applied, its scope has already been reviewed.[1 2 3] The purpose of the present work is to consider, first, the conditions necessary for successful separations on paper chromatograms and, second, some of the various techniques which have been used for the quantitative estimation of the separated substances. For separations to take place the following factors need to be considered.

(*a*) **Choice of Solvent.**—A solvent must be found partially miscible with water giving sufficiently different partition coefficients for the various substances of the mixture to be separated. In practice this can be taken to mean differences of at least 10 %. Where no single solvent is available giving adequate differences of partition coefficient for all the components of the mixture, a second solvent can be subsequently utilized by means of the two-dimensional technique.[4] Although many substances can be separated in systems of this kind in which the solvent held stationary in the filter paper is water, for substances of a very non-polar character, it may be necessary to work in non-aqueous systems. Thus Boldingh [5] by pre-treating filter paper with latex has been able to separate esters of fatty acids by means of methanol. In this system the rubber itself acts as the immobile solvent. Boldingh has also successfully used mixed organic solvents such as methanol, benzene and 1/1 methanol acetone on rubber paper. He remarks that the rubber acts as a carrier for the less polar solvent. This is no doubt true, but as using methanol acetone the R_f values are considerably increased compared with those obtained with methanol alone, the effect of the acetone in the mobile phase must also be important.

The use of aqueous systems employing mixed organic solvents as the moving phase has often proved necessary. Also the partition coefficients of the substances under separation have been modified by suitable additions to the aqueous phase. Systems giving convenient partition coefficients may be obtained in one of the following ways.

(i) The R_f values can be changed by the admixture of a suitable proportion of a second organic solvent. This solvent may be completely miscible with water. So long as not more than enough is used for the formation of two phases, useful results may be expected. The effect of the addition of a more polar solvent is, of course, to increase the water content of the organic solvent phase and thus the R_f values of all types of substance. Results of this kind were obtained by Partridge [6] who measured the R_f values of the sugars in *n*-butanol and in *n*-butanol containing 10 % ethanol. Before the introduction of partition chromatography on paper this principle was used by Martin and Synge [7 8] when they increased the R values of the acetylamino acids on silica gel chromatograms by employing progressively higher concentrations of *n*-butanol in chloroform. A second effect of the alcohol in this system was its virtual elimination of adsorption of the acetylamino acids by the gel. Even though most substances seem scarcely to be adsorbed by filter paper, in those cases, e.g., the chromatography

[1] Consden, *Nature*, 1948, **162**, 359.
[2] Hais, *Chem. Listy*, 1948, **42**, 125.
[3] Hais and Rábek, *Chem. Listy*, 1949, **43**, 80.
[4] Consden, Gordon and Martin, *Biochem. J.*, 1944, **38**, 224.
[5] Boldingh, *Experientia*, 1948, **4**, 270.
[6] Partridge, *Biochem. J.*, 1948, **42**, 238.
[7] Martin and Synge, *Biochem. J.*, 1941, **35**, 1358.
[8] Gordon, Martin and Synge, *Biochem. J.*, 1943, **37**, 79.

of inorganic ions,[9] where such effects may be significant they are no doubt reduced by the admixture of more polar organic solvents.

(ii) If the substances being separated are acids or bases their R_f values can be greatly influenced by adjustment of the pH of the aqueous phase. As an example, the effect of ammonia on chromatograms developed with phenol in decreasing the R_f values of the dicarboxylic and increasing those of the basic amino acids may be mentioned. Conversely, acetic acid has been used by Lugg [10] to assist the separation of certain organic acids by *n*-butanol. Buffer solutions [11] have also been used in a rather similar way both on filter paper and on silica gel chromatograms for the separation of the penicillins. A further advantage of the addition of buffers, at least on silica gel chromatograms, is that partition chromatograms can be run by displacement development and thus much larger amounts can be handled.

In addition, the R_f values of certain compounds can be modified by the addition of substances mainly soluble in the organic solvent phase. Thus Winsten and Eigen [12] have found that the R_f values in *n*-butanol of the various members of the streptomycin group could be markedly increased by the addition of 2 % of *p*-toluenesulphonic acid. The solvent also contained 2 % of piperidine which served partially to de-ionize the basic groups of the streptomycins. The amount of *p*-toluenesulphonic acid used was such that the pH of the system (determined after the addition of an equal volume of water) was on the alkaline side. However, it was noted that separations could still be obtained if so much *p*-toluenesulphonic acid had been used that the pH was acid. It is interesting to compare the effect of *p*-toluenesulphonic acid with that of ammonia in *n*-butanol. Thus if it is assumed that the increased R_f values of the streptomycins were not solely due to the piperidine, then *p*-toluenesulphonic acid, although an acid substance, must have affected the system in the same sense as would be expected for the addition of ammonia. It seems likely that additions of this type have their effect both because they increase the polarity of the organic solvent phase and because as acids they specifically modify the ability of this phase to dissolve the substances being separated. That the effect is not due only to the former cause seems to be indicated by the observation [13] that the addition of *p*-toluenesulphonic acid to collidine increases the R_f values of the basic preferentially to those of the neutral amino acids.

(*b*) **Adsorption by the Paper.**—If the separations are to depend only on partition effects, the substances in the mixture to be separated must not be adsorbed by the paper. Fortunately few substances are strongly adsorbed by untreated filter paper. Perhaps the largest group of substances for which adsorption is likely to be important are the organic dyestuffs. Attempts to separate dyes by partition chromatography on paper often lead to resolution of the mixture. However, closer examination of the system may reveal that partition effects have been secondary (as is the case if the separation can be carried out equally well with the dry solvent). Adsorption chromatography on strips of filter paper of the streptomycins using a 2 % aqueous solution of ammonium chloride as solvent has been described by Horne and Pollard.[14] Unfortunately no resolution of the different streptomycins was obtained.

[9] Arden, Burstall, Davies, Lewis and Linstead, *Nature*, 1948, **162**, 691.
[10] Lugg and Overell, *Nature*, 1947, **160**, 87.
[11] Goodall and Levi, *Nature*, 1946, **158**, 675.
[12] Winsten and Eigen, *J. Amer. Chem. Soc.*, 1948, **70**, 3333.
[13] Gordon (unpublished).
[14] Horne and Pollard, *J. Bact.*, 1948, **55**, 231.

Among large molecules the proteins are also known to be adsorbed, at least from salt solutions, by filter paper. These effects will not be discussed further here as they may be the subject of another contribution. The examples mentioned may be enough to illustrate the possibility of adsorption in paper chromatography. This possibility should not automatically lead to the opposite view that the separations in all systems employing solvents which do not form two phases with water must necessarily be due to adsorption. As was first suggested to the author by Dr. Synge,[15] a system employing, for instance, pyridine and water can be considered to be a partition chromatogram because the effect of the cellulose hydroxyl groups results in the existence of a very different milieu inside the fibre from that of the moving phase.

(*c*) **Chemical Reactivity of Solvents and Paper.** –The substances under separation must not react either with the paper or with the solvents used. For the amino acids and many of the other substances for whose separation paper chromatography has proved applicable, these criteria have proved to be rather easily fulfilled. However, the recent finding of Moore and Stein,[16] that using 1/2/1–*n*-butanol–*n*-propanol–0·1 N HCl on starch chromatograms 6% and 7% of aspartic and glutamic acids respectively became esterified, suggests that closer and more quantitative examination of paper chromatograms in which mixtures of acids and alcohols are used might reveal similar effects. Cellulose itself fortunately shows little or no reactivity towards either the substances separable on paper chromatograms or the solvents used for their separation. The problem thus becomes that of the possible reactions which may occur between the substances under separation, the solvents and impurities present in the paper. This will be further discussed in the next section.

(*d*) **Techniques for Detection and Estimation of Spots.**—Finally for qualitative work a suitable detection technique must be available. In practice the sensitivity, reliability and ease of application of such techniques may be the decisive factor in determining whether paper chromatography is to be used. Certainly the remarkable sensitivity of ninhydrin by which less than 1 μg. of an amino acid can just be made visible has been most useful. The small scale of the method as a whole, e.g., 1–20 μg. per component of the mixture under separation for the amino acids, the sugars and for the purines and pyrimidines, has the advantage that it is usually possible to develop as many chromatograms as there are different detection techniques available. Particularly when dealing with complex biological fluids this type of approach by which several classes of compounds can be simultaneously investigated may be of value.

If quantitative estimations are to be attempted, not only must the substances be located, but sufficiently accurate means must be found for their estimation either *in situ* or after removal from the paper. For quantitative purposes, obviously the most suitable detection techniques are those which do not permanently affect the substances to be estimated. The most generally applicable means by which this can be accomplished is certainly the examination of the spots in u.-v. light. In this way spots of purines, pyrimidines, amino acids and peptides can be shown up. Holiday [17] has reported that as little as 0·5–1 μg. per cm.^{2} of the purines or pyrimidines can be detected if u.-v. light of wavelengths between 230 and 400 mμ is used, the most important wavelengths being in the region

[15] Synge (personal communication).
[16] Moore and Stein, *J. Biol. Chem.*, 1949, **178**, 53.
[17] Holiday and Johnson, *Nature*, 1949, **163**, 216.

of 254 mμ. Although only amounts of more than 3 μg. per cm.^{2} of the amino acids can be detected [18] the use of this technique has been continued by Woiwod.[19] By this means it is possible to avoid the necessity of developing parallel guide chromatograms and also the most inconvenient process of visualizing the spots as their mercury derivatives in the case of the purines and pyrimidines.[20]

The only chemical method so far reported which ultimately leaves the substances unchanged on the chromatogram seems to be that of Brante.[21] This author has sprayed chromatograms of bases such as amino-ethanol, choline and creatine with an alcoholic solution of iodine. Brown spots are formed which disappear on being allowed to stand. Unfortunately this reagent is not very sensitive for the amino acids, but, as noted by Brante, it may prove useful in quantitative experiments.

Turning now to the various methods which have been used for the quantitative estimation of substances separated by paper chromatography, these may be divided into those in which the substances are estimated on the paper, usually as coloured derivatives, and those in which they are first eluted and then estimated in solution. The former approach seems only to have been employed for the coloured substances formed from the amino acids by ninhydrin and for substances containing radio elements. Thus several authors [22 23] have reported that useful rough estimations can be made by visually comparing the spot strengths with those of a series of standard spots. Attempts have also been made to increase the accuracy of this method by the preliminary division of the amino acids into acidic, neutral and basic types.[24] A spectrophotometer was used for estimating the strengths of the spots but as yet no indication has been given of the accuracy thus obtained. A spectrophotometer has been similarly used by Bull [25] who has been at pains to standardize the conditions of colour development. Under the conditions chosen, arginine, serine, valine, glutamic acid, leucine, threonine, alanine and lysine gave the same amount of colour per mole, the probable error for a single determination being almost 9 %. This result is somewhat unexpected as Moore and Stein,[26] who have carefully measured the amounts of colour formed in solution in presence of a reducing agent, have reported, for instance, that threonine forms only 81 % as much colour as does lysine. It may possibly be the case that some of the amino acids form more nearly equivalent amounts of colour in solution than on paper. On the other hand Bull [25] found that glycine and methionine give less colour on paper than does leucine, whereas Moore and Stein [26] indicate that under their conditions these three amino acids yield almost identical amounts of colour per mole. Possibly more thorough investigations of the optimum conditions for the development of the ninhydrin colour on paper may lead to some reduction in the rather large errors at present involved in this technique. Work of this kind may also explain why Pratt and Auclair [27] find that glycine and glutamic acid are the amino acids giving visible spots at minimum strength, whereas Moore and Stein [26] have found lysine to give the strongest colour in solution.

Where substances containing radioactive isotopes have been separated

[18] Phillips, *Nature*, 1948, **161**, 153.
[19] Woiwod, *Biochem. J.* (in press).
[20] Vischer and Chargaff, *J. Biol. Chem.*, 1948, **176**, 703.
[21] Brante, *Nature*, 1949, **163**, 651.
[22] Consden, Gordon, Martin and Synge, *Biochem. J.*, 1947, **41**, 596.
[23] Polson, *Biochim. Biophys. Acta*, 1948, **2**, 575.
[24] Block, *Science*, 1948, **108**, 608.
[25] Bull, Hahn and Baptist, *J. Amer. Chem. Soc.*, 1949, **71**, 550.
[26] Moore and Stein, *J. Biol. Chem.*, 1948, **176**, 367.
[27] Pratt and Auclair, *Science*, 1948, **108**, 213.

the detection technique, i.e., the exposure of successive areas of the chromatogram to the counter, is itself suitable as a quantitative estimation. An apparatus designed for the estimation of spots containing P^{32} has recently been described.[28] In order to estimate the amino acids by means of radioactive isotopes conditions have been worked out for their quantitative conversion into *p*-iodophenylsulphonyl (pipsyl) derivatives containing I^{132}. After chromatography on paper nearly quantitative recoveries were obtained for the few amino acids tested.[29] Further work on this promising method will be awaited with interest.

Although techniques in which the substances are eluted from the paper prior to estimation cannot have the simplicity of those already mentioned, they would seem to offer several compensating advantages. Thus, once the substance is in solution it can be treated with a much wider variety of reagents than while it is still on paper. This should, for instance, allow better control of the conditions under which the ninhydrin colour is formed from the amino acids. Unfortunately, no attempt has been reported to utilize the valuable information on this subject already obtained by Moore and Stein.[26]

The most successful attempts to estimate quantitatively the amino acids involve their conversion into copper complexes. Thus Martin and Mittelmann [30] used what was essentially the method of Pope and Stevens,[31] in which the amino acids are allowed to react with a suspension of copper phosphate in phosphate–borate buffer. After centrifugation from the excess of copper phosphate, the amount of copper in solution was estimated polarographically. No attempt was made to determine the recoveries of the amino acids after elution from the paper, but, since the current increased linearly with the amount used, estimations were shown to be possible if several comparison chromatograms of known amounts of each amino acid were developed simultaneously with each analysis. The results in a test analysis of gramicidin S clearly substantiated the theory that this peptide consists of five different amino acids in equimolar proportions. Only in the case of one of these acids, leucine, did the amount found deviate more than 5 % from that expected.

The copper method has also been used by Woiwod,[19] who has modified the reagent by replacing sodium borate with di-sodium hydrogen phosphate. He has thus obtained a more stable suspension, lower blanks, and a linear relationship between amino nitrogen and copper. The copper has been estimated colorimetrically with sodium diethyldithiocarbamate instead of polarographically. In order to obtain sufficient accuracy for those amino acids present as the weaker components of mixtures, the analyses have been conducted on larger amounts (1–50 μg. amino nitrogen) than those employed by Martin and Mittelmann.[30] In this way similar accuracies seem to have been achieved, and, because of the convenience of colorimetry compared with polarography, this is probably at the moment the method of choice. Generally Woiwod, like Martin and Mittelmann, has employed the comparative method, but in addition he has measured the losses sustained as an amino acid moves down the chromatogram. Thus only 86 % of the original amount of glycine could be recovered after the spot had moved 10 in. down a chromatogram developed with 40 % *n*-butanol–10 % acetic acid–50 % water. In partial explanation of such low recoveries, Woiwod mentions an interesting observation to the effect that fluorescent

[28] Lindberg and Hummel, *Arkiv. Kemi*, 1949, **1**, 17.
[29] Keston, Udenfriend and Levy, *J. Amer. Chem. Soc.*, 1947, **69**, 3151.
[30] Martin and Mittelmann, *Biochem. J.*, 1948, **43**, 353.
[31] Pope and Stevens, *Biochem. J.*, 1939, **33**, 1070.

material can often be seen extending right down to the front of the solvent. He considers that this may be due to failure of the amino acid to reach equilibrium with the mobile phase. However, as the usual treatment with ninhydrin, which is believed to be capable of revealing smaller amounts of amino acids than are visible in u.-v. light, does not show up such streaks, it seems more likely that the amino acid must have been converted into a form no longer capable of giving a colour with ninhydrin. The existence of some substances in certain filter papers capable of combining with the amino acids is already known from the observations of Consden *et al.*[4] on the formation of copper complexes of the amino acids. These are progressively formed as the amino acids move down the chromatogram. These authors [32] have also noted the formation on paper chromatograms of a zinc salt of cysteic acid which like the copper complexes gives a pink colour with ninhydrin. The R_f values of these metal complexes are close to those of the corresponding free amino acids, but there seems no reason why there may not be other impurities in the paper which could combine to form complexes moving with any R_f value. The more the R_f values of such impurity amino-acid complexes differ from that of the amino acid itself, the more difficult it must be to obtain quantitative recoveries from the paper. The solution of these difficulties may either be similar to that adopted by Consden *et al.*[4] who added a reagent to the paper which prevented the action of the copper by itself combining with it ; or else it may be necessary to employ special purification of the paper. If purification of the natural paper making materials proves inadequate, use may in time be made of papers formed entirely from synthetic fibres.

These findings naturally raise the question of the occurrence of similar losses as other substances move down paper chromatograms. Fortunately, very accurate methods of analysis exist both for the purines and pyrimidines and for the sugars after elution from the filter paper. For the purines and pyrimidines it is only necessary to measure their absorption at the appropriate wavelength in u.-v. light. Both Vischer and Chargaff [20] and Hotchkiss [33] have used this method without finding necessary a comparative approach similar to that employed for the amino acids. They report accuracies of ± 4 % and do not mention progressive losses. The figures quoted suggest slight losses for those spots which have moved further down the chromatograms but are hardly sufficient to be decisive. The need for further work on this question is apparent.

Even more accurate analyses of sugars after separation on paper chromatograms have been reported,[34] and again no mention of progressive losses has been made. It may not be accidental that materials similar to the matrix of the chromatogram can be recovered with least loss.

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[32] Consden, Gordon and Martin, *Biochem J.*, 1946, **40**, 580.
[33] Hotchkiss, *J. Biol. Chem.*, 1948, **175**, 315.
[34] Flood, Hirst and Jones, *J. Chem. Soc.*, 1948, 1679.

SEPARATIONS USING ZEOLITIC MATERIALS

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A short account has been given of aspects of zeolitic sorption which are important in separating molecular mixtures. Some natural crystalline zeolites fall into three classes of molecular-sieve sorbent each capable of separating mixtures by selective occlusion if there are sufficient differences in shape and dimensions between the molecules in the mixture. The molecules removed by occlusion must always be comparatively small, but separations may be quantitative after a single exposure to the zeolite.

By cation interchange and by burning out interstitial ammonium ions a diversity of modified molecular-sieve sorbents can be produced with extension of the classification as molecular sieves into numerous classes, the potentialities of which as selectively sorbing media have not yet been fully investigated. Moreover, by operating at low temperatures major differences may develop in the sorption rates of gases all of which are rapidly sorbed at higher temperatures, and in which there are only small differences in molecular dimensions.

Zeolites have sometimes proved very suitable for resolving molecular mixtures.[1,2] Complete separations can often be effected, but to use the crystals effectively one must be acquainted with their sorbent and molecular-sieve properties. In the present summary, factors determining these properties will be described, for natural and artificially modified zeolites.

Some General Properties of Gas-sorbing Zeolites.—Crystalline zeolites act as sorbents only when the water normally present in interstices within the crystals has been removed by heat and evacuation. Not all these minerals are, however, capable of taking up gases and vapours in place of the interstitial water. There are three structural types of importance from the viewpoint of the sorptive property :—

Bonding in two dimensions weaker than that in the third. (Fibrous zeolites.)	Scolecite Natrolite Edingtonite Thomsonite
Bonding in layers stronger than that between layers. (Laminar zeolites.)	Heulandite Stilbite
Bonding strong in all dimensions. (Robust three-dimensional network zeolites.)	Chabazite Gmelinite Mordenite Analcite Harmotome Levynite

Fibrous and laminar zeolites tend to shrink when interstitial water is removed, and this shrinkage may be irreversible if the heating is too severe. If the heat treatment during outgassing is more gentle, water may be resorbed, or its place may sometimes be taken by the small polar molecule NH_3. However, these minerals do not occlude non-polar gases, and have no general power of sorption. Robust network zeolites, on the other hand, may occlude both polar and non-polar gases copiously, although even here

[1] Barrer, *J. Soc. Chem. Ind.*, 1945, **64**, 130 and 133 ; *Brit. Pat.* 548,905 ; *U.S. Pat.* 2,306,610.

[2] Barrer and Belchetz, *J. Soc. Chem. Ind.*, 1945, **64**, 131.

there is a wide variety in the accessibility of the interstitial volume. Harmotome and, as a rule, analcite sorb only small polar gases; mordenite and levynite sorb many small molecules, and gmelinite and chabazite occlude a still greater variety of sorbates.[3 4 5 6]

No zeolite has hitherto been discovered which will occlude really large molecules, and although a number still remain to be investigated it is unlikely that any crystalline zeolite will prove capable of doing so. In order for this to be the case the aluminosilicate framework would have to be so open that instability would almost certainly ensue, with collapse of the whole structure into a crystal more economical of space and so of higher density. Nevertheless the chabazite lattice, based on a comparatively open aluminosilicate framework, is surprisingly stable, and at least one purely synthetic zeolite has been made by hydrothermal methods with a stable framework just as open as that of chabazite.[7 8] This zeolite has no naturally occurring counterpart, and there remains the possibility that other synthetic open framework crystals may be grown, with the limitation on the degree of openness noted above. As a group the gas-sorbing zeolites are therefore capable of sorbing only smaller molecules, and their potential value in chromatography is for removing such smaller molecules from admixture with larger ones.

Molecular-Sieve Properties of Zeolites.—Several researches have been directed towards establishing the molecular-sieve behaviour of some zeolites and also towards the artificial modification of this behaviour.[1 2 8 9 10] The molecular-sieve properties to be described refer to thoroughly outgassed finely powdered zeolites, but with an outgassing temperature inside the range of thermal stability of the crystals. Variable outgassing conditions can strongly influence the molecular-sieve behaviour.

Several sorbate molecules of fairly accurately known shape and dimensions were used to determine the accessibility of the crystalline interstices as sorption sites. *iso*Butane, propane, ethane, methane, argon, nitrogen and oxygen were used for this purpose, and three categories of molecular-sieve crystal were characterized:

Class I (Chabazite, gmelinite, synthetic zeolite $BaAlSi_2O_6.nH_2O$).	Do not sorb *iso*-C_4H_{10} (and other *iso*-paraffins). Sorb C_3H_8 (and other *n*-paraffins) slowly at room temperature or above, and sorb C_2H_6, CH_4 and molecules of smaller cross-section rapidly.
Class II (Mordenite).	Do not sorb *n*- or *iso*-paraffins. Sorb C_2H_6 and CH_4 slowly and N_2 and molecules of smaller cross-section rapidly at room temperature or above.
Class III (Ca- and Ba-mordenites).	Do not sorb *n*- or *iso*-paraffins. Negligible sorption of C_2H_6 and CH_4. Rapid sorption of N_2, O_2 and molecules of smaller cross-section at room temperature or above.

[3] Barrer, *Proc. Roy. Soc. A*, 1938, **167**, 392.
[4] Barrer, *Trans. Faraday Soc.*, 1944, **40**, 555.
[5] Barrer and Ibbitson, 1944, **40**, 195 and 206
[6] Barrer, *Ann. Reports*, 1944, 31.
[7] Barrer, *J. Chem. Soc.*, 1948, 127.
[8] Barrer and Riley, *ibid.*, 1948, 133.
[9] Barrer, *Trans. Faraday Soc.*, 1949, **45**, 358.
[10] Barrer, *Nature* (in press).

It was demonstrated that the *shape* rather than the molecular *volume* of the sorbate exercises the decisive influence upon the sorptive behaviour. The cross-section referred to in the above classification of several zeolites is that normal to the direction of the greatest length of the molecule. For all *n*-paraffins this cross-section is the same in their most extended configuration. Since chabazite, for example, slowly occludes the standard molecule propane, it should slowly occlude all *n*-paraffins. This was verified using *n*-butane, *n*-pentane and *n*-heptane. Increasing the chain length exerted a secondary influence in further slowing down the velocity of intracrystalline diffusion, but as sorption equilibrium was approached large amounts of all the *n*-paraffins studied were occluded. *n*-Heptane has a considerably larger molecular volume but a smaller cross-section than *iso*butane which is completely excluded from the intracrystalline sorption sites.

Again, the standard molecule *iso*butane, $CH(CH_3)_3$ is as noted not sorbed by chabazite; and accordingly other molecules of similar shape and size such as $CHCl_3$ and $CHBr_3$ are also not sorbed. On the other hand the molecules $ClCH_2Cl$, $ClCH_2CH_3$, $BrCH_2CH_3$ and $BrCH_2Br$ which have similar shapes and dimensions to $CH_3CH_2CH_3$ are like propane slowly occluded by chabazite. Many other examples of predictable behaviour were observed and the sorptive action towards a variety of species is summarized in Table I.

Selective Sorption from Mixtures.—The molecular-sieve property of zeolites would suggest that clear-cut separations could be obtained in molecular mixtures of which one constituent was freely occluded by the zeolite, while the other constituent, having the wrong molecular dimensions, was not occluded. Proof of this simple principle does not appear to have been published before 1945, but since then the possibility of such separations has been abundantly verified.[1 2 8] Two methods of verification were employed. In one—the static method—a quiescent gas or liquid mixture was brought into contact with the outgassed zeolite, and after an interval of time the non-sorbed gas or liquid was removed and its composition investigated.* The second procedure—the percolation or chromatographic method—consisted in passing a gaseous or liquid mixture through a column of outgassed zeolite, and investigating the composition of the effluent. This method which was employed only in one or two instances (with C_2H_6–C_3H_8 and CH_2Cl_2–$CHCl_3$ mixtures) can undoubtedly be extended, and chromatograms might be developed for suitable molecular mixtures.

The actual separations carried out have been given elsewhere [1 2 6 8] and we may therefore only make some general comments upon them. Class I molecular-sieve sorbents (chabazite, and synthetic $BaAlSi_2O_6.nH_2O$) can be used to separate *n*-paraffins of lower molecular weights from all *iso*-paraffins, aromatic hydrocarbons or naphthenes. In these same sorbents methane derivatives, in which the substituents are small groups such as $-NH_2$, $-OH$, $-CH_3$, $-CN$, $-Cl$, are rapidly occluded, and similarly substituted ethanes are slowly occluded. Quantitative separations of these species are usually possible from compounds which, like *iso*-paraffins or aromatics, are excluded from the sorption sites (e.g., column 4, Table I).

In mordenite, a Class II sorbent, methane derivatives in which the substituents are as before, $-NH_2$, $-OH$, $-CH_3$, $-CN$, $-Cl$, are slowly occluded, but the similarly substituted ethanes are not sorbed, so that separations were possible of substituted methanes from the corresponding substituted ethanes, as well as from many other species as given in Table I. In the

* The time-intervals ranged from an hour to more than a week and the temperature from 20° to 250° C according to the occlusion velocity of the sorbable component, and its thermal stability.

TABLE I

MOLECULES OCCLUDED OR EXCLUDED BY THREE CLASSES OF MOLECULAR SIEVE

—	Typical molecules rapidly occluded at room temperature or below	Typical molecules moderately rapidly or slowly occluded at room temperature or above	Typical molecules which are not appreciably occluded at room temperature or above
Section (i) Class I minerals	He, Ne, A H_2, N_2, O_2 CO, CO_2 COS, CS_2 H_2O HCl, HBr NO NH_3 CH_3OH CH_3NH_2 CH_3CN HCN Cl_2 CH_3Cl,CH_3Br CH_3F CH_2Cl_2, CH_2F_2 CH_4, C_2H_6 C_2H_2 CH_2O H_2S CH_3SH	C_3H_8 and simple higher *n*-paraffins C_2H_5OH $C_2H_5NH_2$ C_2H_5F, C_2H_5Cl, C_2H_5Br I_2, HI CH_2Br_2 CH_3I C_2H_5CN C_2H_5SH $H.COOCH_3$, $HCOOC_2H_5$ CH_3COCH_3 $CH_3CO.OCH_3$ $NH(CH_3)_2$, $NH(C_2H_5)_2$	Aromatic hydrocarbons cyclo and *iso*-paraffins. Derivatives of these hydrocarbons. Heterocyclic compounds (e.g., thiophene, pyrole, pyridine). $CHCl_3$, CCl_4, $CHCl=CCl_2$, CH_3CHCl_2, $CHCl_2CCl_3$, C_2Cl_6, and analogous bromo and iodo compounds. Secondary straight chain alcohols, thiols, nitriles and halides. Primary amines with NH_2 group attached to a secondary carbon atom. Tertiary amines. Branched chain ethers, thio-ethers and secondary amines
Section (ii) Class II minerals	He, Ne, A H_2, O_2, N_2 CO NH_3 H_2O	CH_4, C_2H_6 CH_3OH CH_3NH_2 CH_3CN CH_3Cl, CH_3F HCN Cl_2	All classes of molecules in columns 3 and 4 of section (i)
Section (iii) Class III minerals	He, Ne H_2, O_2, N_2 H_2O	A HCl NH_3	All molecules referred to in column 4, section (ii) Also CH_4, C_2H_6, CH_3OH, CN_3NH_2, CH_3SH, CH_3CN, CH_3Cl, CH_3F

case of Class III sorbents the separations effected were of small polar inorganic molecules (H_2O, NH_3, HCl) from organic compounds.

Some partial or complete separations were also effected using zeolites in which both components were sorbed but at different velocities. Using chabazite these included the pairs C_2H_6–C_3H_8, CH_3OH–C_2H_5Br, C_2H_5OH–*n*-C_7H_{16}, C_2H_5OH–CH_2Br_2, CH_3CN–CH_2Br_2, the molecule first mentioned being occluded most rapidly. A number of separations were also carried out which could not be effected by direct distillation, either because the mixtures were azeotropic pairs (H_2O–C_2H_5OH, CH_3OH–CH_3COCH_3, H_2O–dioxane, CS_2–CH_3COCH_3, C_2H_5OH–*n*-C_7H_{16}, C_2H_5OH–toluene) or because the constituents had nearly the same boiling points (*n*-heptane-*iso*octane).

Artificial Modification of Molecular-Sieve Sorbents.—Water, which is strongly sorbed by zeolites, by occupying sorption sites which would otherwise be accessible to other sorbates, can greatly diminish not only the

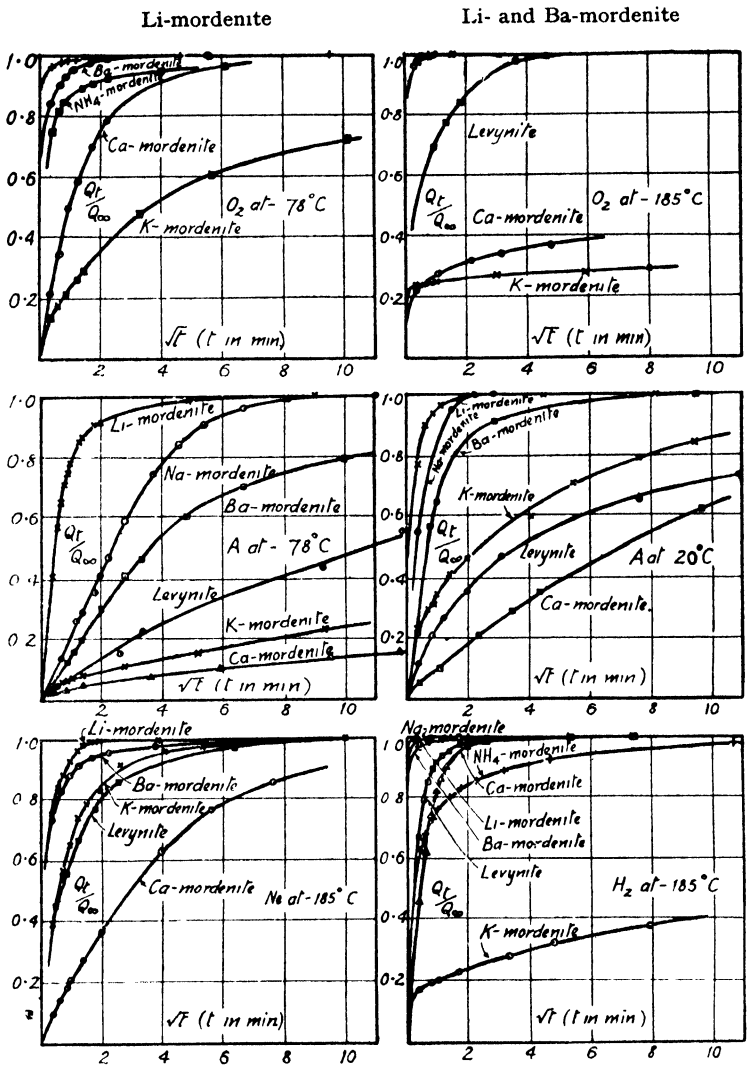

FIG 1.—Relative sorption rates of each of several sorbates in levynite and in a series of mordenites modified by base exchange. A great measure of control over the sorption rate can be exercised by a suitable choice of base-exchange mordenite

amount but also the velocity of sorption and the molecular-sieve character of the crystals. This behaviour has been studied in any detail only with chabazite,[11 12] which was progressively transformed from a Class I sorbent into one whose properties more nearly recalled a Class III sorbent. Although a small amount of water might be a useful modifying agent, larger amounts by depressing the sorptive capacity of the zeolite unduly would have a poisoning effect. Indeed, other strongly occluded polar molecules sometimes appear to act as poisons and limit separations of mixtures which in their

[11] Lamb and Woodhouse, *J. Amer. Chem. Soc.*, 1936, **58**, 2637.
[12] Emmett and DeWitt, *ibid.*, 1943, **65**, 1253.

absence could easily be resolved. In resolving mixtures of *n*- and *iso*-paraffins, for instance, the gases should be free of moisture or other small polar molecules capable of preferential sorption and so of acting as poisons.

Other methods of modifying the zeolites have now been evolved which are free from objections. The first of these is by cation interchange.[9 13] In many zeolites exchange occurs freely although higher temperatures are usually needed than is the case with amorphous or gel zeolites such as Permutit. Thus exchange is best carried out by hydrothermal methods * in the temperature range 150°–250° C using an excess of the exchanging salt. In this way, for instance, the ions in chabazite have been largely replaced by Ca, Sr, Ba, NH_4, K, Cs, Na and Li; and similarly mordenites enriched in Ca, Ba, NH_4, K, Na and Li have been prepared. In analcite exchange is more specific, the replacements Na $\rightleftharpoons$ K $\rightleftharpoons$ NH_4 occurring readily, whereas exchanges which aimed to introduce Ca, Ba, Cs and Li occurred at best only to a very limited extent.

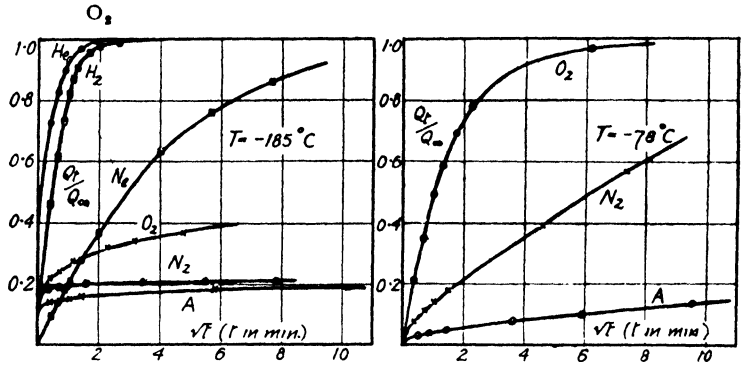

FIG 2.—Examples of the molecular-sieve action of Ba- and Ca-mordenites towards different simple gases, as expressed by selectivity in the rates of sorption at −185° C and −78° C

A study of the cation-exchanged mordenites established that, within limits set by the anionic aluminosilicate framework of the crystal, the exchange process yields a group of sorbents with a range of molecular-sieve properties. The powdered crystals gave very diverse sorption rates as Fig. 1 indicates. Although these rates are liable to be modified by a mean particle size which may have differed from one of these sorbents to another, the range in values of the constant D/a^2 (a = mean radius of particle, D = diffusion coefficient) is greater than anything to be anticipated on this count. In general, in any one sorbent the rate sequence which tended to be preserved was $Kr<A<N_2<O_2$, $Ne<H_2<He$, while the affinity between sorbent and sorbate was approximately in the converse order.

By cooling the sorbents to low temperatures (− 78°, − 186°, − 195° C) one may often greatly alter the sorption rate of one gas relative to another,[9 14] and also augment the amounts of each gas occluded at equili-

[13] Barrer, *J. Chem. Soc.*, 1948, 2158.
[14] Idem, *Nature*, 1947, **159**, 508.

* Except in the case of NH_4^+, when vapour-phase exchange is the simplest procedure, using NH_4Cl as exchanging salt. Exchange by heating the zeolite with fused low-melting salts is also often very satisfactory.

orium. Examples of the differences in sorption rates are indicated in Fig. 2, which illustrates the magnitude of the molecular-sieve effect, even where the dimensions of the diffusing molecules differ only slightly.

A second method of altering the dimensions of the interstitial channels, and hence of modifying the molecular-sieve behaviour of zeolites, is limited to ammonium ion-exchanged crystals which are capable of occluding oxygen gas.[10] It has been found possible slowly to oxidize the ammonium ions with oxygen gas at $\sim 350^\circ$ C:

$$4(-\mathrm{O}\ldots\mathrm{NH_4^+}) + 3\mathrm{O_2} = 4(-\mathrm{OH}) + 6\mathrm{H_2O} + 2\mathrm{N_2}.$$

Since the $-$ OH group occupies a smaller volume than the $-\mathrm{O}\ldots\mathrm{NH_4^+}$ group this method, as far as it has been investigated, has led to crystals with more readily accessible or more open intracrystalline channels than occurred in the parent NH_4-zeolites. A great deal of study remains to be done before the potentialities of the ion-exchange and oxidation methods are fully evaluated in the production of special purpose sorbents for effecting molecular-sieve separations of particular mixtures. It has, however, already been shown possible to produce a great diversity of graded molecular sieves, and that quite small modifications may result in important variations in relative sorption rates, variations which may often be augmented still more by an appropriate choice of the experimental temperature. The production of modified zeolites already seems to have reached a point permitting separations of species in which only minor differences in dimensions arise (N_2–O_2; A–O_2; A–N_2; Ne–H_2, etc.) although actual separations have not yet been carried out.*

Activation and Poisoning of Zeolites.—It has already been pointed out that the zeolites should be used as finely divided powders which are well outgassed, because the interstitial water may modify the amounts and velocities of occlusion. Several attempted separations using chabazite and involving acetone proved to be unsuccessful, perhaps because acetone which was sorbed only excessively slowly nevertheless blocked the entrance to the interstitial channels and prevented uptake of species which in absence of acetone would have been fully occluded. Little systematic work has yet been done on interference effects which may arise between individual pairs of molecules both of which are sorbed but at different velocities. In general one would expect such interference to be less important if the rapidly diffusing species were more polar than the slowly diffusing molecule because the polar species should show a higher affinity for the intracrystalline sites and so a greater interstitial concentration. The zeolites can also be poisoned when one constituent of the molecular mixture tends to decompose at the temperature of the experiment giving carbonaceous deposits on the surface of the sorbent particles. Such deposits can be burnt off using air or oxygen and the sorptive power of the zeolite restored at least in part.

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* Work is now in progress concerned with this aspect.

MODIFIED ACTIVATED CARBON ADSORBENTS FOR CONTINUOUS FRACTIONAL ADSORPTION

By D. E. Weiss

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The properties of H and L carbons are briefly discussed and summarized, and a distinction drawn between the short- and long-range adsorptive forces of activated carbons. Adsorption of electrolytes on L carbons is due to electrovalent and short-range forces in alkaline solutions, and to short-range forces alone in acid solutions. The addition of some non-electrolytes increases the selectivity of the adsorbent and a theory is proposed ascribing the effect to a change in the dielectric constant in the vicinity of the carbon surface. Fatty acid films on L carbon behave as non-electrolytes in acid solutions, but form salts with cations in alkaline solutions. Cations may be salted out onto an L carbon surface at a low pH by the addition of suitable counter ions to the solution. A new technique for continuous fractional adsorption is briefly described.

It is the purpose of this paper to describe the preparation, properties and use of activated carbons modified in such a manner that they are suitable for use in chromatographic processes and in a new continuous fractional adsorption column technique at present under development by the author. The proposed mechanism of adsorption on modified adsorbents may be useful in interpreting reported anomalies encountered in partition chromatography.

Theories of Adsorption on Activated Carbon

Michaelis and Rona [1] proved the adsorption of electrolytes on activated carbon to be an exchange phenomenon. In 1929 Kruyt and de Kadt [2] suggested, and Schilow and Tschmutow [3] developed the oxide theory according to which activated carbons adsorb cations and anions by salt formation with surface acidic and basic oxides; however, basic oxides of carbon of a corresponding stability are unknown in organic chemistry. Frumkin and co-worker [4] proposed an electrochemical theory in which an aqueous suspension of carbon in the presence of molecular oxygen acts as an oxygen electrode, becomes positively charged, and permits the adsorption of anions. Objections to this theory are that a completely outgassed carbon activated at 1000° C can adsorb hydrochloric acid even in the absence of molecular oxygen, and that an exponential relationship between the partial pressure of the oxygen in the system and the adsorption of anions cannot be experimentally demonstrated.

Bartell and Miller,[5] King [6] and Steenberg,[7] have shown that activated arbons may be classified as either L or H carbons. Steenberg, who confirmed

[1] Michaelis and Rona, *Biochem. Z.*, 1920, **102**, 268.
[2] Kruyt and de Kadt, *Kolloid-Beih.*, 1931, **32**, 249.
[3] Schilow and Tschmutow, *Z. physik. Chem. A*, 1930, **148**, 233.
[4] Bruns and Frumkin, *Z. physik. Chem. A*, 1929, **141**, 141.
[5] Bartell and Miller, *J. Physic. Chem.*, 1924, **28**, 992.
[6] King, *J. Chem. Soc.*, 1937, 1489.
[7] Steenberg, *Adsorption and Exchange of Ions and Activated Charcoal* (Alinquist and Wiksells, Uppsala, 1944).

their results for H carbons, defined an L carbon as one that could, and an H carbon as one that could not adsorb sodium hydroxide from an aqueous solution.

The Properties of H Carbons.—Steenberg showed that anions were adsorbed by van der Waals' forces on H carbons whereas cations were adsorbed electrovalently by L carbons and distinguished between ions primarily and secondarily adsorbed on H carbons. Primarily adsorbed ions are those held to the carbon surface by the short-range adsorptive forces and which attract a diffuse ionic atmosphere of secondarily adsorbed counter ions. The secondarily adsorbed inorganic ions, but not the primarily adsorbed ions, can be exchanged for other inorganic ions depending on their position in the lyotropic series and the concentration of the exchanging ions. Application of the Boltzmann distribution law to the adsorption of

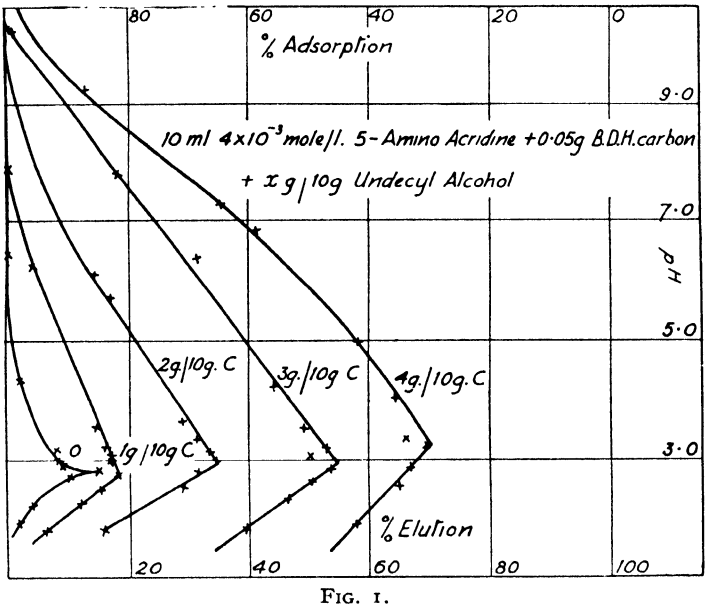


FIG. 1.

ions on H carbons yields a relationship between the primary adsorption of an ion and the concentration and valency of its counter ions. Similar ideas were independently advanced by Weiss,[8] who was unaware of the work of Steenberg, and who showed that an activated carbon containing primarily adsorbed oleic acid functioned as an ion-exchange adsorbent for 5-amino-acridine cations.

Steenberg has also shown that all inorganic anions (with the exception of the hydroxyl ion), but no inorganic cations (with the exception of the hydrogen ion) can be primarily adsorbed ; and thus the adsorption of hydrochloric acid by H carbons is due to the primary adsorption of hydrogen ions, and the secondary adsorption of chloride ions. Similarly the weak primary adsorption of chloride ions accounts for the weak adsorption of sodium chloride by an H carbon although it cannot adsorb sodium hydroxide. Primary adsorption of inorganic ions is reduced by the adsorption of organic ions, irrespective of their electrical charge.

[8] Weiss, *Nature*, 1948, **162**, 372.

The pH variations [5] associated with adsorption of salts on H carbons can be interpreted in terms of the Donnan equilibrium. When the activity of the secondarily adsorbed or counter ions is comparable with that of the hydrogen or hydroxyl ions of water, competitive secondary adsorption with the counter ions occurs, with a resultant alteration in the pH of the original aqueous suspension. If the salt concentration of the suspension is increased this competitive effect diminishes.

The Properties of L Carbons.—L carbons may be prepared at lower activation temperatures than H carbons as shown by King [6] and Steenberg.[7] Low activation temperatures produced L carbons, the alkali-adsorbing capacity of which increased with decrease in activation temperature and which was shown by Weller and Young [9] to vary inversely with its ability to adsorb acid. The pH of aqueous suspensions of L carbons so prepared

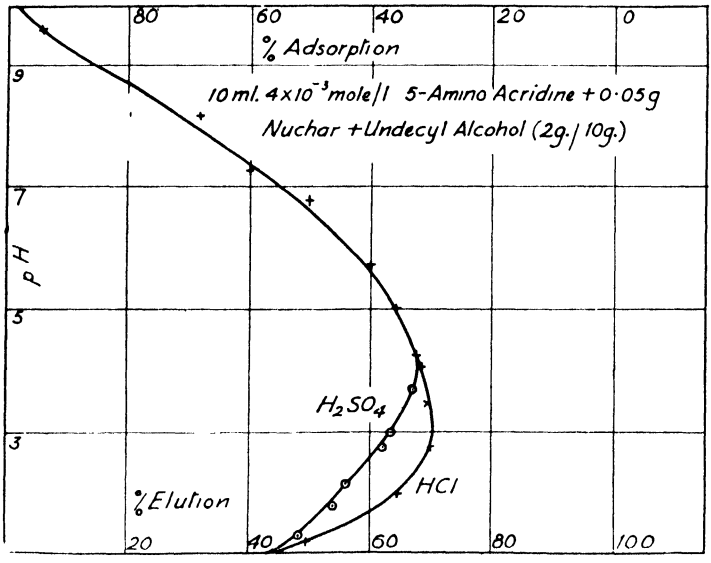


FIG. 2.

decreased with decrease in activation temperature and suggests that the degree of surface oxidation increases with decrease in activation temperature, an assumption supported by the analytical data of Weller and Young.

Hauge and Willaman [10] and Sastri [11] showed that typical activated carbons, presumably L carbons, exhibited maximum adsorption of electronegative substances from aqueous solutions at a low pH, and of electropositive substances at a high pH. The adsorption of these electrolytes was a minimum in the region of a pH zone termed the isoelectric zone but the adsorption of electrically neutral substances was unaffected by pH. Olin, Lykins and Munro [12] deduced from catophoretic experiments that L carbons are electrokinetically negative at high pH values but electropositive at low pH values.

[9] Weller and Young, *J. Amer. Chem. Soc.*, 1948, **70**, 4155.
[10] Hauge and Willaman, *Ind. Eng. Chem.*, 1927, **19**, 943.
[11] Sastri, *Quart. J. Indian Sci.*, 1942, **5**, 107.
[12] Olin, Lykins and Munro, *Ind. Eng. Chem.*, 1935, **27**, 690.

The Adsorptive Forces on Activated Carbons.—It is important to distinguish between the short- and long-range forces of adsorption operating at a carbon surface. The short range or van der Waals' forces only extend a distance of about 5 Å from the surface and are highly specific, being greatly influenced by the molecular configuration of the adsorbate and adsorbent. The long-range or electrovalent forces of adsorption, which arise from ionization of a chemically bound radical on the carbon surface or from primarily adsorbed ions, extend to distances up to 100 Å from the carbon surface and are relatively non-specific.

Modified Activated Carbon Adsorbents

The properties of activated L carbons may be utilized to prepare versatile modified adsorbents. A film of non-electrolyte or fatty acid, defined as the modifying agent, is adsorbed on the carbon. The adsorption and desorption

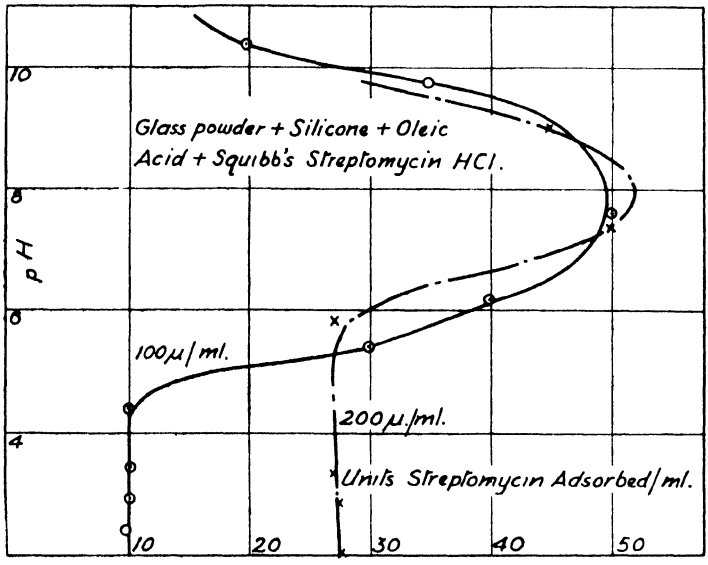


FIG. 3.

of electrolytes from aqueous solutions may then be closely controlled by pH adjustments and the possibilities of preferential adsorption are enhanced. The adsorption of 5-aminoacridine, a conjugated basic molecule, and streptomycin, a non-conjugated basic molecule, has been studied on these modified adsorbents. The addition of undecyl alcohol to an L carbon enables strong electrovalent adsorption of 5-aminoacridine to occur at pH 9, but the adsorption decreases with decrease in pH to a minimum value below which adsorption increases with further decrease in pH. Adsorption in this low pH region is probably due to the short-range forces, a view supported by comparison of the adsorption of 5-aminoacridine and streptomycin on modified adsorbents in this pH region. Adsorption of streptomycin on films of oleic acid supported on hydrophobic glass or H carbon has shown that the acid film behaves as a cation exchange adsorbent over a limited range of pH, but that at low pH values the oleic acid film behaves in an essentially similar manner to the carbon modified with undecyl alcohol. Similar behaviour occurs when the oleic acid is supported on an L carbon but is complicated by the electrovalent forces arising from dissociation, under alkaline conditions, of the surface acidic oxides of the carbon.

Addition to Non-electrolytes to L Carbons.—Fig. 1* shows the relation between pH and the adsorption of 5-aminoacridine hydrochloride on an L B.D.H. carbon and on this carbon modified with undecyl alcohol. The curves show that modification with undecyl alcohol reduces the adsorption of the organic cation, particularly at pH 2·8, the pH of minimum adsorption. At this pH the surface oxides of the carbon are only slightly ionized, and the carbon behaves as an H carbon.

The effect of the alcohol is considered to be threefold; it competes with the cations for adsorption by the short-range forces, it acts as a solvent for the cation, and it reduces the dielectric constant in the immediate vicinity of the carbon surface. Since the alcohol may form a hydrogen bond with the heterocyclic nitrogen atoms of the 5-aminoacridine to produce an alcohol-acridine complex at the carbon surface, carbons modified by the addition

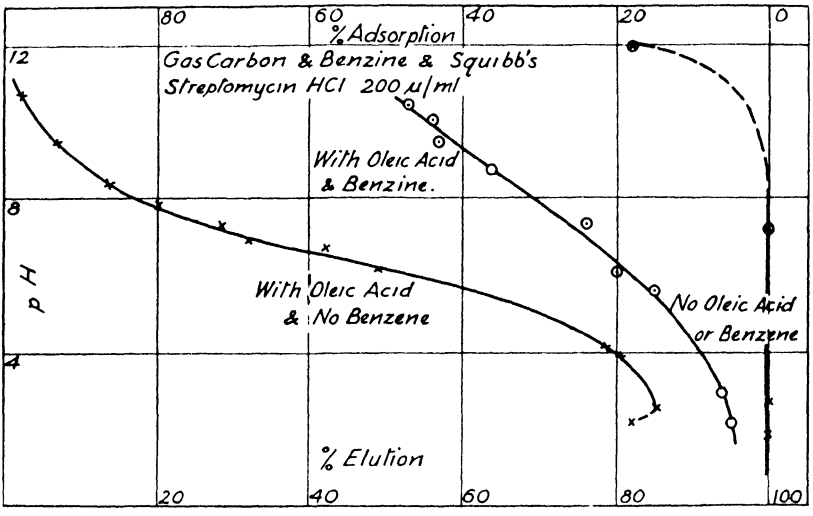


FIG. 4.

of small amounts of undecyl alcohol may be pictured as consisting of primarily adsorbed acridine ions surrounded by alcohol molecules which prevent the further approach of hydrated acridine ions from the solution. Equilibrium is reached when the tendency for the primary adsorption of the 5-aminoacridine ions is counterbalanced by the electrostatic repulsive forces of the interfacial potential produced by adsorption, and the electrically charged carbon surface may be regarded as one plate of a condenser. Addition of undecyl alcohol diminishes the dielectric constant in the vicinity of the carbon surface, so reducing the primary adsorption of ions necessary to maintain the equilibrium electrostatic repulsive force. Increasing the alcohol film thickness reduces the effect of lowering the dielectric constant in the vicinity of the carbon surface since a greater proportion of the approaching ions will be surrounded by a liquid of the same dielectric constant.

In addition to their effect on the dielectric constant in the vicinity of the carbon surface, the alcohol molecules probably competitively displace some of the adsorbed 5-aminoacridine ions from the carbon surface. If the

* The curves were obtained by equilibrating 0·05 g. samples of the modified adsorbent with 10 ml. aliquots of the solution, centrifuging, and estimating the 5-aminoacridine in the supernatant liquid at 390 mμ in a Beckman spectrophotometer.

effect of the undecyl alcohol were only to produce a film in which the cations were soluble, so that adsorption of the cations were simply a partition between two solvents, the adsorption of 5-aminoacridine should increase with increase in alcohol concentration whereas the experimental results show that the reverse actually occurs. When the adsorbed ions are completely immiscible with the modifying adsorbate film, zero adsorption of the ions will occur if the modifying adsorbate is more strongly adsorbed.

These concepts are supported by some observations of Steenberg [7] who showed that Nile Blue adsorbed on an H carbon was desorbed on shaking with a benzene-water emulsion since Nile Blue is insoluble in benzene. When atabrin was primarily adsorbed instead of Nile Blue, no desorption occurred after shaking with benzene and water, since atabrin is soluble in benzene.

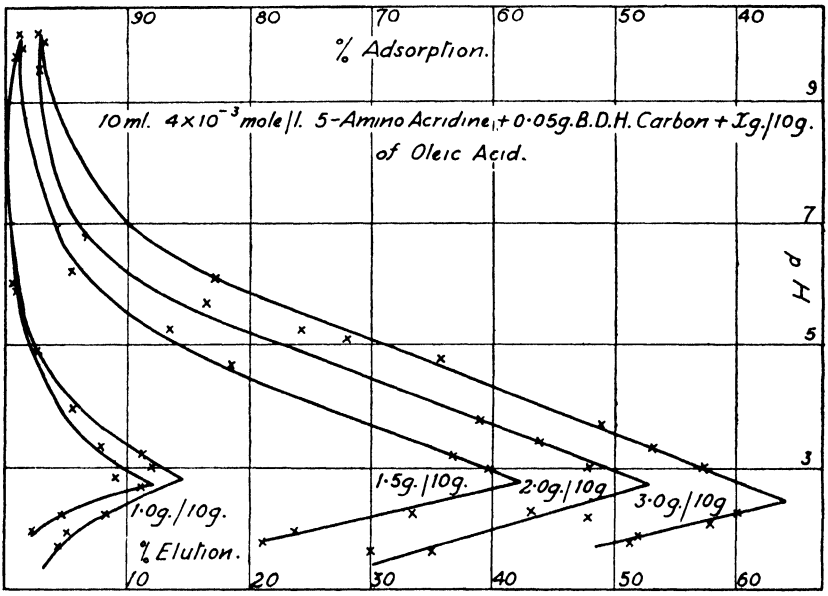


FIG. 5.

The increase in adsorption of 5-aminoacridine as the pH is raised from the minimum at 2·8 results from ionization of the acidic surface oxides, which increases the electrostatic negative charge, so attracting additional 5-aminoacridine ions to the carbon surface where some are adsorbed primarily by the short-range forces, and others are adsorbed electrovalently to form an ionic atmosphere in the vicinity of the ionized oxide groups. Hence the adsorption of 5-aminoacridine will increase with increase in pH to a maximum, where complete ionization of the surface oxides occurs. The primarily adsorbed ions will in turn attract an ionic atmosphere of chloride or hydroxyl ions.

The observations of Steenberg with H carbons show that the primary adsorption of an ion can be increased by increasing the valency or concentration of its counter ions. Increasing the chloride ion concentration by the addition of hydrochloric acid increases the primary adsorption of the 5-aminoacridine hydrochloride below pH 2·8: here the carbon behaves essentially as an H carbon. Fig. 2 compares the adsorption of 5-aminoacridine on the L carbon (Nuchar), modified with undecyl alcohol, when the pH is adjusted with hydrochloric or with sulphuric acid, and shows that the same

amount of primary adsorption of 5-aminoacridine occurs with a lower concentration of sulphate than chloride ions. Hence the pH for minimum adsorption of a cation on an L carbon depends on the degree of surface oxidation of the carbon, and on the nature of the anions present in the solution.

These findings are in agreement with the experimental results of previous investigations on the adsorption of electrolytes on activated carbons: the addition of suitable modifying adsorbates merely increases the selectivity of the adsorption. Further, these conclusions account for the observations of Olin, Lykins and Munro,[12] mentioned above, on the electrokinetic properties of L carbons, which are governed by the net electrical charge at the carbon surface. Increase in the valency or the concentration of the counter ions will decrease the positive electrokinetic potential by reducing the extent of the diffuse double layer.

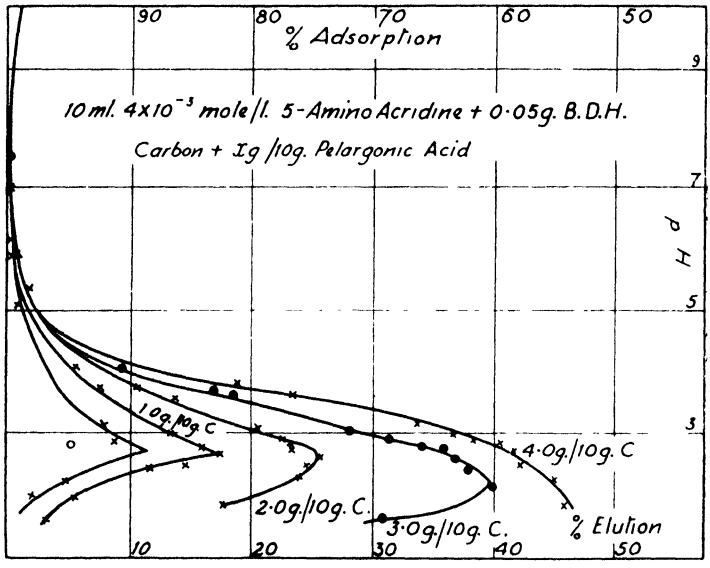


FIG. 6.

The Adsorption of Cations on Fatty Acid Films on Glass and H Carbons.—Fig. 3 shows the relation between pH and the adsorption of Squibb's streptomycin hydrochloride * on a hydrophobic glass powder † modified with oleic acid and shows that oleic acid adsorbed at a hydrophobic surface can act as a cation exchange adsorbent for streptomycin over the pH range in which oleic acid is ionized. The weak adsorption of streptomycin below pH 4 may be due to hydrogen bonding between the hydroxyl groups of the streptomycin and the undissociated carboxylic groups of the acid.

Fig. 4 shows the relation between pH and the adsorption of streptomycin on a steam-activated H carbon modified with oleic acid. Unlike the hydro-

[13] Titus and Fried, *J. Biol. Chem.*, 1948, **174**, 57.

* The streptomycin solutions were assayed by the maltol method of Titus and Fried.[13]

† In the experiments discussed in this section hydrophobic glass powder was prepared by treating glass powder with a benzene solution of Drifilm, a silicone preparation.

phobic glass powder modified with oleic acid, adsorption is almost complete in the higher pH range although streptomycin should be present as the free base in this pH region. Exposure of the H carbon to benzene vapour before modification with oleic acid resulted in a relatively high adsorption of streptomycin at pH 8–10 although it might be expected that the surface would behave in a similar manner to that of the hydrophobic glass modified with oleic acid.

The Adsorption of Cations on Fatty Acid Films on L Carbons.—Fig. 5 and 6 show the relation between pH and the adsorption of 5-aminoacridine hydrochloride on B.D.H. L carbon modified with a fatty acid. At pH 2·8 the unionized fatty acid functions similarly to undecyl alcohol and the increased adsorption of 5-aminoacridine with decrease in pH is due to the effect of the anions added during pH adjustment. The effect of increasing the pH on the adsorption of 5-aminoacridine is twofold: as the

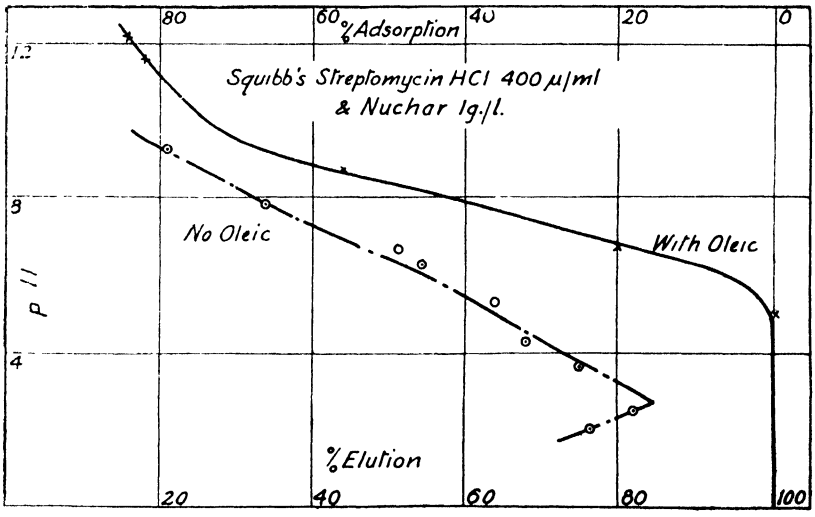


FIG. 7.

pH is increased above 2·8, 5-aminoacridine is adsorbed electrovalently both by the oleic acid and the surface oxides. Above pH 9–10 the ionization of the 5-aminoacridine is reduced, and the free base is precipitated onto the carbon surface. Fig. 7 and 8 show that the adsorption of the streptomycin cation on Nuchar and B.D.H. carbon treated with oleic acid does not increase with decrease in pH, possibly since the streptomycin cation with its carbohydrate structure has no affinity for, and cannot be primarily adsorbed in the hydrophobic oleic acid film.

Discussion

The foregoing results demonstrate that the adsorptive properties of activated carbons may be modified in a great many ways by the addition of a suitable modifying adsorbate. An H carbon can be converted to a cation or anion exchange adsorbent with an exchange capacity comparable with that of the synthetic ion exchange resins. Many substances other than oleic acid may be used as modifying adsorbates. Thus the modification of an H carbon with picric acid, methyl dioctylamine, or a quaternary ammonium dyestuff produces an exchange adsorbent capable of removing inorganic

cations, acids, or anions from aqueous solutions. Application to the adsorption of organic ions is more limited since a modifying adsorbate must be chosen which is not desorbed by the organic ions to be exchanged. Exchange of organic ions is greatly influenced by the short-range forces of adsorption, and hence by the molecular structure of the modifying adsorbate. A judicious choice of modifying adsorbates permits the preparation of ion exchange adsorbents of great versatility and selectivity, and which, unlike the synthetic ion exchange resins, do not swell upon change of pH, a feature of considerable practical interest.

The difficulty of desorbing a material after adsorption on activated carbons may be overcome by either of two general methods. The carbon containing the adsorbate can be shaken with an emulsion in water of an organic liquid in which the adsorbate is insoluble, so expelling the adsorbate into the aqueous solution. This technique, which can be used for non-electrolytes as well as for electrolytes, enables highly concentrated eluates to be produced

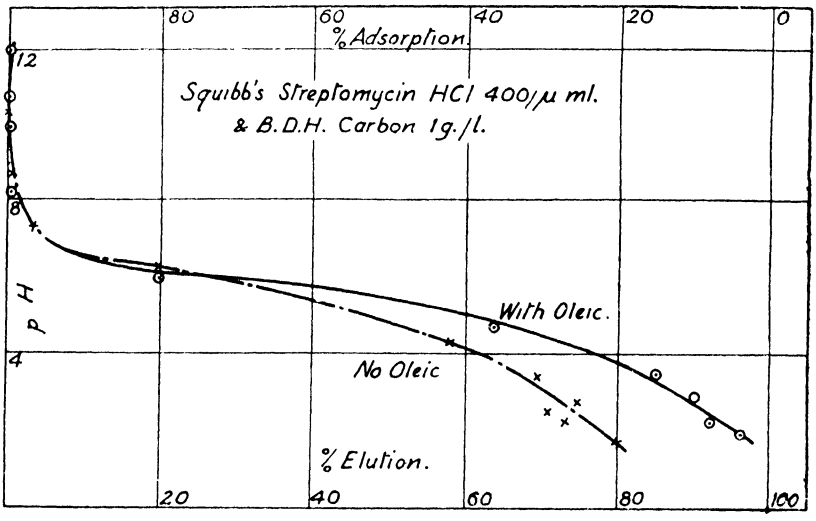


FIG. 8.

with the use of a very small volume of organic solvent, but has the disadvantage that the carbon is not simultaneously regenerated. The alternative procedure is to modify an L carbon with a suitable adsorbate film with which the materials to be adsorbed are partially miscible. Adsorption and desorption of electrolytes on such an adsorbent can then be controlled by adjusting the pH or ionic strength of the solution. Adsorption at a high pH takes advantage of both the short- and long-range adsorptive forces of an L carbon surface, and can be used to separate cations from anions and non-electrolytes. Adsorption at a low pH in the presence of a suitable modifying adsorbate utilizes the more selective short-range forces. For example, 5-aminoacridine can be adsorbed in the presence of oleic acid at pH 2, whereas streptomycin is completely desorbed at this pH, although both are strongly adsorbed at pH 9.

These adsorbents possess two important properties; desorption by pH adjustment results in simple regeneration of the adsorbent and a suitability for use in a continuous fractional adsorption technique at present being developed in this laboratory. For use in this technique, modifying adsorbates must be chosen which not only confer the desired adsorptive properties

on the carbon surface but also condition it for froth flotation. The "floating" adsorbent may be contacted continuously in a column with a descending liquid stream containing the substances to be fractionated. The feed solution is introduced midway in the fractionating column, and divides it into rectifying and stripping sections. Rectification is obtained by maintaining a pH gradient over the rectifying section, in which a partial stripping of the adsorbate occurs as the adsorbent ascends. The adsorbate retained by the adsorbent at the top of the rectifying section is desorbed by a further pH adjustment, enabling the whole operation to be conducted in an aqueous solution and the adsorbent to be re-cycled.

The author gratefully acknowledges the able assistance of Miss H. Doery * with the experimental streptomycin work and A. L. Odgers with the remaining experimental work. The work described in this paper is part of the research programme of the Chemical Engineering Section of the Division of Industrial Chemistry, C.S.I.R.O., Australia.

Division of Industrial Chemistry,
C.S.I.R.O.,
Melbourne,
Australia.

* An officer of the Commonwealth Serum Laboratories.

GENERAL DISCUSSION *

Dr. A. J. P. Martin (*Medical Research Council Unit, London*) said: The easy separation of *cis* and *trans* isomers of carotenoids on adsorption columns contrasts with the almost complete identity of behaviour of oleic and elaidic acids on partition columns. Is information available as to partition coefficients of the carotenoid isomers? Since all the isomers would seem to allow easy approach of solvent molecules from all sides, little difference in partition coefficient would be expected.

Prof. L. Zechmeister (*California*) said: Dr. Martin has asked the question whether classical chromatography or partition chromatography is more efficient in the separation of *cis-trans* isomers This question cannot be answered at the present time, since, because of the high efficiency of ordinary chromatography, the partition method has not yet been tested. However, it seems to be useful to make the following comments.

We may differentiate between (*a*) morphologically sensitive stereo-isomeric sets (for example, a single *trans*→*cis* rearrangement may thoroughly alter the molecular shape of a polyene), and (*b*) morphologically less sensitive sets (for example, the overall shape of a sugar molecule is not essentially altered by epimerization). It is suggested that the chances for successful resolutions of stereo-isomeric mixtures by classical chromatography are greater for type (*a*) than for (*b*).

Prof. A. Tiselius (*Uppsala*) said: The results reported by Prof. Zechmeister and Prof. Brockmann appear to me to be particularly valuable as an illustration of what factors are of importance for the selectivity of adsorption chromatographic methods. In this connection I would like to raise the question which of the two phenomena, adsorption or partition (and one ought also to include ionic exchange), is the most specific—a question which should be of great practical importance in the planning of work in this field.

Dr. A. Klinkenberg (*The Hague*) said: In the paper by Stewart data have been given on the band-widening caused by the passage of a fluid through

* On remaining papers of Section I.

a packed column. I should like to draw attention to the following way of approach to a description of this phenomenon, worked out for the case of a linear isotherm.[1]

The authors of theoretical papers on chromatographic adsorption and other percolation processes, such as transient heat transfer in a packed bed, ignore in their calculations the disturbing effect of non-uniformity of forward velocity and then develop a differential equation involving a constant value of this velocity. Of course the velocity in reality varies from wall to axis in any capillary and it will also differ for pores of different radii.

I separated this effect from the other cause for the levelling-off of concentration gradients, viz., the finite rate of mass transfer (non-attainment of equilibrium), by studying cases, where mass transfer was definitely ruled out. To this end solvent was displaced by solution, or vice versa, in a packing of non-porous particles of 80–100 mesh. The effect was quite considerable. The plot of the effluent concentration against volume collected resembled the S-shaped curve of the probability integral.

This can be understood with the aid of the following model. The packing is assumed to consist of layers of particles, the passage through each of which producing a certain distribution of times of passage. Complete mixing occurs between layers, so that successive distributions are uncorrelated. The distribution of times of passage for the aggregate of many layers will then approach a Gaussian probability curve, i.e., an instantaneous introduction of solute will give rise to a Gauss curve in the effluent and a concentration jump to the probability integral. The same of course applies to distributions in terms of volumes of effluent.

Another approach is obtained by introducing an apparent diffusivity

$$D_{\text{app.}} = cD_p v ,$$

where D_p = particle diameter,
v = mean linear velocity calculated on free space,
c = dimensionless constant, characterizing the packing,

as has been made by Schmidt and Damköhler [2] for radial conduction of heat and Bernard [3] for radial transport of solute.

This again leads to Gaussian distributions, provided the column is long enough. But for the interpretation of the dimensionless constant the equations are identical. The value of v is found to have no effect on the distribution, and the standard deviation of the distribution, expressed in volumes of solution, is proportional to the square root of height H over particle size D_p.

Some very fine examples of Gauss curves are indeed to be found in literature, e.g.,

(i) Weil-Malherbe,[4] benzpyrene in light petroleum containing 3 % of benzene, percolated through silica.

(ii) Moore and Stein,[5] partition chromatography of amino acids on starch.

The values of the constant c calculated from these curves were of the same order as, and certainly not much higher than, the c values obtained from displacement experiments in non-porous beds, packed with good care. It was therefore concluded that the disturbing effect, caused by the variations in forward velocity may be an important contribution to the total band-widening.

It is recalled that the stage concept (Martin and Synge [6]) and the concept of finite rate of mass transfer (Schumann-Furnas theory) for long columns also lead to Gauss distributions, so that it is impossible to decide on a mechanism on the strength of a single observed curve. The number of stages calculated according to Martin and Synge is closely related to the number of layers of particles, H/D_p; in fact, it is found to equal $H/2cD_p$.

Trends other than those given by Levi have been developed in Holland since 1943, when an independent discovery of partition chromatography was

[1] To be published in *Trans. Faraday Soc.*
[2] Damköhler, *Z. Elektrochem.*, 1936, **42**, 859.
[3] Bernard, *Thesis* (Princeton Univ., 1948).
[4] Weil-Malherbe, *J. Chem. Soc.*, 1943, 303.
[5] Moore and Stein, *Ann. N.Y. Acad. Sci.*, 1948, **49**, 161.
[6] Martin and Synge, *Biochem. J.*, 1941, **35**, 1358.

made by Van Dijck and a patent application filed. Subsequently through references, Martin and Synge's work became known.

We tried to do away with adsorption as a disturbing factor more radically than did Martin and co-workers, and used non-porous solids with adhering liquid, e.g., in a typical experiment a packing consisting of 222 ml. of sand (70–120 mesh), 27·5 ml. water and 104 ml. of an ether-pentane 50/50 mixture. With this packing hydroquinone and pyrocatechol were separated successfully. The packing was obtained from a water-filled sand by displacement by the organic liquid.

In order to have the polar liquid moving, the oleophilic solid carborundum was used, which is commercially available in carefully screened fractions Benzene in this case was the stationary fluid. With suitable buffer mixtures as the moving fluid the system was used for some separations of alkaloids.

Mr. C. D. Cook (*Widnes*) said : I would like to confirm certain observations made by Mr. Stewart on the subject of band widening as the solute travels down the column. As Mr. Stewart has pointed out, theory has it that the volume of the band should not increase during elution, but, using compounds with linear isotherms and the partition systems, (*a*) *n*-hexane-nitromethane-silica, and (*b*) chloroform-water-silica, I have found that the rate of increase of the volume of the band in the percolate was proportional to the volume required to elute the maximum concentration of the solute. Although the volume of the band was dependent on the weight of the solute in the sample, it was independent of the partition coefficient.

It was found possible to derive a simple, linear expression, based on experimental work, to calculate the volume of the band in the percolate when (*a*) the volume required to elute the maximum concentration of the solute, (*b*) the weight of the solute present, and (*c*) certain characteristics depending on the silica and solute are known.

The volume of the band was found to increase slightly when the rate of flow of the eluent was caused to decrease from 5 ml./min. to 50 ml./hr. A rapid increase in the band width was observed when the rate of flow was further diminished from 10 ml./hr. to 1 ml /hr. This rapid band widening was, I believe, partially due to vertical diffusion. The volume of the band, likewise, increased with increasing volume of the solution of the sample when loaded on to the column.

By varying the weights of silica, nitromethane and using components of differing partition coefficients, the following expression was derived :

$$V_E = V_g + N(\alpha - 1) - \frac{w}{\rho}, \qquad (1)$$

where V_E = the volume required to elute the maximum concentration of the solute,
V_g = the gel volume,
w = wt. of silica,
ρ = density of silica,
N = volume of immobile phase,
α = the partition coefficient.

This expression, which has been found to be obeyed with considerable accuracy, is identical to the Martin and Synge expression : [7]

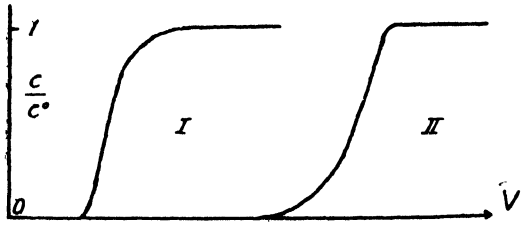
$$\alpha = \frac{A}{RA_s} - \frac{A_L}{A_S},$$

but I believe it is in a more useful form.

Since the volume of the band is dependent on V_E (eqn. (1)), it is essential that a silica of low gel volume be used to effect a maximum resolution of two components whose partition coefficients do not differ appreciably. We, therefore, consider that the gel volume of silicas of similar sieve range is one of the more important characteristics of a silica used in partition chromatography. I hope to have more to say on the characterization of silicas used in partition chromatography at a later date.

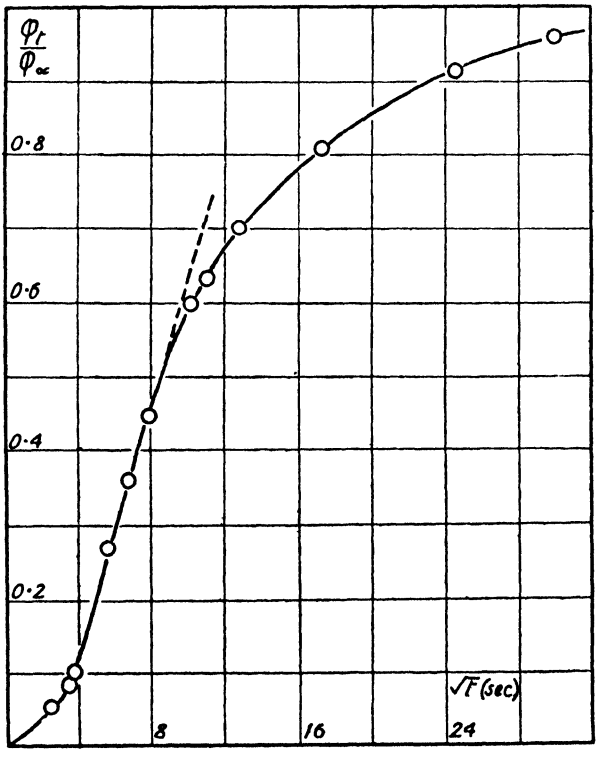
[7] Martin and Synge, *Biochem. J.*, 1941, **35**, 1358.

Dr. E. Glueckauf (*A.E.R.E., Harwell*) (*communicated*) : It usually requires a fair amount of kinetic experimental data to decide, in the case of non-equilibrium, whether grain-diffusion (I) or diffusion through a liquid film (II) is the rate-determining factor. It may therefore be of interest to know that in the case of isotherms concave against the c-axis the form of a self-sharpening front boundary obtained during frontal analysis has a different form, according to which of these processes is predominant. It follows from eqn. (3), (3c) and (4) of our paper [8] that in these two cases this boundary has the forms I and II (see Fig.) respectively, which should make recognition easy. We were able to confirm this by experiments with ion-exchangers using greatly different concentrations. Using all possible starting points $C_1°$ $C_2°$ we get thus two families of curves in a C_1–C_2 diagram. Examples in the case of two isotherms are shown [8] in Fig. 2 and 3.



Elution boundary in the case of non-equilibrium due to I grain-diffusion, II liquid-film diffusion.

Mr. B. A. J. Lister and Dr. J. F. Duncan (*A.E.R.E., Harwell*) said : The results of Mr. Kressman and Dr. Kitchener suggest that under a given set of conditions one mechanism of diffusion only is operative and that film-diffusion



Particle diffusion equation curve for the uptake of cerous ion on Zeo-Karb 215 from 0·1 N $Ce(NO_3)_3$ solution.

[8] Glueckauf, Barker and Kitt, This Discussion.

and particle-diffusion kinetics are sufficiently different to be readily distinguished by conformity to one of two equations.

An examination of the graphs in their paper shows that in almost every case illustrating particle diffusion (Fig. 7 (*a*), 8 and 9) the experimental points at lower values of Q_t/Q_∞ do not fall on straight lines passing directly through the origin but deviate from linearity in a regular manner. Curve 7 in Fig. 6 is described as film mechanism although it does not appear to differ essentially in shape from curves drawn through the experimental points illustrating particle diffusion in Fig. 7, 8 and 9. A completely different mechanism does not appear to be necessary especially since experimental points were determined only over the range $0{\cdot}2 < Q_t/Q_\infty < 0{\cdot}9$.

An examination of some results (B. A. J. L.) on the rate of uptake of cerous ion on Zeo-Karb 215 (among other exchangers) shows a similarly shaped curve (see Fig.). Here a linear portion in the particle diffusion curve was obtained but this was not directed immediately towards the origin. The slower initial uptake has also been found by Kressman and Kitchener although not emphasized in their graphs. It may be due to a variety of causes such as swelling, variation in the diffusion constant, slow saturation of the surface with cations or simultaneous occurrence of film and particle diffusion. Further experimental work is necessary to distinguish between these. It appears more difficult, therefore, than is suggested to differentiate between the two mechanisms and it seems to us that they may overlap more generally than has been supposed.

Dr. J. A. Kitchener (*London*) said : In reply to Mr. Lister's comments on our paper, we cannot agree that there is any real difficulty in practice in differentiating unambiguously between the particle- and film-diffusion mechanisms in a given case. (The only possible exception to this statement is in those rare circumstances where the transitional region between the two mechanisms happens to be met. Even then, the *I*-mechanism is clearly indicated since neither *P*- *nor* *F*-kinetics fit.) In every case so far investigated the form of the kinetics—which is used as the first test of mechanism—has proved to have given the correct indication of mechanism when interruption tests have been applied.

For example, consider the case questioned by Mr. Lister, namely, the $NH_4^+ - Na^+$ exchange at 25° C. If it is not immediately apparent that curve 7 in Fig. 6 (*b*) is of distinctly different type from those normally obtained for particle diffusion (which should be clear since curve 7 *cuts* curve 8), at least when the alternative graph is plotted for the same data (see line 4, Fig. 7 (*b*)) the excellent straight line now obtained *over the whole range of the exchange* gives conclusive indication that the data conform to *F*-kinetics.

If further proof should be required, examine the results of interruption tests on the same system. These are included as crosses in curve 2, Fig. 5. Interruption is seen to be entirely without effect. Consequently, the process is unquestionably controlled by film diffusion.

There are thus always *three* criteria, not one, namely, (*a*) the results must conform to one set of kinetics, (*b*) they must deviate from the other, and (*c*) they must behave appropriately in interruption tests. In some cases, too, as we have shown, there is also the added confirmation—though never really needed to prove the mechanism—of the influence of stirring. All these criteria must lead to mutually consistent conclusions, which can therefore be accepted with complete confidence.

With regard to the fact that exchanges of the *P*-type have always tended to give a slow beginning (as can be seen in all our $\sqrt{t}$-graphs in Fig. 7 (*c*), 8 and 9 and by the departure of the results from Paterson's theoretical equation, mentioned in the paper), there is now no doubt that the effect is due to slow swelling of the initially air-dried resin grains when they are first immersed in the solution. Measurements of the mean grain cross-sectional area have been made by a photo-electric method, and have shown a slight swelling which is practically complete in about two minutes. This value is qualitatively consistent with the amount of " lag " revealed by the $\sqrt{t}$-graphs.

It is interesting that this extraneous complication of swelling provides unexpected additional evidence in support of the two clear-cut mechanisms. This comes about as follows. *All* those results which indicate *P*-mechanism

show the above-mentioned slow beginning with gradual acceleration as the particle swells. In contrast, all those exchanges which conform to *F*-kinetics (Fig. 6 (*a*) and 7 (*b*)) show no sign whatever of acceleration : even the earliest points lie well on a straight line passing through the origin. This is exactly what is to be expected, since changes of rate of diffusion *inside* the particles should have no effect on the kinetics when the rate-controlling process is diffusion through a bounding Nernst film. The influence of swelling therefore provides a *fifth* line of evidence in determining the mechanism of exchange.

Dr. J. F. Duncan and Mr. B. A. J. Lister (*A.E.R.E., Harwell*) said : Since our paper was written further information has become available concerning the results of other workers who have determined mass products in ion exchange systems. These are summarized :

(*a*) Davidson, Argersinger, Stoenner and Lowen [9] have obtained a curve of similar shape to Fig. 6 for the sodium-hydrogen exchange system, the maximum occurring at $X_{Na_R} = 0{\cdot}23$, and the slopes on each side of the maximum being somewhat greater.

(*b*) Strickland [10] has also obtained a maximum for the sodium-hydrogen system using Dowex-50, and a coarse Zeo-Karb, but not with a finely divided Zeo-Karb.

(*c*) Reichenberg [11] has obtained a falling value of K_a for large values of X_{Na_R} (using a sulphonated polystyrene), but did not obtain a maximum.

(*d*) Marinsky and Coryell [12] observed values of K_a which rose from $X_{Na_R} = 0$ to about 0·1 (using 40–100 mesh Dowex-50) in agreement with our observations (using 100–250 Dowex-50) but no decrease in K_a was observed at higher values. The cerium-hydrogen mass-product plots obtained by these workers (using a molar fraction concept) were similar in form to that obtained in the lanthanum-ammonium system (Fig. 5), constant values of K_a being obtained over the range $10^{-3} < X_{Ce_R} < 10^{-5}$. Similar curves were also obtained for the barium- and calcium-hydrogen systems. The barium system was quite different from the curve of Fig. 4.

For the copper-hydrogen system, we have found that the value of the mass product (using both eqn. (3) and (10)) slowly approaches a constant value as x (or X) becomes zero. If the barium-hydrogen system were studied at a low enough concentration presumably the K_a value would behave similarly.

In view of these discrepancies, it is reasonable to suppose that the conditions, or materials used by various workers differ in some unknown manner. In particular, behaviour at low values of the molar fraction of the metal ion seems anomalous, and in this respect the results of Strickland are significant. That changes in the volume of the exchanger are a contributory factor we do not doubt, since swelling of the exchanger has been shown to take place in the zinc-uranyl exchange system (see below). We do not, however, consider that this is the whole explanation of the anomalies reported above.

Investigations of the effect of swelling have been made on the zinc-uranyl exchange system, for which values of

$$K_c = \frac{X_{Zn_R}}{X_{Zn_S}^{++}} \times \frac{X_{UO_{2S}}^{++}}{X_{UO_{2R}}}$$

(where the symbols are the same as described above) have also been determined by gravimetrically estimating the loss of uranium from a mixture of zinc and uranyl nitrates of known total concentration when equilibrated with an exchanger of known capacity. The mass product in solutions of 0·2 N was found to vary from 0·6 to 1·90 ($0{\cdot}10 < X_{Zn_S}^{++} < 0{\cdot}90$) and passed through a maximum about $X_{Zn_S}^{++} = 0{\cdot}25$. A curve of similar shape was also observed at a concentration of 2 N.

The size of spherical pieces of the exchanger was observed under the microscope after allowing them to stand for one hour in solutions containing different ratios

[9] *Techn. Report Office Naval Res. N.R.* 057158 (University of Kansas, February, 1949).
[10] Private communication.
[11] Private communication.
[12] Private communication.

of zinc and uranyl ions at the same total concentrations. Although a wide scatter was observed it is clear that the volume of the exchanger also passes through a maximum at roughly the same values of $X_{Zn_S}^{++}$ as the maximum obtained in the mass product plot. In solutions of 0·2 N the volume varies by about 5 % over the whole range of $X_{Zn_S}^{++}$

If one assumes the exchanger to consist of free cations bound by a solid anion in an aqueous phase, volume changes of this order would produce a concentration change which would seem to be quite adequate to account for the observed variation of the mass product, even if it is valid to assume that the thermodynamic equilibrium constant is independent of the ion concentration in the exchanger Small changes of dilution of the ions in the exchanger will cause large changes in the activity coefficients (since the solutions in the exchanger will be greater than 4 N) and it is unlikely that the ratio of the activity coefficients of the ions would be constant.

Mr. J. D. H. Strickland (*Royal Arsenal, Woolwich*) said : May I take this opportunity to mention briefly some of the results that we have recently obtained during an investigation of monovalent ion exchange (chiefly K^+, NH_4^+ and H^+ ions) on a sulphonated phenol formaldehyde and a sulphonated cross-linked polystyrene resin. Our findings largely support those of Duncan and Lister and in addition may throw some light on the apparent anomalies in the results of various workers in this field.

The expression

$$\frac{X_R}{1 - X_R} \times \frac{1 - X_S}{X_S}$$

should be equal to the mass law product K of an exchange such as :

$$K^+ + H_R \rightleftharpoons K_R + H^+, \quad . \quad . \quad . \quad . \quad (1)$$

or

$$K^+ + NH_{4R} \rightleftharpoons K_R + NH_4^+, \quad . \quad . \quad . \quad (2)$$

provided that the solutions are sufficiently dilute for activities to be equated to concentrations in the aqueous phase. Nothing definite is known at present concerning activities of the ions in the resin phase and any variations of these are best included in the value for K.

This expression for K can be very dependent upon what experimental data are used in its evaluation, especially when X_R is very small or very large or when the total ionic concentration of the solution is small in comparison with the exchange capacity of the resin. When X_R is small we find indications of the 'hump' in the plot of K against X_R that is shown in Fig. 6 in the paper by Duncan and Lister. We, like they, used an experimental technique which measured X_R directly and it is assumed that X_S, etc., are known. These experiments not only measure 'true' exchange but also any adsorption of the exchanging ion (e.g., K^+) that may have taken place. Such adsorption can have a very marked effect on the constant when X_R is small, even although the adsorption does not amount to more than about 1 % of the exchange capacity of the resin.

When we made measurements of K at $X_R = 0{\cdot}1$ to 0·2, using a technique which measured the change of H^+ or NH_4^+ in *solution*, the constant again appeared to have exceptionally high values but these could only result from either true exchange or true exchange plus *desorption* of traces of the exchanged ion (e.g., H^+ or NH_4^+) from the resin. When solutions of known composition were equilibrated with a resin and then analyzed we found (at $X_R = 0{\cdot}2$) that slightly more H^+ or NH_4^+ came off the resin than K^+ went on. In short, during ion exchange both true exchange and the adsorption and desorption of traces of cationic material are probably occurring. Adsorption phenomena of any likely magnitude will generally speaking have little effect on K but under some circumstances can cause apparently large and contradictory variations, according to the experimental conditions and data used.

For the rest of the $K - X_R$ plot, we find that with eqn. (2), K is remarkably constant as shown by Kressman and Kitchener,[18] but that with exchanges

[18] Kressman and Kitchener, *J. Chem. Soc.*, 1949, 1190.

involving hydrogen ions (e.g., eqn. (1)) this is not the case. K decreases with increasing X_R (even with the Li^+ exchange) and with the phenol formaldehyde resin this is particularly marked at X_R values exceeding 0·9.

We consider that the relative availability of exchange sites on the resin to each of the exchanging ions has bearing on these phenomena and that although with certain exchanges a mass-law product may be valid over most of the isotherm, this will not in general be the case. I fully support the plea of Dr. Duncan for more work in this field and for more agreement upon definitions. As things are, only a very guarded and critical use should be made of so-called thermodynamic equilibrium constants.

Prof. C. W. Davies (*Aberystwyth*) said : Before examining special explanations for the variations found in the K values for exchange equilibria, it is worth looking in more detail into the simplest possible picture of the process, and its consequences. If the resin is to be regarded as a homogeneous phase in Duncan and Lister's experiments, the usual Donnan equations [14] for the distribution of the diffusible ions lead to

$$\left(\frac{a_{Ba}}{a_H^2}\right)_R = \left(\frac{a_{Ba}}{a_H^2}\right)_S ;$$

on this basis the K of Duncan and Lister's eqn. (2) would be unity.

If now v is the volume in litres of resin phase containing one equivalent of exchanging groups, and X is the fraction of these associated with barium,

$$(a_{Ba})_R = f'_{Ba}X/2v,$$

where f'_{Ba} is an activity coefficient (volume concentration scale) for the ion in the resin phase. Writing the activity of the hydrogen ion in the same way we get

$$\frac{X}{2(1-X)^2}\left(\frac{a_H^2}{a_{Ba}}\right)_S = \frac{f'^2_H}{f'_{Ba}} \cdot \frac{1}{v} .$$

This equation is similar to Duncan and Lister's eqn. (6) and (10), and clearly a mass-action "constant" is going to vary with changes in v and in the activity coefficient ratio ; v will undergo large changes with alterations in the extent of swelling of the resin ; and the activity coefficient ratio is also a much more important term than has often been supposed, for no matter how dilute the solution may be the ionic strength of the resin phase will be high.

If we traverse Duncan and Lister's Fig. 2 from right to left, the substitution of hydrogen for barium, other things being equal, will almost certainly cause the activity coefficient ratio, and therefore K, to rise ; on the other hand, a substitution of two diffusible H ions for one Ba ion will increase the swelling and cause K to fall ; and in addition to these two opposing effects, the ionic strength of the resin phase will change enormously as we traverse the diagram, causing further unpredictable changes in the activity coefficient ratio. The experimental curves do not therefore appear surprising.

Incidentally, we can get an approximate idea of the magnitude to be expected for K on this simple picture. At an ionic strength of 3·0, the mean activity coefficient of barium chloride is 0·389,[15] whereas that of 0·01 N HCl in barium chloride solution is 0·823.[16] So for this comparison

$$\gamma^4_{HCl}/\gamma^3_{BaCl_2} = 7{\cdot}8.$$

If we take this as a very rough indication of conditions in the resin phase, and as the value of $1/v$ is about 4 g.–equiv./l. a K value of around 20 to 30 for this bi-univalent exchange appears quite reasonable.

Dr. J. F. Duncan (*A.E.R.E., Harwell*) said : Since the value of K_a may vary by 50 % or more with concentration and the ratio of the exchanging ions, it becomes difficult to assess the affinity of any ion for a given exchanger without comparing the values of K_a at corresponding points on the mass-product plot. Values of K_a quoted in the literature hitherto should therefore be considered as

[14] Cf. Bauman and Eichhorn, *J. Amer. Chem. Soc.*, 1947, **69**, 2830.
[15] Tippetts and Newton, *J. Amer. Chem. Soc.*, 1934, **56**, 1675.
[16] Harned and Geary, *J. Amer. Chem. Soc.*, 1937, **59**, 2032.

approximate only, unless the range and concentration in which K_a has been determined have been quoted. To avoid further confusion in this matter in the future it seems logical to compare the affinities of ions by quoting the value of K_a with respect to a constant reference ion (say, hydrogen) at infinite dilution of both ions, with one of the ions at infinite dilution in the exchanger. Thus, for copper we propose to quote the value of K_a with respect to hydrogen obtained when the concentrations of copper and hydrogen in solution are zero, and x_{Cu_R} and $X_{Cu_R} = 0$. This requires considerable experimental effort to determine and it is possible that at low concentrations adsorptive phenomena become significant, but we consider that this mass product at least has some meaning being unconfused by such variables as concentration, ion ratio and swelling.

Dr. J. A. Kitchener (*London*) said: According to the evidence presented by Dr. Duncan and Mr. Lister, no authors have yet found a method of calculating thermodynamic activity ratios for ions in the resin phase; mass-action "constants," by whatever means calculated, are apparently not *accurately* constant over the entire range of an exchange.[17] While sympathizing with the desire which has been expressed to find some standard condition and expression for presenting equilibrium results, I do not favour the choice of "infinite dilution" as the standard state. A non-thermodynamic mass-action product is not fundamentally sounder at infinite dilution than elsewhere, and the very dilute range is experimentally less accessible and practically less useful. For purposes of tabulation, the "constant" for half-exchange might be the most sensible.

It is still true that the mass-action product is *approximately* constant for a limited range. It therefore has some practical utility for that range. For the present, one cannot do better than to present results as some kind of clearly defined "constant" with careful statement of the experimental conditions to which it applies (e.g., a mean over a stated range of resin or solution composition). Where the accuracy of the data justify, the variation of this "constant" with conditions can be recorded as in the paper of Duncan and Lister. Such "constants" are obviously of limited theoretical value, but they still provide the most concise means of recording the experimental results for purposes of comparison and practical calculation.

Dr. R. W. Richardson (*Braintree, Essex*) said: The paper of Kunin and Myers has shown the part played by the microstructure of the resin in ion-exchange and I would like to emphasize the importance of this point as it seriously limits the practical usefulness of the method in some cases.

Studying the absorption of the free sulphonic acids of azoic direct cotton dyestuffs (preliminary note submitted for publication elsewhere) I have found that the largest dye acids are rapidly absorbed by Deacidite B to a low final equilibrium figure, this presumably being absorption at the surface of the resin. With a decrease in the molecular size a point is reached at which a slow diffusion into the structure of the resin begins. Progressively smaller dye acids from this point show an increasing rate of absorption to equilibrium figures which rise correspondingly.

If sulphuric acid is taken as completely saturating all the available ion-exchange centres then there exists a decrease by a factor of $\sim$ 1000 in the extent of absorption in the case of the large Sky Blue FF (Colour Index No. 518) sulphonic acid, a dyestuff which it is assumed reacts only at the surface, 100 for the substituted naphthalene-azonaphthalene and 15 for the benzene-azobenzene molecules

For the larger dyestuffs, the acidic properties of the sulphonic acid groups were shown to be little affected by the presence of the weakly basic aromatic amino groups. The extent of sulphonation did not appear to alter the relative positions of equilibrium absorption within the series studied; presumably the size of the molecule plays a more significant part in determining the absorption. It is of the utmost importance therefore that new exchange materials be developed with as porous internal structures as are compatible with practical hydraulic properties.

[17] Cf. Kressman and Kitchener, *J. Chem Soc.*, 1949, 1190.

Dr. A. Wassermann (*London*) said: The study of adsorption properties of highly swollen alginate gels, from a thermodynamic and kinetic point of view, is of interest because it indicates suitable methods of an investigation into the adsorption properties of other biologically important materials of similar degree of swelling.[18] The paper by Shepard and Tiselius describes experiments in which proteins are taken up by an inorganic adsorbent. I believe that it is also of importance to measure the adsorption of simple inorganic and organic substances on proteins, under continuous flow conditions, similar to those prevailing in chromatographic analysis. Such experiments involving fully swollen myosin gels of specified nitrogen and potassium or calcium content are carried out by Mrs. M. L. R. Harkness, who could show that this muscle protein is a typical cation-exchange material, the overall effect depending *inter alia* on the relative rate of inter- and intramolecular ionic metatheses.[19] We intend to carry out experiments with a view of finding out (*a*) whether these cation-exchange reactions at constant pH give rise to alterations in the molecular shape of the adsorbents similar to those observed with fully swollen alginate fibres,[20] and (*b*) whether these protein gels can be utilized to separate mixtures of certain substance the analysis of which cannot easily be carried out by the conventional chromatographic methods.

Mr. D. K. Hale and Mr. D. Reichenberg (*Teddington*) (*communicated*): We would like to draw attention to the important question of the extent to which anions can diffuse into a cation-exchange resin. This has been investigated by Bauman and Eichhorn,[21] who concluded that the concentration of excess electrolyte within the resin was considerably less than the concentration outside, although the results were not in complete agreement with the simple Donnan theory. We are indebted to Dr. W. C. Bauman for pointing out to us that the increase in rate of exchange observed with increasing hydroxyl ion concentration might be accounted for by the diffusion of hydroxyl ions into the resin and the neutralization of hydrogen ions within the resin phase. We do not consider our own experiments decisive on this point but the agreement between the results obtained with N NaOH and 2 N NaOH solutions (Fig. 8) suggests that, with the resin we used, a limiting rate of exchange is reached at a hydroxyl ion concentration of M or below. Whether this limit is due to the elimination of hydrogen ion film diffusion as we have suggested, or to some other cause remains an open question. The extent of diffusion of excess electrolyte into the resin phase must also be considered in the determination and interpretation of affinity constants.

Dr. A. Wassermann (*London*) said:* In collaboration with Mr. Mongar a microcalorimetric method has been developed [22] which enables an estimation to be made of heat effects accompanying the interaction between filter paper strips, fully swollen with water (the dry weight of the paper was about 6 mg.) and 0·02–0·04 cm.^{3} of the electrolyte solution. It could be established that the heat of swelling of the filter paper in water is markedly different from the heat of swelling of the same paper [23] in the electrolyte solution, there being no irreversible reaction between the cellulosic material and the salts.

[18] Cf. Wassermann, *Nature*, 1946, **158**, 271; 1948, **161**, 562. *Ann. Botany*, 1949, **13**, 79.

[19] A description of this work will be given in the near future, preliminary reports having been submitted on 21st September, 1949, to the Secretary of the Department of Scientific and Industrial Research.

[20] Mongar and Wassermann, *Nature*, 1947, **159**, 746. MacArthur, Mongar and Wassermann, *Nature*, 1949, **164**, 110.

[21] Bauman and Eichhorn, *J. Amer. Chem. Soc.*, 1947, **69**, 2830.

[22] The technique is a modification of that used by Hill (see e.g., *Proc. Roy. Soc. B*, 1938, **126**, 136) for a study of heat effects accompanying electrically stimulated contractions of frog sartorii.

[23] The carboxyl value of the paper was so small that ion exchange did not occur to any appreciable extent.

* In connection with a remark by Prof. Tiselius relating to filter-paper partition chromatography.

Dr. E. Heymann (*Melbourne, Australia*) (*communicated*) : The information obtained from investigations of equilibria between cation exchange resins and electrolytes in solution can be supplemented by investigation of the electric conductivity of swollen plugs of resin containing various cations.[24] We use a conductivity cell in which the resin is packed between perforated platinum electrodes. This cell allows of replacement of one ionic species by another in the same plug. We investigate sulphonic acid resins.

The hydrogen resin and the alkali metal resins are fairly good conductors of electricity, of the same order as dilute solutions of strong electrolytes. The conductivity of these resins is roughly proportional to the mobility of the respective cations in water. This suggests that these cations are weakly bound in the resin.

Resins containing bivalent cations show much lower conductivities than the first group, and there is no proportionability between resin conductivity and mobility of the cation in water. Resins containing cations of higher valency are poor conductors Obviously these ions are bound more strongly than the univalent ones. Our investigations suggest the following series of strength of binding in the resin Amberlite IR–100 :

$$Th^{4+} > La^{3+} \gg Ba^{++} > Ag^{+} > Ca^{++} > Mg^{++} \gg \text{alkali metal ions} > H^{+}.$$

This series is similar to that obtained by Boyd, Schubert and Adamson [25] from equilibrium studies.

It is probable that the conductivity of the swollen resin plugs is not solely determined by the strength of binding, but different degrees of swelling of resins containing various cations may also have an effect. These effects are under investigation at present.

Dr. D. J. Crisp (*I.C.I. Paints Division*) (*communicated*) : It is worth drawing attention to the phenomenon of exchange in ionized surface films, which represent in certain respects the most simple of ion-exchange systems. Hitherto when experiments have been described in which the ionic composition of the substrate below the film has been changed, emphasis has been placed upon the resulting changes in surface pressure and surface potential, rather than upon the exchange of small quantities of ions between the monolayer and the solution. As a result, processes which are strictly ion exchanges have not been presented from this standpoint, but have been termed " adsorption " (in the case of simple and polyvalent ions) or " penetration " (with organic ions) as though the addition or entry of a new ionic species were the only process taking place.

The use of charged monolayers to elucidate details of ion-exchange phenomena may prove very useful by making possible the measurement of certain characteristics of the system which are not readily observed with solid ion exchangers. For example, the phase-boundary potential indicates that as the concentration of one ionic species of opposite sign from that of the monolayer is increased, there is at first a simple exchange of ions followed by a reduction in the thickness of the double layer ; the relative concentrations at which exchange occurs with different monovalent organic ions is simply related to the van de Waals' interaction of the ion with the film.[26] The advantages of using such systems may be summarized thus :

(*a*) Virtually instantaneous equilibration between the monolayer and solution.

(*b*) Exchanges between the film and solution do not sensibly affect the concentration of the latter.

(*c*) The monolayer represents a two-dimensionally homogeneous exchange system in which all sites are equivalent to one another.

(*d*) The capacity of the film may be regulated by altering its specific area.

(*e*) Changes in surface pressure or surface area resulting from ion exchange are two-dimensional analogues of swelling phenomena in solid exchangers, and are easily measured.

(*f*) The phase-boundary potential can be determined.

[24] Heymann and O'Donnell, *J. Colloid Sci.*, 1949.
[25] Boyd, Schubert, and Adamson, *J. Amer. Chem. Soc.*, 1947, **69**, 2818.
[26] Crisp, *Surface Chemistry* (Butterworths, 1949), p. 65.

As a useful cation exchange monolayer, the α-halogenated fatty acids with 16–18 carbon atoms may be recommended. These are well ionized in neutral solution—as distinct from the unsubstituted fatty acids—and are not excessively soluble as are the sulphonic acids—and therefore produce well-ionized but quite stable monolayers.

Prof. A. Tiselius (*Uppsala*) said: It seems to me that the combination of partition and displacement methods applied by Dr. Levi offers very great promise on account of its extremely high specificity. The losses due to irreversible adsorption (for example on charcoal) have somewhat limited the use of the displacement development so far, but one may perhaps hope that such losses would be considerably less on a partition column.

A similar procedure applied in filter-paper partition chromatography might offer a means of making this method quantitative, as in displacement development, so that the length of a zone is proportional to the amount of substance which it contains.

It also seems to me that, as filter paper originally has not been manufactured for chromatographic purposes, much improvement could be hoped for by close collaboration with the manufacturers, especially as regards homogeneity, purity and possibly also elimination of the causes of losses due to tailing or irreversible adsorption.

Dr. J. Boldingh (*Zwijndrecht, Holland*) said: With regard to the rubber-coated paper strips for fatty-acid analyses additional information about the quantitative separation of the even numbered saturated fatty acids may be given. A column of powdered rubber (Mealorub) which has been swollen with benzene is used; the mobile phase consists of a mixture of methanol-acetone (3/1) with varying proportions of water. By decreasing the water content of the mobile phase at the right moment separation of the C_6–C_{18} series has been made possible with a recovery of 95 % to 100 %. More recent experiments with another solvent combination have made it possible even to separate mixtures containing the series C_6–C_{24} in one straight run. With a column of 15–20 cm. length and a diameter of 1·2 cm., a mixture containing 2–10 mg. of each acid can be analyzed. Mealorub may be obtained from the "Rubber Stichting" at Delft (Holland). Details will be published later.

Prof. R. M. Barrer (*Aberdeen*) said: Crystalline silicates exist which provide regular networks of channels with diameters no bigger than those of quite small molecules. Such crystals could act as sieves and might bring about separations of molecular species by occluding the smaller molecules while acting as non-sorbents towards other larger or wrongly shaped molecules. Among zeolites there are a number of species which are capable of producing molecular sieve separations in this manner, and I have in my paper indicated some of their properties which are relevant to the separating of molecular mixtures.

When a gaseous mixture is passed through a porous zeolitic bed one may have Poiseuille flow or Knudsen flow in macroscopic spaces between crystal grains together with intracrystalline diffusion into the individual crystallites. The rate of transpiration must be adjusted so that sorbable species may be occluded during the time of transit, and so removed from the gas stream. In the region of molecular streaming (or Knudsen flow) the diffusion coefficient in absence of sorption effects in a cylindrical macroscopic capillary is

$$D_1 = \frac{4r}{3\sqrt{\frac{\pi m}{2kT}}}, \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

while the diffusion coefficient of sorbate into the individual crystallites is

$$D_2 = D_0 \, e^{-E/RT}. \quad . \quad . \quad . \quad . \quad . \quad (2)$$

In many cases E is small and the condition for a good separation by gaseous chromatography may be fulfilled (i e, that rate of uptake inside crystallites is at least comparable in velocity with the time of transit through the powder).

Either occlusion or adsorption, by immobilizing a proportion of the molecules, can prolong the time of transit of one species compared with another non-sorbed

molecule. In the low-pressure range (molecular streaming) one has for the sorbable component

$$\frac{\partial c}{\partial t} = D_1 \frac{\partial^2 c}{\partial x^2} - \frac{\partial S}{\partial t} - \frac{\partial S_1}{\partial t} \quad . \quad . \quad . \quad . \quad (3)$$

where S and S_1 are the amounts adsorbed and occluded respectively per unit volume of the sorbent bed. When both adsorption and occlusion are rapid enough, and at low pressures,

$$\left.\begin{aligned} S &= Ck \text{ (linear adsorption isotherm)} \\ S_1 &= Ck_1 \text{ (,, occlusion ,,)} \end{aligned}\right\} \quad . \quad . \quad . \quad . \quad (4)$$

and substituting in eqn. (3) gives

$$\frac{\partial c}{\partial t} = \frac{D_1}{(1 + k + k_1)} \frac{\partial^2 c}{\partial x^2}, \quad . \quad . \quad . \quad . \quad (5)$$

and the occlusion process reduces the diffusion coefficient of the sorbable component in the ratio $1/(1 + k + k_1)$. In such a system good separations may occur should k and k_1 be large enough. In zeolites this is often the case. Moreover in the steady state of flow

$$P = \frac{D_1(c_1 - c_2)}{(1 + k + k_1)l} \qquad (l = \text{bed thickness, } c_1, c_2 = \text{ingoing and outgoing concentrations}),$$

and the permeability P is no longer proportional to $\frac{1}{\sqrt{m}}$ for a series of such gases as in Knudsen flow in absence of sorption effects.

Dr. R. L. M. Synge (*Rowett Res. Inst.*) said: Without wishing to discuss the mechanisms proposed by Weiss for the interactions which he has observed on charcoals doped in various ways, I would like to emphasize that the modification of the adsorptive properties of charcoal for chromatographic purposes by adsorbing on it substances not significantly eluted by the developing solvent deserves further study. Recent work at Uppsala [27] showed that by doping charcoal with stearic acid a product was obtained which, though of lower adsorptive capacity, had more nearly linear isotherms with amino acids and peptides in aqueous solution, and showed greater specificity in separating aromatic from aliphatic compounds.

Mr. A. Stewart (*Grangemouth*) said: The slurry method for packing columns with a fairly granular adsorbent is preferred as entrapped air escapes in the initial mixing and the columns are substantially free from air bubbles. Closest packing is obtained by tapping uniformly, preferably stopping and restarting the solvent flow at the same time. These columns show greater band movement in the middle than at the periphery. Dry packing of such adsorbents may give columns containing small air bubbles which cause the movement at the band edges to be somewhat irregular; the tamped dry-packed columns also have a greater resistance to solvent flow.

Mr. R. H. S. Robertson (*Glasgow*) (*communicated*): Although in chromatography it is usual to separate a mixture of dissolved substances by means of a standard adsorbent, the opposite procedure of using a standard mixture of solutes to examine an unknown adsorbent has been found to be a useful diagnostic test.

Half a gram or less of powder is squashed into a smooth concave surface with a spoon, and three drops of the standard mixed solution are rapidly poured into the basin so formed. The development of the colour rings is observed and recorded. Standard solutions can be made up to give an indication of pH, of chemical reactions and of physical adsorption, according to the nature of the substances in the powder. The method has proved of value in the examination of diatomites, cosmetic powders and clays.

[27] Synge and Tiselius, *Acta Chem. Scand.*, 1949, **3**, 231.

SUMMARIZING PAPER

By R. L. M. Synge

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In this first section of the Discussion we were expected to deal with the basic principles of chromatography, including the mathematics and the physical chemistry. The original invitations to contributors were distributed in the hope of obtaining reviews covering the whole field more or less systematically. However, many of the contributions received and printed are accounts of original work. This is an indication of the active and healthy development which the subject is undergoing. In our discussion of the printed communications we have ranged over most of the ground to be covered, though not in any orderly manner. This makes the task of summing up the Discussion both more difficult and more necessary. Perhaps it can conveniently be done under four heads, as follows:

(1) Mathematical treatment;
(2) Operating procedures;
(3) Techniques for analysis and visualization;
(4) Physical and chemical mechanisms of the equilibria determining separations.

(1) **Mathematical Treatment.**—Those who have engaged in mathematical analysis of chromatographic operations have not always stated clearly what simplifications and assumptions they have made and at what points in their argument physical principles are left behind and purely mathematical manipulations embarked on. These errors of presentation have made the subject a particularly difficult one to disentangle for those of us who are not mathematicians, and have helped to encourage a widespread but erroneous idea that there are a number of "rival theories" of chromatography. In fact, the general nature of the relevant processes going on at different points in a chromatographic column—diffusion, streamline flow, adsorption, desorption, transfer between phases, ionization and so forth—is well known to all of us, and in most cases these processes obey laws capable of expression in rather simple mathematical terms. However, the multiplicity of points in space and instants in time that have to be considered makes the full mathematical treatment impossibly complicated, and pending developments in mathematical technique it is necessary to make simplifying assumptions and to consider particularly simple operating conditions. Two main types of assumption have proved useful. One is the "theoretical plate" concept of conventional counter-current theory, where the operation of all such factors as diffusion, slowness in attaining equilibrium and microscopic irregularities of flow is abstracted into the simple unit H.E.T.P. (height equivalent to a theoretical plate). The behaviour of substances having linear and mutually independent distribution isotherms between stationary and moving phases is then capable of simple treatment. The other assumption is to postulate instantaneous local equilibrium, that is, to put H.E.T.P. = 0, and to dismiss the processes abstracted into that concept as "disturbing factors." The behaviour of substances with non-linear distribution isotherms can then be treated, as can the interaction

of such substances. The treatment is particularly simple for the cases of frontal analysis and of completed displacement development, as shown in Prof. Claesson's contribution. Of course it is not fortuitous that the most effective chromatographic operating procedures lend themselves to particularly simple mathematical treatments, since in them steps are taken to minimize effects of processes that do not contribute to the desired result. Even in the intermediate cases where mathematical treatment becomes difficult, it is often possible to visualize qualitatively what the effect of a change in working conditions will be, and one can also check in practice what factors are contributing to the result by varying one factor at a time, maintaining other conditions constant. In fact, such " simple-minded " approaches have probably contributed more to the understanding of chromatography than have the more intricate mathematical analyses.

(2) **Operating Procedures.**—Prof. Tiselius has distinguished three main methods by which chromatograms may be developed ; these are :

(*a*) FRONTAL ANALYSIS, where the original mixed solution is passed continuously through the column.

(*b*) ELUTION DEVELOPMENT, where the original mixed solution is placed on the column in small volume and washed through with pure solvent.

(*c*) DISPLACEMENT DEVELOPMENT, where the original mixed solution is placed on the column in small volume and washed through with a solution of some displacing agent.

There would be advantages in adopting this nomenclature, and using it consistently. (The symbols used in connection with chromatography could also with advantage be standardized. It is particularly confusing that the symbol for band-rates R adopted by LeRosen [1] and used by some subsequent authors is not defined in the same way as R of Martin and Synge [2] but is the same as R_f of Consden, Gordon and Martin.[3])

As regards these operating procedures two points seem to require emphasizing. One is the special value of frontal analysis for analyzing unknown mixtures of substances having unknown properties. The other is that for displacement development to be effective the necessary condition is not that the substances undergoing separation should exhibit curved distribution isotherms, but that they should compete with and displace one another on the column. Should this not be so, no amount of curvature of the isotherms will lead to effective separations by displacement development.

It is good that Prof. Claesson has raised the question of the analogy between chromatographic and electrophoretic processes. Consideration of the electrical migration of substances in a stirred many-compartment diaphragm cell, where the pH and conductivity of each compartment is maintained constant and all the diaphragms are thin and of the same material, shows that substances will migrate from one compartment to the next at rates determined by their relative transference through the membrane, the amount of electro-endosmosis and so forth. However, the amount leaving a given compartment will always be directly proportional to the amount in that compartment. Thus conventional counter-current theory can be applied to such a set of compartments—the relative transference and electro-endosmosis of the substances determining the enrichment factors and the linear relationship between amount present and amount migrating giving the mathematical equivalent of a linear distribution

[1] LeRosen, *J. Amer. Chem. Soc.*, 1942, **64**, 1905.
[2] Martin and Synge, *Biochem. J.*, 1941, **35**, 1358.
[3] Consden, Gordon and Martin, *Biochem. J.*, 1944, **38**, 224.

isotherm. Each compartment corresponds to one theoretical plate, and substances initially present in one compartment will distribute themselves as they migrate approximately according to the Gaussian curve. Migration in a jelly can then be viewed as the juxtaposition of a number of small compartments separated by diaphragms. The enrichment factor between two migrating substances is then simply the ratio of their net mobilities, after allowing for electro-endosmosis, and the H.E.T.P. is determined by the diffusion constants for the substances in the jelly. In electrophoresis in free solution, H.E.T.P. is likewise an expression of the diffusion constant.

This way of looking at electrophoretic phenomena clears up a point that had puzzled me for some time—the justification of the use of the H.E.T.P. concept by Brewer, Madorsky and colleagues [4-7] for the electrical migration of ions in a tube packed with sand against a stream of water. Westhaver [7] gives a detailed analysis of the role of diffusion and lack of uniformity of flow of solvent in the pores in determining the H.E.T.P. Much of this treatment can be used directly for considering factors determining H.E.T.P. in ordinary chromatograms. Dr. Klinkenberg has already emphasized in discussion the importance of flow mechanisms in determining column efficiency.

It is very valuable that electrical migration is compatible technically with chromatography. Some obvious applications are :

(*a*) The interpretation of adsorption effects in jelly ionophoresis.

(*b*) The use of electro-endosmosis for moving liquids through a jelly and observing molecular sieve effects, as mentioned by Prof. Tiselius in his Introductory Paper.

(*c*) The combination, in suitable instances, of chromatographic and electrical procedures, as done by Strain,[8] to economize the number of operations in a separation.

There are certain operating procedures that do not at first sight fall into any of the categories so far discussed. One of these is the stationary type of chromatogram, such as those on alumina described by Prof. Flood or with penicillins on alkali-impregnated silica, described by Catch, Cook and Heilbron.[9] These are really frontal analyses, but the mutual competition of the solutes for stoichiometric combination in the stationary phase is so intense that what is virtually a displacement chromatogram results, and the different band lengths may be used for quantitative analysis just as in displacement development.

Another, and very important, operating procedure is development with successive changes of solvent. Very valuable results have been obtained with this procedure from the earliest days of chromatography and numerous examples of its use are given in this Discussion. It may perhaps be regarded as a form of displacement development where the solvent or eluting agent displaces some components, but not others, of the mixture being separated. This leads to the introduction of a spatial gap between the components in question, which facilitates their separation from each other. They can usually be separated from the solvent or eluting agent quite easily, taking advantage of differences in physical or chemical properties such as volatility. In simple displacement development there is no spatial gap between successive components, and this leads to technical difficulties in cutting.

[4] Brewer, Madorsky and Westhaver, *Science*, 1946, **104**, 156.
[5] Brewer *et al.*, *J. Res. Nat. Bur. Stand.*, 1947, **38**, 137.
[6] Madorsky and Straus, *J. Res. Nat. Bur. Stand.*, 1947, **38**, 185.
[7] Westhaver, *J. Res. Nat. Bur. Stand.*, 1947, **38**, 169.
[8] Strain, *J. Amer. Chem. Soc.*, 1939, **61**, 1292.
[9] Catch, Cook and Heilbron, *Nature*, 1942, **150**, 633.

Chromatograms in which there is a continuous change in operating conditions along the length of the column (such as would be caused by evaporation from flowing mixtures of solvents) do not seem to have received much consideration, though I have more than once heard Prof. Tiselius discuss their possibilities. There seems no reason for expecting such systems to be useful when completeness of separation is the main consideration. However, one can imagine a dilute mixed solution entering such a chromatogram continuously, the result being an accumulation of different solutes at different points. Such systems must be of common occurrence in nature, responsible for some of the concentrations of substances observed in rocks and soils, and possibly capable of being employed in technology.

(3) **Techniques for Analysis and Visualization.**—This subject is, in a sense, a subsidiary aspect of chromatography. One can note a general tendency away from the practice of extruding and cutting up chromatograms, and in favour of observation and analysis of the effluent. When dealing with unknown mixtures, there is a great deal to be said for taking numerous arbitrary fractions, and analyzing these retrospectively, since it may not be apparent at the start what properties should be taken into account in making the cuts, whereas if the fractions are small enough they can later be pooled in the groups found most suitable. Chromatographers owe a debt to Dr. Stein and Dr. Moore [10] for introducing an automatic fraction-cutting technique, which enormously reduces the labour involved.

Numerous authors in this Discussion have used radioactive substances as markers on chromatograms, and this procedure certainly has very general significance for observational purposes. Prof. Brockmann's new fluorescence procedures also deserve further study.

(4) **Physical and Chemical Mechanisms of the Equilibria Determining Separations.**—Several contributors have described kinetic studies in this Discussion, and it has been suggested that differences in kinetics of approach to equilibrium could be utilized for separations in addition to differences in equilibrium. This is true, but it should be emphasized that such separations would not be chromatography in the usual sense but would require a quite different type of apparatus, perhaps similar to that used by Signer and colleagues.[11]

The exploration of different mechanisms determining equilibrium distributions in chromatographic systems, and of the specificity of these mechanisms for different substances, is at present in a phase of rapid development and is daily yielding valuable results. Prof. Tiselius in introducing the Discussion dealt with this topic at some length and there is little to add. The contributions of Prof. Barrer and of Dr. Myers and Dr. Kunin suggest that, before long, molecular-sieve properties will be utilized for chromatographic separations of compounds extending over a fair range of molecular sizes. There seem also to be possibilities, so far little explored, for increasing the specificity of adsorption processes. In this connection, the proposal of Dr. Wassermann, mentioned in discussion, to study the adsorptive properties of proteins has special interest. In this group of substances we know, from the data of serology and enzymology, that specificity of adsorption is capable of being very great. There seems also every reason for hoping that chromatography will prove a useful technique for effecting separations of high molecular substances.

In concluding, I would like to stress how much we can hope to learn by further detailed studies of the ultramicroscopic structure and properties

[10] Stein and Moore, *J. Biol. Chem.*, 1948, **176**, 337.
[11] Signer, Hänni, Koestler, Rottenberg and v. Tavel, *Helv. chim. Acta*, 1946, **29**, 1984.

of the substances used for making chromatographic columns. Until we have much more accurate ideas on this subject, the chromatographic art will remain largely empirical, and our interpretations speculative. We shall learn much by trying to interpret in physico-chemical terms the practical achievements of chromatography that will be presented in the second half of this Discussion. However, other techniques than chromatography will also be required for developing this much-needed body of knowledge.

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II. APPLICATIONS

Introductory Paper

By F. A. Robinson

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My first impulse on being invited to introduce the section dealing with the applications of chromatography was to relate some of the more important advances that have resulted from the use of this new tool. On reflection, however, I decided that it would be more profitable to spend the few minutes at my disposal in attempting to assess the suitability for different purposes of the various forms of chromatography now available. I have been encouraged to follow this line of thought because at each of our sessions several speakers have asked whether there is any method of predicting the most favourable conditions to use in any given circumstances. I therefore wondered if it would be possible to obtain any guidance from an analysis of the data presented at this meeting; for I think we can take the contributions to this Discussion as fairly representative of trends in recent research in this field.

We have already heard much about the three main branches of chromatography—adsorption, partition and ion-exchange chromatography—and the three main methods of development—displacement development, elution development and frontal analysis—the relative advantages and disadvantages of which have been so admirably summarized by Dr. Synge. Now suppose someone without previous experience of chromatography were asked to separate two or three substances chromatographically, how would he proceed? How would he find his way amongst the "enormous number of alternatives possible," as I believe Prof. Tiselius expressed it? In the early days when I prepared my first chromatogram the procedure was simple; one used alumina or lime or magnesia, and most probably it would have been an unsuitable grade at that, simply because no other was available. Nowadays, however, the position is very different; there is a choice of three fundamentally different techniques and within each of these techniques a fairly wide choice of column materials, eluting agents and methods of manipulation. Which of the possible permutations and combinations should one select?

In the first place, of course, the answer will depend on how much or how little the experimenter knows about the properties of his substances. He

may know nothing, in which event he must try various techniques in turn. But he may know that his substances are neutral, acidic or basic; he may know something of their relative strengths or partition coefficients or even something about their adsorption isotherms. This is unusual, and more often than not he will only have a very general idea of their chemical behaviour. Even this, as I hope to show, should help in making a choice of techniques.

Now the papers prepared for this Discussion refer to the use of adsorption chromatography for the separation of the following substances—metals, hydrocarbons, fats and fatty acids, steroids, high polymers, proteins and carotenoids. The significant point about this list to my mind is that with the exception of cations, which are separated by ion exchange anyway and not by true adsorption, and fatty acids, these substances consist solely of organic compounds free from functional groups, suggesting that these are the substances for which adsorption chromatography is nowadays considered most appropriate.

The list of substances for the separation of which partition chromatography has been used is rather different. True, it includes metal ions and fatty acids, but for the rest it comprises sugars and sugar derivatives, amino acids and peptides, penicillins and vitamin B_{12}, all of which are organic compounds with well-defined polar groups.

The list of substances to which ion-exchange chromatography has been applied has much in common with the last list. It includes anions and cations, penicillins, purine bases, nucleic acids and amino acids. This means, of course, that ion-exchange chromatography is most appropriate for the separation of inorganic and organic ions.

I would therefore suggest that the chances are that adsorption chromatography is likely to give the best results with non-polar molecules, partition chromatography with organic compounds containing polar groups and ion-exchange chromatography with inorganic ions and organic acids and bases.

Like most sweeping generalizations, mine has many exceptions—I am sure that each of you will be able to think of several—and I will immediately qualify my statement by suggesting that adsorption chromatography offers many advantages compared with the newer techniques and may retain this advantage for some time to come.

In the first place it is a much older technique and therefore more is known about it. Useful empirical rules have been prepared that simplify the task of selecting the most suitable adsorbents and eluents; these are to be found in the text-books of chromatography. In the second place, adsorption chromatography is the method that lends itself most readily to the application of frontal analysis, which gives the maximum of information about the qualitative nature of a mixture, and displacement development, which gives the maximum amount of quantitative information. However, we must not lose sight of the fact which has emerged during the Discussion that displacement development can be applied both to partition chromatography and to ion-exchange chromatography, so that this particular advantage may not long remain peculiar to adsorption chromatography.

One further reason for the retention of adsorption chromatography for the separation of organic compounds containing functional groups is that colourless substances of this type can often be converted into coloured or fluorescent derivatives that are readily located on the column. Another factor that may for the time being favour the retention of adsorption chromatography in fields where one might expect it to be superseded by partition and ion-exchange chromatography is that at present it is possible to obtain standardized aluminas and, in some degree, standardized carbons,

but it is not yet possible to obtain standardized carriers for holding the static phase in partition chromatography; satisfactory grades of silica gel are notoriously difficult to prepare and both starch and filter papers are variable substances, although not everyone may subscribe to this view. Nor can the present range of ion-exchange materials be regarded as entirely satisfactory, although there are indications that new and, one hopes, better products may soon become available.

I think that in the past workers have been negligent in failing to publish fuller details of the properties of the chromatographic agents they have used, and it is no use bemoaning the fact that manufacturers will not supply suitable reagents unless potential users will go to a little trouble in defining in unambiguous terms the properties they regard as desirable. Ideally, a specification should be prepared for each chromatographic agent, whether required for adsorption, partition or ion exchange, and it might then be possible for manufacturers to supply standardized materials that will give reproducible results not only in the hands of the same workers, but also in different laboratories.

I must confess that I find myself unable to fit Mr. G. Robinson's method into my classification and I am not sure whether to regard the 8-hydroxyquinoline column as a special example of adsorption chromatography in which separation depends on differences in the adsorption affinities of oxine salts for oxine, or as an example of partition chromatography in which separation depends on the relative solubilities of oxine salts in water, or as the first example of an entirely new type of technique to be called perhaps 'reagent chromatography,' in which separations are effected by differences in the rates of reaction with a selective reagent. It would be interesting to hear of other types; I know only of this and of violuric acid and 5-oxo-4-oximino-3-phenyl-isoxazoline, but the principle should clearly be capable of further extension.

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II(A). INORGANIC

NATURE AND APPLICATIONS OF INORGANIC ALUMINA CHROMATOGRAPHY *

By George-Maria Schwab

Received 21st June, 1949

The optical spectra of cations adsorbed by sodium exchange on alumina confirm the view, derived from chemical evidence, that the adsorption zones contain basic double aluminates. Inorganic chromatographic analysis is essentially improved by combining it with the usual group separation reactions. The microchemical sensitivity of chromatographic cation detection is about equal to that of spot tests. The chromatographic separation of the platinum metals is described.

* Contribution from the Department of Inorganic, Physical and Catalytic Chemistry of the Institute Nicolaos Canellopoulos, Piræus, Greece.

The chromatographic adsorption method of Tswett was first applied to the analytical separation of inorganic cations by Schwab and Jockers [1] and of inorganic anions by Schwab and Dattler.[2] These authors, using alumina containing exchangeable sodium for the cation separations and acidified alumina containing exchangeable anions for the anion separations, established the basic principles of ion-exchange chromatography which in the following years acquired so great an importance for amino acid separations [3] and, after the introduction of organic polymer ion exchangers, for the separation of rare earths and pile products.[4] In the present communication, some new progress in inorganic alumina chromatography of practical interest will be summarized. It has been published in periodicals rather difficult to obtain at present.

The Nature of the Ion Adsorption.—In the first publications [1 2] it was stated that the adsorption of ions on alumina columns takes place by the displacement of ions of equal sign contained in the alumina surface, i.e., sodium by cations, and nitrate or chloride by anions. The specificity of the adsorption, which is manifested in a reproducible adsorption series, together with the fact that adsorbed cations are accompanied by a certain amount of anions, lead to the conclusion that the adsorption complex, especially in cation chromatography, is a basic double salt, e.g., a basic aluminate-sulphate. Doubts about this result have been expressed by Siewert and Jungnickel [5] who assumed that the adsorption and the adsorption series could be explained by the precipitation of carbonates on the column by the sodium carbonate present. However, this opinion, based on an incorrect interpretation of the solubility product and of titration results, has been revised in a joint publication [6] in favour of the original theory.

This latter has been directly substantiated by Schwab and Issidoridis [7] by the measurement of the light absorption of the adsorbed ions by reflected light. It was shown that with cupric ions a new absorption band at 602 mμ appears on adsorption lower than that of the hydroxide and characteristic of Cu-O bonds. With chromic ions a shift of the 575 mμ band to longer wavelength occurs, dependent on the anion present, this being less with chromite but greater with the hydroxide. $CrAlO_3$ shows the opposite shift. In nickel adsorption the 680 mμ band is shifted to shorter waves, the displacement being less than with the hydroxide. The uranyl ion gives a shift of the 660 mμ band towards the violet end, this being less than with sodium uranate. All these facts show that the state of the adsorbed layers is intermediate between the free hydrated ion and hydroxide, and this is compatible with the assumption of basic salts for copper, nickel and uranium, and of a chromite-like structure for the strongly adsorbed chromium.

Chromatography in Qualitative Analysis.—It had been found that a full qualitative cation analysis by alumina chromatography is rendered difficult by the coincidence of certain zones and the chemical interference of others (Ag-Mn, As-Fe, Cu-Al). Schwab and Ghosh [8] found that these shortcomings could be overcome by combining the usual analytical group separations with a chromatographic identification within each group.

[1] Schwab and Jockers, *Angew. Chem.*, 1937, **50**, 646.
[2] Schwab and Dattler, *Angew. Chem.*, 1937, **50**, 691.
[3] Kuhn and Wieland, *Ber.*, 1940, **73**, 968.
[4] A series of papers in *J. Amer. Chem. Soc.*, 1947, **69**.
[5] Siewert and Jungnickel, *Ber.*, 1943, **76**, 210.
[6] Schwab, Siewert and Jungnickel, *Z. anorg. Chem.*, 1944, **252**, 321.
[7] Schwab and Issidoridis, *Z. physik. Chem. B*, 1942, **53**, 1.
[8] Schwab and Ghosh, *Angew. Chem.*, 1939, **52**, 389.

Thus, the hydrochloric acid group $Pb^{\cdot\cdot}$, $Ag^{\cdot}$, $Tl^{\cdot}$ is accessible to such identification. From nitrate solutions, lead is adsorbed at the top, silver in the middle and thallium at the bottom of the column. With potassium chromate as developer, the lead zone turns yellow, the silver zone red and the thallium zone yellow. With ammonium sulphide the lead zone is black, the silver zone grey and a black thallium zone is washed down the column. Mercurous ion must not be present, as mercury is generally unfit for chromatography.

The hydrogen sulphide group is also well split into zones, provided that $Hg^{\cdot\cdot}$ and $Sn^{\cdot\cdot}$ are absent and that the solution, the column and the washing water contain some tartaric acid. With a hydrogen sulphide solution as developer one obtains at the top an orange antimony zone, and below a yellow arsenic zone, a black lead zone, a brownish-black copper zone and a yellow cadmium zone.

TABLE I

No.	Cation	Reagent or Developer	Colour of Zone	Sensitivity in γ	
				Spot-test	Chromatographic
1	$Fe^{\cdot\cdot\cdot}$	$Fe(CN)_6^{\prime\prime\prime\prime}$	Blue	0·04	0·01
2	$Cu^{\cdot\cdot}$	$Fe(CN)_6^{\prime\prime\prime\prime}$	Brown-red	—	0·02
	$Cu^{\cdot\cdot}$	H_2S	Brown-black	—	0·4
	$Cu^{\cdot\cdot}$	Thio-oxamide + NH_3	Black	0·006	0·005
3	$Co^{\cdot\cdot}$	α-Nitroso-β-naphthol	Brown	0·05	0·06
	$Co^{\cdot\cdot}$	Thio-oxamide + NH_3	Brown-black	0·03	0·04
	$Co^{\cdot\cdot}$	$(NH_4)_2S$	Brown-black	—	0·2
4	$Ni^{\cdot\cdot}$	$(NH_4)_2S$	Green-black	—	0·3
	$Ni^{\cdot\cdot}$	Thio-oxamide + ammonia	Violet	0·012	0·04
5	$Tl^{\cdot}$	$(NH_4)_2S$	Black	—	1·0
	$Tl^{\cdot}$	KI	Yellow	0·6	0·12
6	$UO_2^{\cdot\cdot}$	$Fe(CN)_6^{\prime\prime\prime\prime}$	Faint yellow	0·92	0·45
7	$Ag^{\cdot}$	$(NH_4)_2S$	Black	—	1·0
	$Ag^{\cdot}$	*p*-Dimethylamino-benzylidene rhodamine	Violet	0·02	0·02
	$Ag^{\cdot}$	$Mn^{\cdot\cdot} + OH'$	Black	2·0	0·11
8	$Pb^{\cdot\cdot}$	$(NH_4)_2S$	Dark black	—	0·54
	$Pb^{\cdot\cdot}$	$CrO_4^{\prime\prime}$	Yellow	—	0·54
9	$Cd^{\cdot\cdot}$	$(NH_4)_2S$	Yellow	—	0·54

The most important possibility is the separation of the ammonium sulphide group in one operation. With ammonia as developer one obtains a brown iron zone, a green chromium zone, a yellow uranyl zone, a white zinc zone, a rose-colour cobalt zone, a blue-green nickel zone and a nearly colourless manganese zone. This latter, on drawing air through the column, turns dark brown. Only $Al^{\cdot\cdot\cdot}$ cannot be detected because its colourless zone is situated between uranyl and zinc and cannot be differentiated from the colourless zinc zone.

Microchemical Chromatography.—The cumulative property of the adsorption column which gathers ions present in high dilution in large liquid volumes in a narrow zone makes the method very suitable for the microchemical detection of elements. In the first experiments [1] iron and copper could be detected in amounts down to 1 γ. Schwab and Ghosh [9] by using alumina micro columns of 1–2 mm. diam. and some specific microchemical reagents, were able to raise the sensitivity of the method to that

[9] Schwab and Ghosh, *Angew. Chem.*, 1940, **53**, 39.

of Feigl's [10] well-known spot-test method. The advantage of the chromatographic method is that these limits of sensitivity can be attained even at the highest dilutions. Table I (opposite) summarizes the results. Generally, the sensitivity runs parallel with that of the spot test, because it depends on the same factors, viz., colour and solubility of the precipitate. Elements having a low place in the adsorption series, such as $Ag^{\cdot}$ or $Tl^{\cdot}$, show a relatively lower sensitivity because at high dilution the adsorption of these elements may be, in part, reversible. In mixtures, forming double rings, the sensitivity is less: in an equivalent mixture, Fe and Cu can be detected by means of $Fe(CN)_6^{\prime\prime\prime\prime}$ down to 0·1 γ, and in a mixture Fe/10Cu to 0·5 γ Fe and 6 γ Cu.

Separation of the Platinum Group.—The same authors [11] in a recent paper applied the alumina chromatography to the separation of the metals of the platinum group. Here, considerable difficulties are encountered, not specific to the method but to the chemistry of these elements. Formation of complex cations and anions, hydrolysis and hydrate isomerism and the ageing of the solutions due to these reactions affect the reproducibility of the results. Osmium has not been included because it is easily removed by the tetroxide distillation. No general developer is available nor is, in fact, needed, as the colours of the different zones are sufficiently characteristic: Ir blue-green, Ru green, Pt faint yellow, Pd brown, Rh orange. The adsorption series is as above from top to bottom, provided that an aged $IrCl_3$ solution and a fresh $RuCl_3$ solution are used. The rhodium solution must not be freshly prepared nor older than a few weeks, otherwise the series is seriously disturbed.

The above colours of the single zones are not changed by the presence of more than one platinum metal and thus are reliable means of detection. This has been proved by isolating the free metals from the different zones of the chromatograms and identifying them by measuring their lattice constants in precision X-ray work.

The platinum metals are easily separated from lead, copper, zinc and nickel, but not from iron, because of their higher adsorbability. With iron present, the platinum metals form very complicated chromatograms, due probably to the formation of mixed complexes.

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[10] Feigl, *Qualitative Analyse mit Hilfe von Tupfel-Reaktionen* (Leipzig, 1938).
[11] Schwab and Ghosh, *Z. anorg. Chem.*, 1949 (O. Hahn vol.), **258**, 323.

THE MECHANISM OF INORGANIC CHROMATOGRAPHIC ADSORPTION ON ALUMINA

By Luigi Sacconi

Received 8th August, 1949

Adsorption of inorganic salts on activated alumina is considered as a hydrolytic adsorption associated with $Al^{\cdot\cdot\cdot}$ ion–$H^{\cdot}$ ion and $Na^{\cdot}$ ion–$H^{\cdot}$ ion exchange processes. Alumina, in this way, exhibits a surface buffering action. The adsorption affinity is proportional to the polarizing power exerted by the ions as well as to the polarizability of the groups co-ordinated around the ions. The co-ordinated water molecules of aquo-ions play a peculiar role in connection with surface hydrolysis and with the high tendency of alumina to hydrate itself. The heat of hydration of alumina is a measure of its adsorptive capacity.

Several hypotheses concerned with inorganic chromatography using alumina ascribe the adsorption of electrolytes to the following: (*a*) $Na^{\cdot}$ ion–cation exchange associated with the presence of sodium aluminate as an impurity [1]; (*b*) precipitation of basic salts caused by the alkaline reaction of technical alumina [1]; (*c*) precipitation of basic carbonates due to alkali carbonates and bicarbonates present in the alumina [2]; (*d*) $Al^{\cdot\cdot\cdot}$ ion–cation exchange.[1] (This latter hypothesis has now been rejected by the author himself. Thus, the fact that the position of Al in the chromatographic series has been located between Pb and Cu means that the adsorption of ions following the $Al^{\cdot\cdot\cdot}$ ion cannot be explained.) (*e*) Amphoteric adsorption by alumina which engages in both $H^{\cdot}$ ion–cation and OH'–anion exchange; (*f*) preferential adsorption of ions which then bind the ion of opposite charge by equivalent secondary adsorption; (*g*) molecular adsorption.[3]

It has been shown [4] that Na-free alumina, such as that obtained from amalgamated aluminium, activated by heating for 10 hr. at 650° C, adsorbs cations, generally in the form of the basic salts, though pure alumina is not basic. Thus, solutions of $Hg(NO_3)_2$ and $TlCl_3$, poured down a column of pure alumina, produce coloured bands (yellow-ochre and brown) of the respective basic salts. It must be emphasized that the filtrate obtained by prewashing the column of technical alumina with water precipitates yellow basic salts from $Hg(NO_3)_2$ solutions, but that the wash water coming from pure Al_2O_3 has no effect. We can conclude therefore that the adsorption mechanisms (*a*), (*b*) and (*c*) are not essential.

Surface Buffering Action of Alumina.—Interesting information about the nature of the adsorption process has already been obtained [5] *.

(1) Pure activated alumina, added to solutions of acids and salts, lowers the hydrogen ion concentration. For instance, the addition of Al_2O_3 to a 0·1 N HCl solution until a thick suspension is obtained raises the pH above 5·6. Similar additions to 0·2 M $CuSO_4$ and 0·2 M $NiCl_2$ solutions (pH = 3·45 and 4·22 respectively) raises the pH above 5·8.

(2) Using as a developer aluminon (NH_4 aurin tricarboxylate), which does not produce a characteristic reaction either with technical or pure alumina, evidence is obtained that in both static and chromatographic adsorption of acid solutions (e.g., 0·1 N HCl), $Al^{\cdot\cdot\cdot}$ ions are displaced. These are subsequently adsorbed on the column and produce, with aluminon, a scarlet band at the top of the column. During static adsorption the displaced $Al^{\cdot\cdot\cdot}$ ion can be detected in the solution; on the column adsorption is complete. Even solutions of salts generally employed in inorganic chromatography can displace $Al^{\cdot\cdot\cdot}$ ions which are then adsorbed at the top of the column. Thus, pouring $CuSO_4$ or $Fe_2(SO_4)_3$ solutions through a column of alumina and developing with aluminon, the white ring situated above the bands of Cu (blue) or Fe (brown) becomes scarlet red. The adsorption band of $Al^{\cdot\cdot\cdot}$ and other ions, however, are partially mixed.

The results of measurements of the width of Al bands obtained on passing H_2SO_4 and $Al_2(SO_4)_3$ solution through columns of pure alumina (diam. 3·5 mm.) are reported in Table I.

The lower part (one-third of the length) of the red zone obtained using H_2SO_4 is pale scarlet. The colour thus seems to depend on the different degree of basicity of the basic aluminium salts adsorbed on the column.

(3) In the static adsorption of solutions of acids (e.g., HCl) or hydrolyzable salts

[1] Schwab and Jockers, *Angew. Chem.*, 1937, **50**, 546. Schwab and Dattler, *ibid.* 1937, **50**, 691; *ibid.*, 1938, **51**, 709.
[2] Siewert and Jungnickel, *Ber. B*, 1943, **76**, 210.
[3] Jacobs and Tompkins, *Trans. Faraday Soc.*, 1945, **41**, 388, 395, 400.
[4] Sacconi, *Gazz. chim. ital.*, 1948, **78**, 583.
[5] Sacconi, *Gazz. chim. ital.*, 1949, **79**, 141; *Nature*, 1949, **164**, 70.

* Technical alumina employed in these experiments was activated by heating for 10 hr. at 600° C; pure alumina obtained from amalgamated aluminium was activated by heating for 10 hr. at 650° C.

on pure Al_2O_3, the concentration of anions is lowered. On an adsorption column, in which the amount of Al_2O_3 is in excess of that of the adsorbate, anion adsorption is complete, i.e , anions cannot be detected in the filtrate coming from the column.

From such experiments the following conclusions are deduced : (*a*) $Al^{\cdot\cdot\cdot}$ ions are displaced from alumina by $H^{\cdot}$ ions present in solutions of acid or hydrolyzable salts ; (*b*) $Al^{\cdot\cdot\cdot}$ ions are subsequently adsorbed, not between the Pb and Cu bands, but together with the most strongly adsorbed ions. This accounts for the absence of $Al^{\cdot\cdot\cdot}$ ions in solutions coming from the adsorption column Alumina therefore by lowering the $H^{\cdot}$ ion concentration effects a surface buffering action

TABLE I

Solution	Vol (ml.)	Vol of wash water (ml)	Vol of developer (Aluminon soln) (ml)	Width of red band (mm)
0·1 N H_2SO_4	0·5	2	2	14·5
	0·25	1	1·5	11
0·1 N $Al_2(SO_4)_3$	0·5	2	2	11·5
	0·25	1	1·5	7·5

Hydrolytic Adsorption on Activated Alumina.— The adsorption of inorganic salts on an alumina column can thus be regarded as a hydrolytic adsorption which takes place according to the general relation

$$M^{\cdot\cdot} + H_2O \rightleftharpoons M(OH)^{\cdot} + H^{\cdot}, \quad . \quad . \quad . \quad . \quad (1)$$

which is displaced towards the right-hand side because of the lowering of hydrogen ion concentration due to $Al^{\cdot\cdot\cdot}$ ion–$H^{\cdot}$ ion exchange. The aluminium salt liberated is then adsorbed as a basic salt .

$$\left.\begin{array}{l} 6\ M^{\cdot\cdot} + 6\ H_2O \rightarrow 6\ M(OH)^{\cdot} + 6\ H^{\cdot} \\ Al_2O_3 + 6\ H^{\cdot} \rightarrow 2\ Al^{\cdot\cdot\cdot} + 3\ H_2O \\ 2\ Al^{\cdot\cdot\cdot} + 2\ H_2O \rightarrow 2\ Al(OH)^{\cdot\cdot} + 2\ H^{\cdot}\ \text{etc} \end{array}\right\} \quad . \quad . \quad . \quad (2)$$

Since alumina is in excess, the process continues until adsorption is complete. In support of an $Al^{\cdot\cdot\cdot}$ ion–$H^{\cdot}$ ion exchange, attention is drawn to Wagner's conclusions [6] according to which the solution of Al_2O_3 in acids is a surface reaction depending not only on the hydrogen ion concentration but also on the concentration of other electrolytes

In technical alumina $Na^{\cdot}$ ion–$H^{\cdot}$ ion exchange also takes place , this accounts for the excess of acid employed in direct neutralization of alumina [7] as well as for the excess adsorption of cations.[1]

The evidence of the different buffering actions of both kinds of alumina can be obtained in the chromatographic adsorption of a $CuSO_4 + K_2Cr_2O_7$ solution (orange) [5] A brown band, corresponding to basic copper chromate, is formed on a pure alumina column ; a light green band, corresponding to more basic copper chromate, is formed on technical alumina.*

$CuPtCl_6$ solution (yellowish green) forms on a pure alumina column two partially mixed zones, the upper yellow, the lower blue-green, corresponding to the basic chloroplatinates of Al and Cu respectively. No effect is observed on washing with water , elution with NaOH solution causes the yellow zone to flow down the column (Na_2PtCl_6 is not adsorbed) and the green band to turn blue because of the formation of $Cu(OH)_2$. On a column of technical alumina, an upper yellow ring and a blue-green band are likewise formed ; on washing with water, the excess of Na_2PtCl_6 (yellow), produced by the $PtCl_6''$ and $Na^{\cdot}$ ions present in technical alumina, flows down the column.

[6] Wagner, *Z. Elektrochem.*, 1938, **44**, 511.
[7] Schwab, Siewert and Jungnickel, *Z. anorg. Chem.*, 1944, **252**, 321

* Basic copper chromates, e g., 7 CuO . CrO_3 . 5 H_2O (greenish yellow), 7 CuO 2 CrO_3 5 H_2O (yellow), 3 CuO CrO_3 . 2 H_2O (brown), have been reported. Cf. Gmelin-Kraut *Handbuch der anorganischen Chemie*, Band. V, Abt. 1, 1909, pp. 1188–91.

Further evidence of hydrolytic adsorption is the following: the easily hydrolyzable Na_2PdCl_4, Na_3RuCl_6 and Na_3RhCl_6 salts are strongly adsorbed on both pure and technical alumina; Na_2PtCl_6, Na_2IrCl_6 and Na_2OsCl_6, which are stable towards alkali, are not adsorbed. This behaviour provides a method for the complete separation of Pd, Ru, Rh from Pt, Ir, Os.

The decrease of cations adsorption with increasing acidity observed by Jacobs and Tompkins [3] can be explained by the above hypothesis, since an increase in the concentration of hydrogen ion causes the reaction (1) to shift to the left.

The hypothesis (*e*) that the hydroxyl groups are responsible for adsorption is at variance with the adsorptive capacity exhibited by activated alumina which has been heated at 600°; this treatment probably causes the bonding of surface hydroxyl groups with elimination of water.[7b] Experimental evidence,[1] furthermore, shows the inability of any cation and anion to replace H^+ ion and OH' ion respectively. On the contrary, cation–H^+ ion and anion–OH' ion exchanges are always observed

The hypothesis of hydrolytic adsorption appears to be in harmony with Schawb's spectrographic observations,[8] according to which the wavelengths of the bands and the adsorption coefficients of the adsorbed salts agree with those of the corresponding basic salts This hypothesis is consistent moreover with the experimental evidence of incomplete separation of ions, probably due to co-precipitation phenomena as well as to the fact that the pH values at which the precipitation of several basic salts begins are close to one another

It must also be remembered that the diffusion coefficient of hydrolyzable salts decreases with the increasing pH of solutions The diffusion coefficient of $Al(NO_3)_3$, for instance, becomes zero [9] at pH = 4·5 Thus the decrease of the diffusion coefficient of a salt solution which is passing through the interstices in the alumina column causes the corresponding basic salts to be adsorbed

Relation Between Adsorbability, Ionic Potential, Diffusion and Dialysis Coefficient of Ions.—Despite the fact that cations are, in general, not completely separable, and that mixed zones are produced on the column, from my experiments as well as from those already reported by Schwab, the order of cation adsorption on an alumina column is as follows:

$$\text{Th, Zr, } Fe^{III}, \text{ Al, Cr, } Ti^{IV}, Hg^{II}, UO_2^{II}, \text{Pb, Cu, Ag, Zn, } \begin{matrix} Fe^{II}, Ni \\ Co, Cd \end{matrix}, Tl^{I}, \text{Mn.}$$
$$U^{IV}, Ce^{IV}, Ce^{III}$$

If we consider the degree of hydrolysis h[10] of chlorides, nitrates and sulphates of many elements above, we observe that, as a general rule, h values decrease from Th to Mn (cf. Table II).

TABLE II

Salt	h (%)	pH
Th sulphate	46–5	1·8
Th nitrate	5·7	
Zr chloride	35	
Fe chloride	37	
Fe sulphate	11·7–22·1	
Cr sulphate (green and violet)	28·7–2·2	1·94–3·2
Cr chloride (green and blue)	16·4–1·6	2·2–3·2
Al chloride	2·8–4·06	2·76–2·9
Al sulphate	1·16–2·01	2·84–2·9
Al nitrate		3·17–3·38
Hg chloride	0·26	
Pb nitrate	0·069–0·084	4·07–4·28
Cu sulphate	0·072	4·14
Zn sulphate	0·0046–0·0042	5·34–5·28
Cd sulphate	0·0016	5·8

[7b] Montoro and De Angelis, *Ricerca sci.*, 1942, **23**, 186.
[8] Schwab and Issidoridis, *Z physik Chem.*, 1942, **53**, 1.
[9] Jander and Winkel, *Z. anorg. Chem.*, 1930, **193**, 1; *ibid.*, 1931, **200**, 257
[10] Landolt-Börnstein, *Physikalisch-Chemische Tabellen*, Hw. II 1165, Eg. I 661, Eg. II*b* 1104, Eg. III*c* 2130–33.

Moreover, if we consider the values of ionic potential $v = z/r$, dialysis coefficient [11] λ and diffusion coefficient [12] D, we observe that, in general, the increase of adsorbability runs parallel with increase of v, $1/\lambda$ and $1/D$.[13] In addition, more highly hydrated cations [14] are the most highly adsorbed.[13] This is shown in Table III.

TABLE III

Ion	$v = z/r$	$1/\lambda$	$1/D$	n H_2O
Ti^{IV}	6·25			
Al	5·27	3·29	2·78	57
Cr	4·69	3·20	3·22	49
Zr	4·60			
Fe^{III}	4·49	3·37	2·70	59
Ce^{IV}	3·92			
U^{IV}	3·81			
Th	3·64	3·62	3·45	61
Tl^{III}	2·86			
Ce^{III}	2·54	2·86	2·78	33
Hg	1·78			
Pb	1·52			
Ag	0·85			
Cu		2·14	2·32	24
Zn	2·41		2·50	
Ni	2·56	2·15	2·38	24
Co	2·44	2·17		24
Fe^{II}	2·41	2·18		24
Cd	1·94			
Tl	0·67		0·83	
Mn	2·20	2·20		24

This behaviour is consistent with the recent views on hydrolysis and its correlation with ionic hydration.[15] Thus experiments have shown that the cobaltamines have no water of hydration [16] except that which is held within the co-ordination shell; in this case the co-ordination water is stoichiometrically determined.

On technical alumina cobaltamine cations are adsorbed in the following order; the ionic potential, calculated on the Stokes radii,[17] is also reported. It must be noted that the separation of cobaltamines, when it takes place, is complete. Developers have not been employed except for the $[Co(H_2O)_6]^{++}$ ion.

(1) $[Co(NH_3)_3(H_2O)_3]^{+++}$; $[Co(NH_3)_4(H_2O)_2]^{+++}$, $v = 1{\cdot}09$; $[Co(NH_3)_3(H_2O)_2Cl]^{++}$
(2) $[Co(H_2O)_6]^{++}$, $v = 0{\cdot}58$
(3) $[Co(NH_3)_6]^{+++}$, $v = 1{\cdot}02$
(4) $[Co(NH_3)_5Cl]^{++}$, $v = 0{\cdot}77$; $[Co(NH_3)_4CO_3]^{+}$, $v = 0{\cdot}37$
(5) $[Co(NH_3)_4Cl_2]^{+}$; $[Co(NH_3)_5NO_3]^{++}$, $v = 0{\cdot}72$

It is evident that cations with co-ordination water are the most highly adsorbed even when they possess a lower ionic potential. It is therefore concluded that the water of hydration plays an important part in adsorption on alumina.

Relation Between Hydration and Adsorption.—It must be emphasized that in inorganic chromatography, where the solvent (water) is highly polar, there are three interacting substances: alumina, ions and water. It is well known that the co-ordination water of aquo-ions (because of the action of the

[11] Brintzinger, Ratanarat and Osswald, *Z. anorg. Chem.*, 1935, **223**, 101.
[12] Spandau, *Z. physik. Chem.*, 1943, **192**, 211.
[13] Sacconi, *Gazz. chim. ital.*, 1949, **79**, 152.
[14] Brintzinger and Ratanarat, *Z. anorg. Chem.*, 1934, **222**, 113.
[15] Emeléus and Anderson, *Modern Aspects of Inorganic Chemistry* (Routledge, London, 1947), p. 148.
[16] Sutra, *J. chim. phys.*, 1946, **43**, 205. Brintzinger, *Z. anorg. Chem.*, 1935, **225**, 221. Brintzinger, *Z. Elektrochem.*, 1945, **51**, 10.
[17] Sutra, *J. chim. phys.*, 1946, **43**, 193, 194.

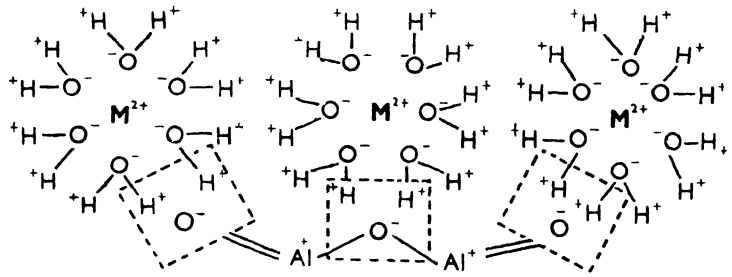
electrostatic field of cation) is polarized and the molecules are oriented with the H atoms directed outwards. Analogous polarization but with the opposite orientation is assumed by water when attracted by alumina which is itself highly polar. In this case the H atoms of water are directed toward the negative ends (oxygen) of the molecules of the adsorbent. Consequently, the polarized water molecules of the aquo-ions will exhibit a tendency to be adsorbed more firmly on alumina than the bulk water in which the molecules are neither polarized nor oriented Therefore alumina will exhibit a greater adsorption potential towards the water molecules of aquo-ions and in this way adsorb the ions attached to their own water of co-ordination

Evidently, the greater the potential of ion, the greater is the polarizing action on molecules of water of co-ordination and the greater is the adsorptive capacity of alumina towards the ion itself. If molecules or ions such as H_2O, NH_3, CO_3'', Cl', etc , are contained in the co-ordination shell, the adsorption affinity will depend upon the size, dipol moment and polarizability of the co-ordinate groups In this manner a reason is given for the fact that the aquo-cobaltamine cations are more strongly adsorbed than cobaltamine cations (O–H distance = 0·955 Å ; N–H distance = 1·08 Å ; $\mu_{H_2O} = 1{\cdot}8$; $\mu_{NH_3} = 1{\cdot}48$).

The Mechanism of Surface Hydrolysis.—It is clear that compounds containing co-ordinated water are essentially acids in the sense defined by Brönsted,[18] i.e., the polarizing action of the cation creates a tendency to split off hydrogen ions, according to the scheme :

$$[Al(H_2O)_6]''' \rightarrow \left[Al \begin{matrix} (H_2O)_3 \\ (OH)_3 \end{matrix} \right] + 3H^{\cdot} .$$

In this way the phenomenon of hydrolysis is explained.[19] In the case of aquo-ions adsorbed on alumina, it is evident that, because of the additional polarization induced by alumina in the co-ordinated water molecule, the production of hydrogen ions and the subsequent salt hydrolysis takes place more easily. The hydrogen ions thus liberated will displace Al''' ions from alumina according to a scheme of the type :



$$+ 9\,H_2O$$

$$\rightarrow 3\,[M(H_2O)_4(OH)_2] + 2\,[Al(H_2O)_6]''',$$

$$2\,[Al(H_2O)_6]''' + 2\,Al_2O_3' + 18\,H_2O \rightarrow 6\,[Al(H_2O)_4(OH)_2]^{\cdot} \quad . \quad (3)$$

The same must be true for aquo-cobaltamine complexes. These, unlike the cobaltamine complexes, exhibit a well-defined tendency to produce $H^{\cdot}$ ions [18 20] by the reaction :

$$[Co(NH_3)_5(H_2O)]''' \rightarrow [Co(NH_3)_5(OH)]'' + H^{\cdot}.$$

This provides an additional reason why aquo-cobaltamine cations are more strongly adsorbed than cobaltamine cations.

[18] Cf. Emeléus and Anderson, *loc. cit.*, pp. 147, 230.
[19] Rice, *Electronic Structure and Chemical Binding* (McGraw Hill, New York, 1940), pp. 422, 423.
[20] Pauli and Valkó, *Elektrochemie der Kolloide* (Springer, Wien, 1929), pp. 46, 51.

In this connection attention is directed to Kolthoff's conclusions [21] concerning the adsorption of polar molecules on polar surfaces, according to which the dissociation of the adsorbate takes place when the adsorption energy is greater than the dissociation energy. In the adsorption of aquo-ions on alumina we conclude that, on account of the polarization produced by the electrostatic field of the cations on the co-ordinated water molecules, the adsorption energy necessary to dissociate the latter is lowered and, therefore, the dissociation is facilitated.

Finally it must be noted that, since the adsorption band of any ion is always a mixed zone of both cation and $Al^{\cdots}$ ion, measurement of the actual width of the adsorption zone of a single ion is impossible.

The Heat of Hydration of Activated Alumina.—Confirmation of the view that inorganic chromatographic adsorption is mainly based on the preferential adsorption by alumina of the polarized water molecules of aquo-ions is provided by the values of the heats of hydration of both technical and pure alumina.[13]

Technical alumina, which is the more active form, has a higher heat of hydration, i.e., a greater tendency to hydrate itself.

The experimental values of the heats of hydration Q are :

Technical alumina : $Q = 8{\cdot}44$ cal./g.
Pure alumina : $Q = 3{\cdot}85$ cal./g.

Since the particle size of pure alumina was about 1/10th that of the technical material, the specific heat of hydration of the latter, referred to unit surface, is 20 times that of pure alumina.

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[21] Kolthoff, *J. Physic. Chem.*, 1936, **40**, 1027.

INORGANIC CHROMATOGRAPHY ON CELLULOSE

Part III.* The Use of Columns of Cellulose for the Separation and Determination of Metals

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A process is described for the separation of inorganic materials on columns of cellulose adsorbent with the aid of organic solvents. A method of preparation of a suitable cellulose pulp is given, together with a general description of the practical details of the process. The scope and applicability of the method are illustrated by a variety of separations which have been achieved and some of the factors influencing and producing the separations are considered.

The separation of a number of groups of inorganic radicals on strips of filter paper have been described [1-7] and reference has been made to the use

[1] Arden, Burstall, Davies, Lewis and Linstead, *Nature*, 1948, **162**, 691.
[2] Burstall, Davies, Linstead and Wells, *ibid.*, 1949, **163**, 64.
[3] Arden, Burstall and Linstead, *Chem. Soc. Symposium* (Chemistry of the Heavy Elements), March, 1949.
[4] Lederer, *Nature*, 1948, **162**, 776.
[5] Lederer, *ibid.*, 1949, **163**, 598.
[6] Pollard, McOmie and Elbeih, *ibid.*, 1949, **163**, 292.
[7] Lacourt, Sommereyns, Degeyndt, Baruh and Gillard, *ibid.*, 1949, **163**, 999.

* A New Method for the Detection and Determination of Uranium by T. V. Arden, F. H. Burstall and R. P. Linstead (in press) should be taken as Part I of this series of papers, Part II, The Separation and Detection of Metals and Acid Radicals on Strips of Absorbent Paper by F. H. Burstall, G. R. Davies, R. P. Linstead and R. A. Wells (in press).

of columns of cellulose for separating larger amounts of material.[3 6] This paper is intended to give a general account of the technique of separating inorganic salts, using columns of cellulose in conjunction with organic solvents, together with an outline of some separations already achieved. Detailed accounts of the individual separations mentioned will be published elsewhere.

The maximum amount of material involved in separations on paper strips is of the order of 1 mg.; larger quantities exceed the capacity of the cellulose and clear-cut separations are not achieved. It is possible, however, to deal with very much larger amounts of material by using columns of cellulose pulp and separations employing up to 250 g. of material have been successfully carried out.

Experimental

General Technique.—A column of cellulose pulp is prepared in a glass tube containing the organic solvent to be used for the separation. An aqueous (usually dilute acid) solution of the mixture to be separated is prepared and transferred to the top of the column. The solution is either poured directly on to the top of the prepared column or is first absorbed on a wad of cellulose, which is then transferred to the top of the tube, broken up with a glass rod and then packed down to form a continuous portion of the column. More regular and concentrated bands are obtained by the latter method. Solvent is then passed down the column and the eluent collected. The material being separated is dissolved, moves down the column and finally emerges in the eluent. Recovery of the extracted material from the solvent eluent is made in one of several ways: (*a*) washing with dilute acid, (*b*) addition of water and evaporation of the solvent and (*c*) precipitation in the organic solvent solution followed by filtration.

Preparation of Cellulose.—Various types of cellulose materials including wood pulp, cotton linters and filter paper have been investigated for suitability in pulp preparation. In all cases degradation of the cellulose to a suitable form of pulp is achieved by boiling with an aqueous solution containing 5 ml HNO_3 ($\rho = 1{\cdot}42$)/100 ml. The time of boiling required varies from 2 to 20 min. according to the nature of the raw material. The type of pulp used is suited to the separation to be carried out, for a number of routine estimations pulp prepared from Whatmans No. 1 Waste Paper Clippings is satisfactory, but when a higher degree of purity is required Whatmans Ashless Tablets are used. On boiling with water these tablets break down to a pulp suitable for many separations, but a more finely divided and retentive pulp is obtained by boiling with 5 % v/v nitric acid for 2 min.; excess acid is then removed by washing with water, alcohol and finally ether. The boiling with dilute nitric acid breaks down the cellulose fibres and increases the specific surface of the cellulose. The number of active end-groups in the cellulose is also increased and this improves the adsorptive powers of the pulp. The reducing property of a cellulose pulp is an important contributing factor in the separation of inorganic materials since polyvalent metals tend to give, in their lower valency forms, salts which are less soluble in organic solvents.

Preparation of the Glass Extraction Tube.—It is necessary to treat the inside surface of the glass extraction tube to prevent 'wall' effects due to creep of aqueous solution from the wad of cellulose containing the sample, down the wall of the absorption column. This wall effect has been overcome by the use of dichlorodimethyl silane ($(CH_3)_2SiCl_2$), which confers very strong water-repellent properties to the glass surface. A small amount of the organic silicon derivative is shaken in the tube until the whole surface has been treated, the excess removed and the tube washed with alcohol. In the absence of fluoride the silicone-treated tube retains its water-repellent properties for a large number of separations.

Results

In the separation of inorganic materials by the column method a number of unusual chromatographic conditions are experienced. For this reason although

separations carried out by the paper strip technique provide a useful guide as to the best solvent to use in a column separation, the conditions of water and acid concentration are not usually the same for both types of separation. The column process appears normally much more sensitive to small changes in acidity and water content of the solvent than does the strip technique. The following examples will give some account of the separations to which the technique has been successfully applied and on which work is still proceeding.

A mixture of methyl propyl ketone, water, HCl and acetone has been used in the separation and estimation of Ni, Co and Cu in samples of steel and minerals. A solution of the sample (0·2 g.) is prepared in dilute hydrochloric acid (2–5 ml.) and transferred to the top of a cellulose column packed in methyl propyl ketone. The cellulose pulp used is prepared by nitric acid treatment of Whatmans Ashless

TABLE I

ESTIMATION OF CU, NI AND CO IN VARIOUS SAMPLES

Material	Results by column process %	Mean of column results %	Results by normal analysis %
Nickel-chrome steel	Cobalt 10·00, 10·10, 10·00	10·03	10·02
	Nickel 12·85, 12·75, 12·75	12·78	12·90
Tungsten steel	Cobalt 4·36, 4·40, 4·31	4·36	4·35
	Nickel 0·49, 0·44	0·46	0·43
Iron pyrites	Copper 2·65, 2·71, 2·68	2·68	2·69
	Cobalt 0·13, 0·12, 0·11	0·12	0·10
Copper nickel speiss	Copper 17·16, 17·02, 17·16	17·11	17·04
	Nickel 22·86, 23·00, 22·86	22·91	22·80

Tablets. The chlorides of Fe, Cu and Co are extracted from nickel using methyl propyl ketone containing 1 % v/v HCl ($\rho = 1{\cdot}18$) as solvent, but clear separation of Fe, Cu and Co is not effected. If the acid concentration, however, is kept low and the water content of the solvent is increased, separation of Co, Cu and Fe is achieved. Thus, using a solvent consisting of 100 parts of methyl propyl ketone, 30 parts of acetone, 4 parts of water and 1 part of HCl ($\rho = 1{\cdot}18$), iron and copper are extracted from cobalt and nickel. The iron is first removed as a yellow band followed by an amber band of copper, while cobalt remains with the nickel. After the copper band has moved completely out of the column, the solvent is changed and the extraction continued with methyl propyl ketone containing 2 % v/v HCl ($\rho = 1{\cdot}18$); this solvent extracts cobalt from nickel. The copper is extracted from the requisite eluent fraction by several washings with 2 N HCl. The combined washings are evaporated to dryness, fumed with

HNO_3 to remove organic matter, then evaporated twice with HCl to remove nitrate. The copper is estimated colorimetrically with rubeanic acid on a Spekker absorptiometer. The cobalt fraction is diluted with acetone and the cobalt estimated by means of the colour developed with ammonium thiocyanate. The nickel is extracted from the cellulose column by washing with dilute HCl. The solution is evaporated to dryness fumed with HNO_3 and then evaporated twice with HCl The nickel is then determined with dimethylglyoxime. Some typical results obtained by this process are shown in Table I.

Mercury as mercuric chloride is extracted quantitatively from copper, bismuth, lead and cadmium using pure dry methyl acetate as solvent. If a normal cellulose column is employed the diffuse rear boundary of the mercury band and the forward boundary of the bismuth band overlap, with consequent contamination of the eluent by traces of bismuth. This effect has been overcome by including in the column a layer of pulp impregnated with sodium phosphate solution The phosphate does not effect the extraction of mercury but successfully retains the bismuth. By this method 0·2 g. quantities of mercuric chloride have been separated from an equal weight of each of the chlorides of lead, copper, cadmium and bismuth The mercury is recovered by passing H_2S gas through the solvent eluent and filtering off the precipitated sulphide.

Other separations which have been carried out in chloride solution include the separation of gold from the platinum metals and the separation of the individual platinum metals. Gold is extracted quantitatively from gram quantities of the platinum metals using ethyl ether containing aqueous HCl and some success has been gained in the preparative separation of the platinum metals using acetone or methyl ethyl ketone containing HCl ($\rho = 1{\cdot}18$).

A number of separations have also been carried out in nitrate solution. In consequence of the unusually high solubility of uranyl nitrate in organic solvents the extraction of uranium from many other elements has been carried out very easily.

Using ethyl ether containing 12½ % w/v HNO_3 promising results have been achieved in the separation of hafnium and zirconium. The separation is not yet quantitative but has been the means of obtaining samples of zirconium free from hafnium.

Discussion

The wide applicability of the method has been indicated by the variety of the examples of separation given. It is obvious that there are many factors which can operate to bring about these separations. There are, however, three main factors which contribute to the mechanism of separation to a varying degree with the individual metals.

(*a*) SELECTIVE EXTRACTION OF THE SALTS BY THE SOLVENT.—This effect plays an important part at the top of the column, where the mixture of salts is situated and is approximately proportional to the relative solubilities of the salts in the organic medium. It does not follow, however, that the order in which the salts reach the bottom of the column is proportional to these solubilities.

(*b*) PARTITION.—Partition of the inorganic substances occurs between the organic solvent and the water present in the cellulose. The water content of the pulp varies with the water content of the organic solvent and when inorganic salts are added as an aqueous solution the water content of the cellulose at the top of the column is abnormally high (at least at the beginning of the extraction). Changes in the concentration and composition of the salts in the solvent phase, as it moves downwards, lead to variations in partition due to alterations in the salting-out influence of the various salts on each other.

(*c*) ADSORPTION.—Metals ions are adsorbed to different extents by the cellulose. This factor is affected by activation of the cellulose, for example, by treatment with nitric acid. It is altered by variations in the acid content of the solvent.

In conclusion it is seen that this method of effecting separations opens up a wide field in the analysis (qualitative and quantitative), preparation and even production of inorganic materials.

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THE APPLICATION TO QUALITATIVE ANALYSIS OF THE SEPARATION OF INORGANIC METALLIC COMPOUNDS ON FILTER PAPER BY PARTITION CHROMATOGRAPHY

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An investigation into the separation of inorganic metallic compounds on filter paper by (*a*) aqueous *n*-butanol containing a complexing reagent and (*b*) aqueous collidine, and its general application to the separation of metallic compounds by a two-way process, is described. The detection of the position of the compounds on the filter paper is achieved by the use of ultra-violet fluorescence

A special application of the separation and detection of arsenic from a large number of other metallic compounds is also described

The use of filter paper for separating metallic derivatives in microchemical methods of qualitative analysis has long been practised. Feigl [1] has discussed these methods, and has pointed out that " the separating action of filter paper, due to capillary phenomena alone, is, as a rule, not sufficient to effect an isolation of the inorganic compounds of a solution into distinct zones." Even when this is possible the compounds are not present alone and in a pure state. The advantage of the new method of paper chromatography is that many inorganic compounds can be separated into quite distinct zones, in most cases several inches apart.

The separation of inorganic compounds on filter paper by the method of partition chromatography has so far developed along two main lines using (*a*) solvents containing reagents capable of forming complexes with inorganic ions, such as benzoyl acetone, and (*b*) special solvents, the usefulness of which may depend partly on the formation of complexes by reaction of the solvent with the metal ions under examination.

Linstead and his co-workers,[2] as well as Lederer,[3] have by the use of partition chromatography achieved a large number of important separations, but have tended to concentrate on special separations rather than on general methods of separating ions. Our procedure has obvious advantages in the general field of qualitative analysis. The technique which we have

[1] Feigl, *Specific and Special Reactions* (Elsevier, 1940), p. 162.
[2] Linstead *et al.*, *Nature*, 1948, **162**, 691 ; *ibid.*, 1949, **163**, 64 ; *ibid.*, 1949, **163**, 598.
[3] Lederer, *Nature*, 1948, **162**, 776.

developed makes use of both types of solvents, attention being focused in the first case on *n*-butanol containing one of the several well-known reagents which are known to form complex ions with the metals, and secondly on collidine as a special solvent of type (*b*).

Our general investigations have also led us to achieve the separation of several metals by a two-stage separation process, using first a solvent of type (*b*), and then one of type (*a*) moving at right-angles to the first solvent.

Under special conditions, the separation of only one inorganic compound from a large number of others has particular importance, and Linstead [4] has shown that uranium compounds can be separated in this manner, while we have adapted our technique to separate arsenic from a large number of other elements.

It is very convenient to have some general reagent which can be used to detect the position of the ions after separation is complete. We have found that materials which form with metallic ions compounds which fluoresce or suppress fluorescence in ultra-violet light are of great value. After spraying the paper with the reagent and then exposing it to the radiation from a mercury vapour lamp, the position of the ion, invisible in daylight, is revealed. A number of suitable reagents have been investigated and are described in this paper.

Experimental

Apparatus.—The general arrangement of the apparatus is similar to that already in use for organic chromatography, and consists of a glass trough supported in a glass tank, 10 in. × 13 in × 16 in., the top of which is ground level and covered with a heavy ground glass sheet to ensure that during an experiment the tank is air-tight. In the trough is put the organic solvent layer and at the bottom is a Petrie dish containing the aqueous layer. The tank is placed in an insulated cupboard.

Preparation of Solvents.—Two solvent mixtures have been used throughout.

(*a*) BUTANOL MIXTURE.—*n*-Butanol (100 ml.) is first shaken with the required quantity of complexing reagent, until the latter is dissolved. The solution is then thoroughly shaken with dilute nitric acid (100 ml.). The normality of the nitric acid is varied according to the complexing agent in use. After standing overnight to attain equilibrium, the aqueous layer is run off and put in the Petrie dish, while the upper layer is placed in the trough.

(*b*) COLLIDINE MIXTURE.—2 : 4 : 6-Collidine (100 ml.) is shaken with 0·4 N nitric acid (100 ml.), the heat generated by the mixing causing the temperature to rise to about 30° C. When the mixture cools on standing to 25° C, the layers are separated as already described.

Preparation of the Metal-ion Solutions.—0·1 N solutions of the nitrates of Ag^{+}, Hg^{+}, Pb^{2+}, Hg^{2+}, Bi^{3+}, Cu^{2+}, Cd^{2+}, Al^{3+}, Fe^{3+}, Zn^{2+}, Mn^{2+}, Co^{2+} and Ni^{2+} were prepared, containing sufficient nitric acid to prevent hydrolysis. 0·2 N solutions of the nitrates of Ca^{2+}, Sr^{2+}, Ba^{2+}, Mg^{2+}, K^{+} and Na^{+} were prepared and made slightly acid with nitric acid. 0·2 N solutions of the chlorides of Sn^{2+}, Sn^{4+} and Cr^{3+} were acidified with sufficient hydrochloric acid to prevent hydrolysis. A 0·1 N solution of arsenious oxide in water was slightly acidified with nitric acid. A 0·1 N solution of antimony tartrate was prepared and rendered faintly acid with tartaric acid.

Procedure.—Drops of solution to give a spot of about 1 cm. diam. are placed at distances of about 4 cm. apart on a pencilled line 8 cm. from the top edge of the sheet (27 cm. × 40 cm.) of Whatman No. 1 filter paper. Two similar sheets are placed one on each side of the trough, with the top edge dipping into the liquid. The tank is closed by the ground glass plate, placed in the insulated cupboard, and left until the liquid front is about 5 cm. from the

[4] Arden, Burstall and Linstead, *The Chemistry of the Heavy Elements* (Chem. Soc. Symposium, 1949).

lower edge of the paper. This takes about 20 hr. for the butanol mixtures and 35 hr. for the collidine. If the vessel is quite air-tight, and not exposed to varying temperatures, the liquid front remains horizontal.

The sheets of paper are removed from the tank, dried in a stream of hot air, and then sprayed with a special reagent to reveal as many ions as possible. As the unknown can be placed on the same sheet of paper, there is no need to use a specific reagent for each ion, since the unknown can always be compared with the known ions which have travelled down the paper simultaneously. This procedure also avoids the necessity of knowing exact R_f values. To confirm the identity of the unknown, it is always possible to put an extra spot of the unknown at the side of the paper, a strip of which can be cut off and tested with specific reagents. The position of the unknown will, of course, be obtained from the main strip.

Detection and Identification of the Ions.—A spray of ammoniacal aqueous hydrogen sulphide solution serves to reveal the presence of Ag^+, Hg^+, Pb^{2+}, Hg^{2+}, Bi^{3+}, Cu^{2+}, Cd^{2+}, As^{3+}, Sb^{3+}, Fe^{3+}, Co^{2+} and Ni^{2+} as coloured sulphides.

As indicated in an earlier publication,[5] however, a much more general method is to use organic reagents which, on exposure of the chromatogram to ultra-violet light, reveal the position of the ions as dark or fluorescent spots. The most satisfactory spray for this purpose is a solution of 5 g. 8-hydroxyquinoline and 1 g. kojic acid in 1 l. of 60 % alcohol. After spraying, the paper is exposed to ammonia vapour to make it alkaline. Many other reagents have been tested for this purpose, and some of the more promising results are recorded in Table I.

Results

(*a*) **Separations using a Solvent Containing a Complexing Reagent.**—Experiments were carried out with butanol mixtures prepared with butanol and dilute nitric acid of different normalities, both with and without complexing reagents. In Fig. 1 the R_f values for most of the common ions are plotted as a function of the position of the ion on the original chromatogram, using mixtures prepared with N, 2 N, and 3 N nitric acid without a complexing reagent. It is clear that the relative separation is the same in most cases, but that the ions travel further in the more highly acid medium. Hg^+, Hg^{2+} and Sb^{3+} show pronounced "tailing" under these conditions, and so their positions have not been included.

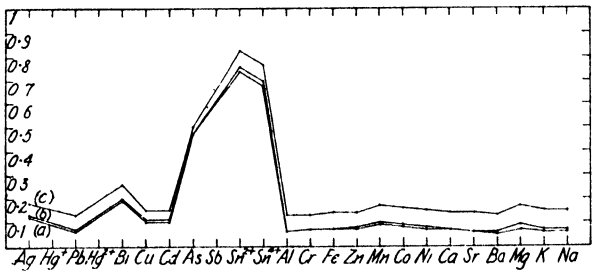

FIG 1.—Separations with *n*-butanol mixture, without complexing reagent, and prepared with (*a*) N HNO_3; (*b*) 2 N HNO_3, (*c*) 3 N HNO_3

It was found that much better separations could be achieved in some cases by the addition to the liquid medium of an organic reagent, capable of forming complexes. Many such reagents were tried under different conditions of acidity, and among the most successful of those yet tried were benzoyl acetone and acetyl acetone, while dibenzoyl methane, acetoacetic ester and dimethyl glyoxime were not, in general, so good. A typical example of the separations achieved is shown in Fig. 2 for benzoyl acetone, which up to the present has given the best separations and most symmetrical spots.

[5] Pollard, McOmie and Elbeih, *Nature*, 1949, **163**, 292.

TABLE I

ORGANIC REAGENTS FOR DETECTING IONS BY ULTRA-VIOLET FLUORESCENCE

Fluorescent Substance	Bright Spots			Dark Spots			Invisible Spots
	+++	++	+	− − −	− −	−	[o]
o-Aminobenzoic Acid ..	—	—	Sb	Ag, Cu, Cd, Mn, Co	Hg^{+}, Pb, Hg^{2+}, Bi, Al, Fe, Zn, Ni	Cr, Ca, Sr, Ba, Mg, K, Na	As, Sn^{2+}, Sn^{4+}
8-Hydroxyquinoline ..	Cd, Al, Zn, Mg	Ag, Sn^{2+}, Sn^{4+}, Ca	Sr, Ba	Fe	Hg^{+}, Hg^{2+}, Bi, Cu, Sb, Mn, Co, Ni	Pb, Cr, K, Na	As
Kojic Acid	Ag, Hg^{+}, Hg^{2+}	Ca	Sr, Ba, Mg	Cu, Fe, Ni	Bi, Al, Mn, Co	Pb, Cd, Sb, Sn^{2+}, Sn^{4+}, Cr, Zn, K, Na	As
Naphthionic Acid ..	—	—	—	Hg^{2+}, Fe, Mn	Ag, Hg^{+}, Pb, Bi, Cu, Cd, Al, Cr, Zn, Co, Ni, Ca, Sr, Ba, Mg, K, Na	—	As, Sb, Sn^{2+}, Sn^{4+}
Morin	Al, Zn	Pb, Cd, Sn^{2+}, Sn^{4+}, Mg	—	Cu, Fe	Ag, Hg^{+}, Hg^{2+}, Bi, Cr, Mn, Co, Ni	—	As, Sb, Ca, Sr, Ba, K, Na

NOTE.—Valencies have been given only to those metals present in more than one valency state.

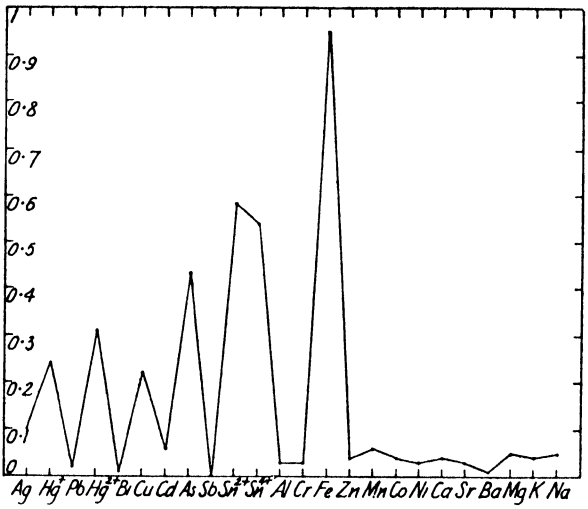

FIG 2.—Separations with *n*-butanol mixture prepared with 0·1 N nitric acid and 0·5 % benzoyl acetone

(*b*) **Separations using a Solvent only.**—The most interesting results with this type of solvent have been obtained with collidine, and the separations are illustrated in Fig. 3. Again it was found that the acidity was important for good results.

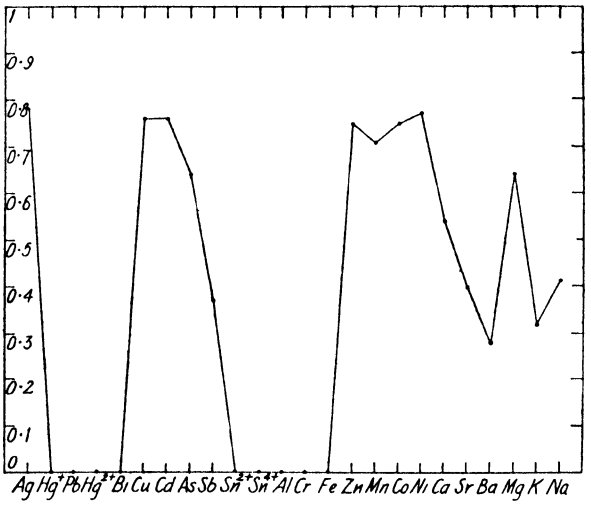

FIG. 3.—Separations with collidine mixture prepared with 0·4 N nitric acid.

(*c*) **Two-way Separations.**—The possibility of separating cations by using the properties of the two types of solvents has been investigated with a mixture containing Al^{3+}, Fe^{3+}, As^{3+}, Cu^{2+} and Cd^{2+}. A spot containing all these ions was put at a point X on the filter paper. The collidine solvent was first used, and the compounds of As^{3+}, Cd^{2+} and Cu^{2+} were thus separated from those of Fe^{3+} and Al^{3+}. The Cu^{2+} and Cd^{2+} moved together as predicted from Fig. 3, and the As^{3+} did not travel so far.

The filter paper was washed with CCl_4 and dried, and then the butanol solvent containing benzoyl acetone was used with the paper at right-angles to its original

position. The iron compound now separated from the aluminium; the copper separated from the cadmium and the arsenic moved to a new position; the copper actually moved with a higher R_f value than predicted from Fig. 2. In this way a clear separation of these five metallic compounds was achieved. Their positions were first detected by fluorescence, and confirmed by hydrogen sulphide, etc. The relative positions of the metallic compounds are illustrated in Fig. 4.

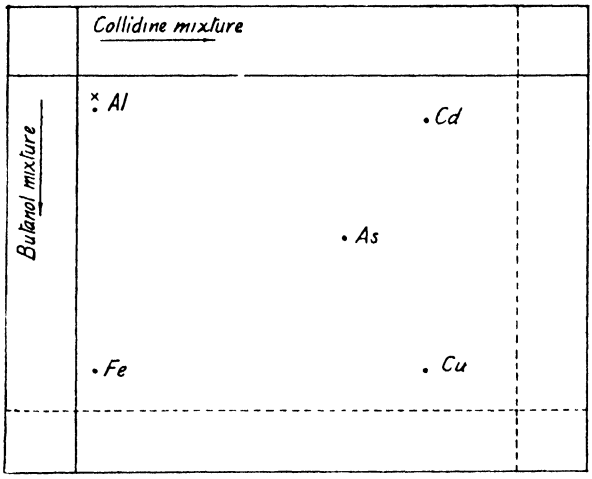

FIG 4.—Example of two-way separation.

(*d*) **Separation of one Ion from a Large Number of Others.**—As an example of the application of this type of separation, arsenic was selected because of its obvious importance. The fundamental technique of using butanol-water mixtures was adopted, and the effect of various substances was investigated to try to obtain large R_f values for arsenic while retaining small R_f values for other cations present. The best mixture proved to be butanol and water prepared with 1 % ammonium borate, 1 % ammonium tartrate, 0·5 % mannitol. The actual R_f values for all the common cations using this mixture are given in Fig. 5, from which the exceptional behaviour of arsenic is obvious.

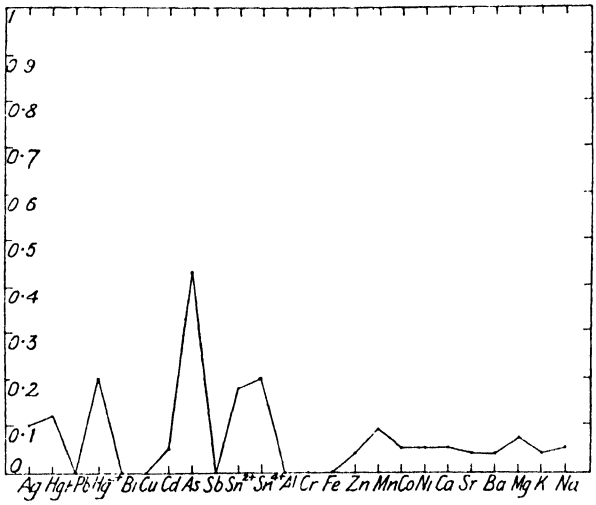

FIG. 5.—Separation of arsenic from other metallic compounds.

A special test for arsenic was developed as follows. The chromatogram was sprayed with a mixture of alcohol containing 1 % conc. nitric acid and 5 % glycerol to make the paper acid, and then, after drying, sprayed with ammoniacal silver nitrate. The arsenic spot is converted, presumably to silver arsenite (yellow), which, on irradiation with ultra-violet light whilst still wet, changes to silver arsenate (brown) and metallic arsenic (black). The spot is then easily visible against the purplish background from the silver nitrate spray. With stationary drops of 0·01 ml. taken by a micrometer syringe from standard solutions of sodium arsenite it was possible to detect 0·1 γ of arsenic; on the chromatogram, however, the limit of sensitivity was 0·3 γ of arsenic. The test can be made quantitative and used for a colorimetric estimation of the arsenic.

Discussion

The illustrations, Fig. 2 and 3, show the kind of separations which are possible for two different types of solvents. It is clear that the cations of their derivatives can be divided into two groups, depending on the movement in the two different solvents.

GROUP A. Cations moving with butanol-water-benzoyl acetone mixture.
Ag^{+}, Hg^{+}, Hg^{2+} Cu^{2+}, Cd^{2+}, As^{3+}, Sn^{2+}, Sn^{4+}, Fe^{3+}.

GROUP B. Cations moving with collidine-water mixture.
Ag^{+}, Cu^{2+}, Cd^{2+}, As^{3+}, Sb^{3+}, Zn^{2+}, Mn^{3+}, Co, Ni, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mg^{2+}, K^{+}, Na^{+}.

While some inorganic compounds move in both solvents the extent of the movement may vary considerably; thus Ag^{+}, Cu^{2+}, Cd^{2+}, As^{3+} derivatives move much further in collidine than they do in butanol. Also, it should be noted that, while copper and cadmium compounds move together in collidine, there is quite a good separation in the butanol mixture. The separation of sodium, potassium and magnesium by the collidine mixture is particularly interesting.

There is some evidence that formation of complexes is essential for rapid movement, if one compares the results of butanol mixtures with and without complexing reagents as illustrated by Fig. 1 and 2. It is noticed that when no complexing reagent is present, movement of several cations, for example, Bi^{3+}, Cu^{2+}, Cd^{2+}, As^{3+}, Sn^{2+}, Sn^{4+}, etc., occurs, and that the movement is greater for the more acidic mixtures. The effect of the increased acidity is probably associated with the prevention of hydrolysis of these particular nitrates, and consequently the nitrates are capable of movement. In the presence of a complexing reagent, and providing the acidity is not too great, that is, for benzoyl acetone not greater than about 0·1 N, the movement of the various ions differs considerably. Under such conditions, one obtains, for example, as has been pointed out already, a separation of copper and cadmium. If, however, the acidity is greater than 0·1 N, for benzoyl acetone the movement of the ions in general resembles that without any complexing reagent being present, and there is no separation of copper and cadmium. Presumably this is due to the instability of the complexes in strongly acid solution, but at the same time it is necessary to have the solution sufficiently acid to prevent undue hydrolysis of some of the original nitrates. For any particular complexing reagent it is found necessary to determine the optimum conditions.

At present there is insufficient evidence for much to be said about the mechanism of these particular chromatographic processes, but it does seem that for their successful operation the formation of complexes is essential. As collidine itself is quite likely to form complexes with the cations, then in this type of solvent complex formation probably plays an important role.

How far true partition between solvents occurs is still uncertain. In our earlier communication [5] we pointed out that for butanol mixtures water alone washes the ions down the paper, while butanol mixture prepared with 10 % acetic acid produces almost no movement. This seemed to indicate that in this case it was essentially a partition process.

The preliminary investigations on two-way separations indicate that our technique is capable of very general application for the separation of the cations common to qualitative analysis, and the special separation of arsenic is being developed for the quantitative estimation of small quantities of arsenic.

Our thanks are due to Dr. J. K. N. Jones for his great interest in this work, and to the Egyptian Education authorities for a grant to one of us (I. I. M. E.).

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INORGANIC CAPILLARY ANALYSES

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The paper deals with chromatographic experiments using porous paper impregnated with $Al(OH)_3$ as adsorbent. The paper is cut in strips, a small amount of the solution is sucked up at one end, followed by a larger amount of pure solvent. The chromatographic zones are developed by brushing with suitable reagents. The method may be used for the analyses of cation or anion mixtures. An anion chromatogram is formed when a strong acid is sucked into the paper prior to treatment with the solution.

The sequence in which the cations are adsorbed is the same as found by Schwab with few exceptions. The effect of the presence of anions and other factors influencing the length of the molar adsorption zone of the cations are discussed. If the less adsorbable M_2^+ ion is added to a solution of M_1^+ ion an increase is observed in the adsorption zone of M_1^+. The reason for this is that a mixed zone of M_1 and M_2 is found. Approximate values of the exchange constant of reactions of the type :

$$M^+_{1(aq)} + M_{2(\text{mixed ads.})} = M^+_{2(aq)} + M_{1(\text{mixed ads.})}$$

have been derived. From the values of these constants the possibility of a quantitative separation of cations in mixtures can be predicted. The effects of complex-forming reagents are also estimated when the complex constants are known. This is demonstrated by the chromatograms of solutions to which glycinate has been added. Addition of complex-forming reagents may also be used for a simple quantitative chromatographic titration.

The investigations reported in the present paper were chiefly carried out during 1938 to 1945. The method itself has been described [1] but as a consequence of the wartime conditions most of the results were either partly published in Norwegian (with only a brief summary in German [2 3 4 5 6]) or remained unpublished.

[1] Flood, *Z. anal. Chem.*, 1940, **120**, 237.
[2] Flood, *Tidsskr. kjemi bergvesen*, 1940, **20**, 111.
[3] Flood and Smedsaas, *Tidsskr. kjemi bergvesen metallurgi*, 1941, **1**, 150.
[4] Flood and Smedsaas, *Tidsskr. kjemi bergvesen metallurgi*, 1942, **2**, 1.
[5] Flood and Smedsaas, *Tidsskr. kjemi bergvesen metallurgi*, 1942, **2**, 17.
[6] Flood, *Tidsskr. kjemi bergvesen metallurgi*, 1943, **3**, 9.

Experimental

The characteristic feature of the method is that porous paper impregnated with $Al(OH)_3$ is used as adsorbent. Some solution, followed by pure water, is sucked up into a strip of the paper. Thick blotting paper is most suitable since it possesses a homogeneous distribution of pores. The impregnation is carried out by dipping the paper in a solution of sodium aluminate (0·1 to 1 mole/l). The paper is then dried and the aluminium hydroxide formed by dipping it into a saturated solution of $NaHCO_3$. The paper is washed for several days in distilled water, dried over a period of some days and finally cut into strips (30 × 1 cm. or smaller).

The solution is sucked up in the one end of the strip to a height of 1–3 cm. (For quantitative purposes the amount ($\simeq$ 0·1 ml.) is measured with a capillary pipette.) The paper is then treated with water until a height of 10 cm. or more is wetted. Two or three times the length of the adsorption zone is sufficient to give a constant length of zone. During the passage of water the adsorption zone does not leave the lower end of the paper. This indicates that the chief adsorption process is an exchange between cations from the solution and sodium ions from aluminate impurities in the aluminium hydroxide. Finally the chromatographic zones are developed by brushing with suitable reagents.

Some advantages of this method compared with powder columns may be noted. The manner of development causes no disturbance of the primary zones. Several developing reagents can be used in the same chromatogram, after cutting up the strip into 2 or more parts. The method is well suited to semi-micro work (1 mg) and can also be modified for micro work (1 γ). Most important, however, is that the results can be reproduced since strips from the same sheet normally show nearly the same adsorptive capacity.

The results are easily reproduced within $\pm$ 5 % and by careful work $\pm$ 2 % may be reached. It must be noted that the height of an adsorption zone is sensitive to depression of the surface tension of the solution. Details, especially concerning the quantitative use of the method, have been given previously.[1]

Results

1. Sequence of Adsorption.—The following sequence of adsorption is found for cations:

$$\begin{matrix} Sb^{III} \\ TiO^{II} \\ Bi^{III} \end{matrix} : \begin{matrix} Sn^{II} \\ As^{III} \end{matrix} : \begin{matrix} Fe^{III} \\ Cr^{III} \\ Hg^{II} \end{matrix} : UO_2^{II} : Pb^{II} : \begin{matrix} VO^{II} \\ Al \end{matrix} : Ag^{I} : Cu^{II} : Zn : Cd : \begin{matrix} Ca^{II} \\ Ni^{II} \\ (Fe^{II}) \end{matrix} : Tl^{I} : Mn^{II}.$$

With few exceptions (Cd–Co, Ag–Cu) this is the same as previously found by Schwab and Jockers [7] on Al_2O_3 columns. Ca, Sr and Ba form mixed zones with the transition elements (Cu, Ni, etc.). Mg apparently is adsorbed, but the zone moves slowly upwards with the washing water (precipitation of $Mg(OH)_2$?)

When some strong acid ($HClO_4$, HNO_3) is sucked into the paper before treatment with the solution, an anion chromatogram may be obtained. The sequence of anion adsorption is found to be:

$$: \begin{matrix} PO_4^{3-} \\ F^- \end{matrix} : \begin{matrix} Fe(CN)_6^{4-}, (CrO_4^{2-}) \\ SO_4^{2-} \end{matrix} : (CrO_4^{2}), Fe(CN)_6^{3-} : Ac^- : Cl^-, CNS, I^- : NO_3^-(: ClO_4^-$$

This too is in accord with previous results of Schwab and Dattler.[8]

Weak effects of secondary anion and cation adsorption are observed. Details have been given previously.[6]

2. The Molar Zone Length.—The zone length h is a linear function of the concentration c of the ion in solution. When h is not too close to zero we may write:

$$h = h' + kc,$$

[7] Schwab and Jockers, *Z. angew. Chem.*, 1937, **50**, 546.
[8] Schwab and Dattler, *Z. angew. Chem.*, 1937, **50**, 691.

(compare Fig. 1); $h' \simeq 0{\cdot}3$–$0{\cdot}5$ mm. seems to be connected with the smaller adsorption capacity of the zone at the lower end where the paper is secured by rubber bands (cp.[1]).

Different ions give different zone lengths shown by the following figures.

TABLE I

Conc.	h in cm. for different cations								
	Cr^{III}	Pb	Cu	Hg	Zn	Ni	Te	Mn	Ba
10^{-6} mole/l ..	4·25	3·3	3·3	2·9	3·6	3·7	3·15	4·1	5·1
2×10^{-6} mole/l. ..	7·7	6·0	5·85	5·1	6·45	6·55	5·3	7·35	8·7

Considering cations of the same valency decreasing adsorption tendency seems to run parallel with increasing molar zone lengths. Increasing valency increases the molar zone length.

The length of a cation zone is to some degree influenced by the anions present in the solution as shown by the following figures.

TABLE II

	h (cm.)			
	SO_4^{--}	Cl^-	NO_3^-	CH_3COO^-
Mn^{++}	6·2	6·4	6·3	6·6
Cu^{++}	6·4	7·7	8·0	7 1
H^+	2·8	3·3	3·5	—

The effects are not very important, but they seem to a certain degree to be specific.

3. Chromatography of Cation Mixtures.—If we add to a solution of M_1 a less strongly adsorbable cation M_2, an elongation of the chromatographic zone of M_1 is observed. The reason is that a mixed zone of M_1 and M_2 is formed. Fig. 2 shows the relative elongation $g = h/h_0$ as a function of the concentration ratio $[M_1]/[M_2]$ in solution. The tendency to form a mixed zone increases with

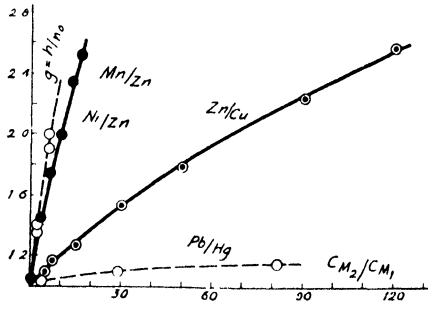


FIG. 2.—The relative elongation of the chromatographic zone as a function of the concentration ratio in the mixture.

decreasing difference in adsorption affinity. Consequently ions such as Ni and Co are completely mixed on adsorption. Only when the difference in adsorption tendency is considerable will the zone length of a cation be apparently independent of the presence of other cations. Analysis of the ion distribution in the zone shows that the ion concentration seems to be constant over the whole zone; this is also the case in an elongated mixed zone.

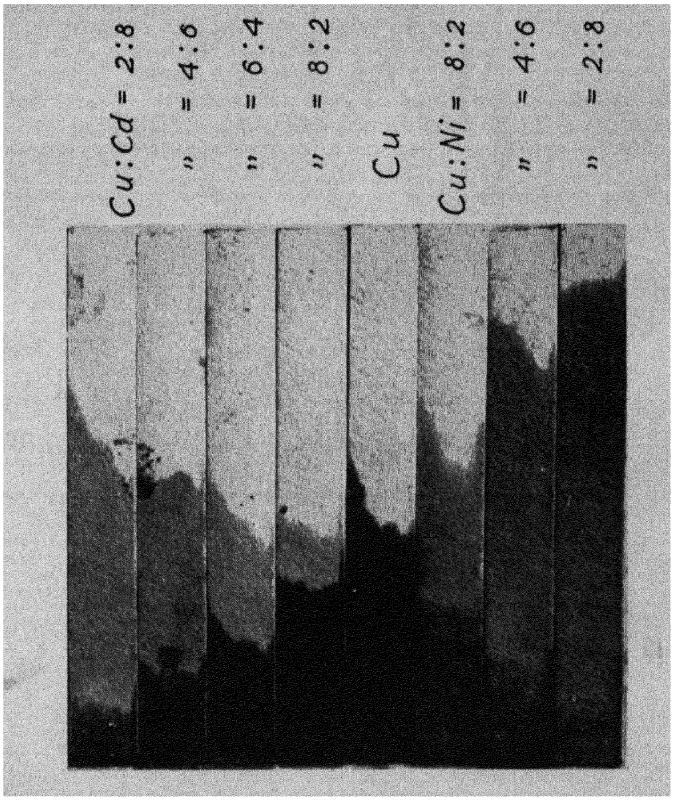


FIG. 1.—Chromatograms of Cu–Cd mixtures (developed with H_2S) and Cu–Ni (develo with dimethylglyoxime).

Cu + Cd = Cu + Ni = 4 $\times 10^{-6}$ mole/l.

Simplified calculations show that this is in accord with the concept that the composition of the mixed zone is determined by an equilibrium of the type:

$$M_1^+ + M_{2(Al_2O_3)} = M_2^+ + M_{1(Al_2O_3)} . \quad . \quad . \quad . \quad (1)$$

We can write:

$$K = \frac{C^+_{M_1} a_{M_2}}{C^+_{M_2} a_{M_1}} = \frac{C^+_{M_1} N_2 f_2}{C^+_{M_2} N_1 f_1},$$

where the C's are concentrations of *the original solution*: a's denote activities, f's activity coefficients, and N's the mole fractions of cations in the absorbed zone.

If we assume $f_1 = f_2$ when $N_1 = N_2 = 0{\cdot}5$ (symmetrical activity functions), it follows that $K \simeq C_1/C_2$, when $h = 2h_0$.

As only rough figures of K are required, they are not corrected for the deviations from proportionality between h and c, and for differences in molar zone length of M_1 and M_2. These two approximations influence the values of K in opposite directions.

Equilibria between ions of different valency may be treated in a similar way by putting, for example,

$$K = \frac{C^2_{M_1+} \cdot N_{M_2} \cdot f_{M_2}}{C_{M_2++} \cdot N^2_{M_1} \cdot f^2_{M_1}} .$$

It follows as before that

$$k \simeq C^2_{M_1+}/C_{M_2++},$$

where $h_{M_2} = 2{\cdot}63 h^2_{M_2}$.

From these experiments we can set up a scale of the adsorption affinity of cations, where the difference between two ions gives an approximate value of $\log_{10} K$ for an exchange equilibria of the type (1). In order to have only positive numbers on the scale we give the relatively weak adsorbable Na^+ ion the arbitrary value of 0.

TABLE III

(Na 0·0); Mn^{II} 0·8; Ni, Co, Fe^{II} 1·1; Cd 1·1$_5$; Zn 1·8; Cu^{II} 3·6; Pb^{II} 4·1; (Hg^{II} 8).

It is evident from Table III that several of the common ions Mn, Ni, Co, Fe, Cd are situated close together, i.e., within 1·0 unit on the log K scale. These ions therefore, in particular, will tend to form mixed zones.

In an earlier communication [4] the alkaline earth metals were included in the scale. Later experiments, however, seem to indicate that the adsorption phenomena of mixtures including these elements are more complicated than was previously thought. For example, Ba forms a mixed adsorption zone with Ni, but this is also the case with the more strongly adsorbable Cu ion.

4. Chromatography of Complexes. Chromatographic Titration.—Electrically neutral molecules or negatively charged complex ions show a negligible adsorption tendency on paper not previously treated with acid. This allows of the possibility of separating cations by addition of complex-forming reagents. When the constants of the complex formation are known, it is possible to predict the chromatographic result by simple calculation using the values of exchange constants given in §3.

This can be demonstrated by the chromatographic behaviour of glycine complexes, where the constants of the stepwise complex formation are known for some of the common ions.[9] Details will be published elsewhere, and only some of the results will be mentioned here.

[9] Flood and Lorås, *Tidsskr. kjemi bergvesen metallurgi*, 1945, **3**, 83.

(*a*) COPPER GLYCINATE COMPLEXES. The calculations show that with a solution of Cu/Gl/Na = 1/1/1, the $CuGl^+$ ion in contact with the adsorbent will disproportionate quantitatively into adsorbed Cu^{++} and unabsorbable $CuGl_2$. (Gl = glycinate ion). Therefore when a solution of Cu^{++} is added to one of NaGl, the mixture behaves as if composed of Cu^{++} and $CuGl_2$. This method, called chromatographic titration, can be used for a quantitative determination of copper. To a series of copper solutions are added known amounts of NaGl and the solutions are chromatographed. The equivalent amount is determined by extrapolation to zero zone length (Fig. 3). The determination is rapidly made and the accuracy obtained is better than ± 5 %. Several other weaker complex-forming cations (such as Cd, Co, Ag, etc.) will not affect the copper determination.

(*b*) Corresponding calculations for Ni glycinate complexes show that the difference in affinity of Ni and Na is not large enough to give more than *ca.* 85 mole % Ni in the adsorption zone in contact with a solution Ni/Na/Gl = 1/1/1. Furthermore, with a solution of Ni/Na/Gl = 1/2/2, an appreciable amount of adsorbed Ni (10 mole %) is obtained, assuming that the adsorption tendency of $NiGl^+$ is comparatively small.

This is in good agreement with the experimental chromatograms (Fig. 3). It is necessary to add a considerable excess of NaGl (Ni/Gl = 1/2·5) before the adsorption of Ni becomes negligible. (The reason for this is the formation of the third complex, $NiGl_3^-$, which has the effect that excess of glycinate >1/2 does not increase the concentration of glycinate ions as much as in the case of copper.) Ni therefore cannot be titrated chromatographically with glycine.

(*c*) CHROMATOGRAPHIC SEPARATIONS BY MEANS OF GLYCINATE COMPLEXES. When NaGl is added to a solution of two cations M_1^{++} and M_2^{++} the result can be easily predicted. When we consider for example the equilibrium,

$$M_1Gl^+ + M_{2(Al_2O_3)} = M_2Gl^+ + M_{1(Al_2O_3)}$$

in a solution containing

$$C_{M_2^{++}} = C_{M_1^{++}} = 2\ C_{Gl^-},$$

we find

$$\frac{N_{M_1}}{N_{M_2}}\ K \cdot \frac{K_{1(M_1)}}{K_{1(M_2)}} = K_0,$$

where K is the exchange constant given in § 4. K_1 is the instability constant for the complex ion MGl^+.

Table IV gives the calculated values of K_0 for some cation pairs. It is evident that the addition of glycinate may effect considerable changes in the normal adsorption sequence.

TABLE IV

M_1	M_2	$\log K_{1(M_1)}$	$\log K_{1(M_2)}$	$\log K$	$\log\left(K_0 = \frac{N_{M_1}}{N_{M_2}}\right)$
Cu	Zn	− 8·2	− 4·8	+ 1·8	− 1·6
Cu	Ni	− 8·2	− 5·8	+ 2·5	+ 0·1
Cu	Co	− 8·2	− 4·6	+ (2·5)	− 0·9
Cu	Cd	− 8·2	− 3·9	+ 2·5	− 1·6
Ni	Co	− 5·8	− 4·6	(0)	− 1·2
Ni	Cd	− 5·8	− 3·9	(0)	− 1·9
Co	Cd	− 4·6	− 3·9	(0)	− 0·7

After glycinate addition, Zn, Co and Cd are more strongly absorbed than Cu, and Ni is as adsorbable as Cu. These results are in good agreement with the experimental results (cp. Fig. where Cu–Co, Cu–Cd and Cu–Ni and Cu–Mg

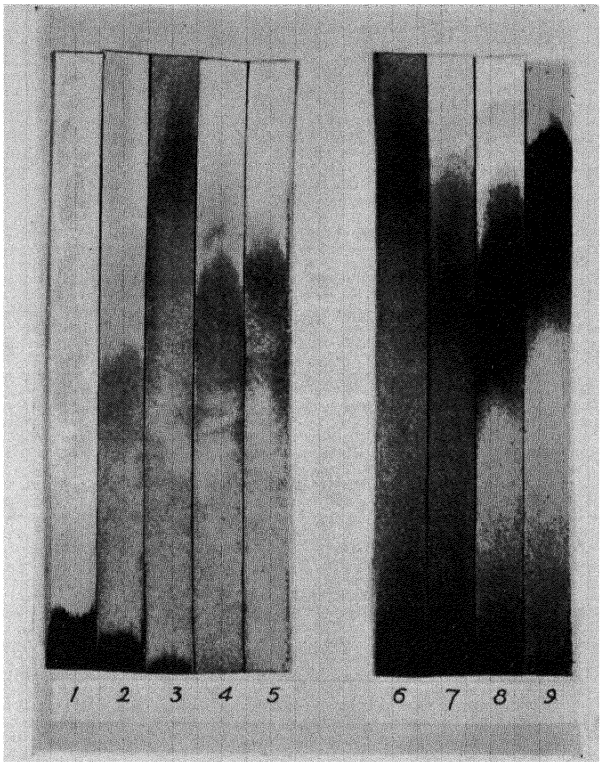


FIG. 3.

(1) Cu/glycinate = 1/0
(2) Cu/glycinate = 1/1·0
(3) Cu/glycinate = 1/1·8
(4) Cu/glycinate = 1/2·0
(5) Cu/glycinate = 1/2·2
(6) Ni/glycinate = 1/1·8
(7) Ni/glycinate = 1/2·0
(8) Ni/gylcinate = 1/2·2
(9) Ni/glycinate = 1/2·4

(1)-(5) Developed with $K_4Fe(CN)_4$. (6)-(9) Developed with rubeane.

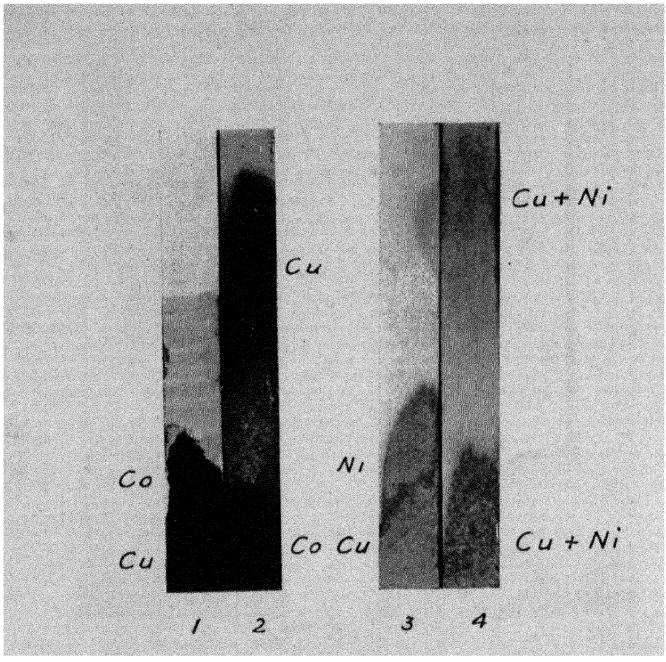
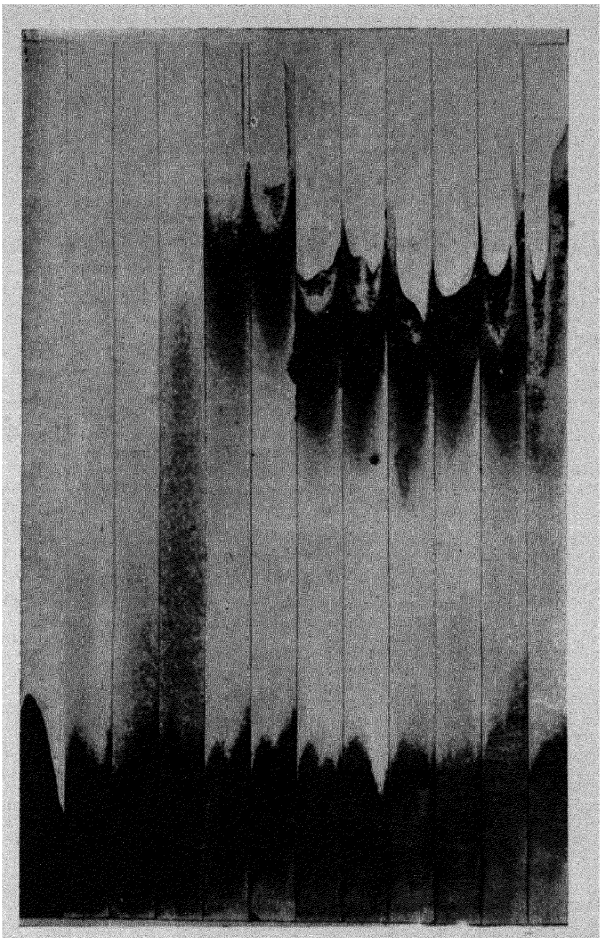


FIG. 4.

(1) Cu/Co/glycinate = 1/1/0
(2) Cu/Co/glycinate = 1/1/1
(3) Cu/Ni/glycinate = 1/1/0
(4) Cu/Ni/glycinate = 1/1/1

(1) and (2) developed with rubeane (dirty green copper zone and brown cobalt zone).
(3) and (4) developed with dimethylglyoxime (grey copper zone, red nickel zone).



1 2 3 4 5 6 7 8 9 10 11 12

FIG. 5.—Chromatograms of Ni-Co mixtures to which increasing amounts of glycine were added. The white zones at the lower end represent hydrogen ions which are formed by complex formation: $Ni^{++} + HGl = NiGl^{+} + H^{+}$, etc.

The zones are developed with rubeane (brown cobalt and blue nickel zones).

The separation is very good in the strips (5)–(8) where the ratio Ni/Co/glycine = 1/1/5–10.

At larger glycine concentrations the complex formation of cobalt becomes marked.

(1) Ni/Co/glycine — 1/1/0
(2) Ni/Co/glycine — 1/1/0·6
(3) Ni/Co/glycine — 1/1/1·2
(4) Ni/Co/glycine — 1/1/2·4
(5) Ni/Co/glycine — 1/1/5·0
(6) Ni/Co/glycine — 1/1/7·0
(7) Ni/Co/glycine — 1/1/9·3
(8) Ni/Co/glycine — 1/1/11
(9) Ni/Co/glycine — 1/1/13
(10) Ni/Co/glycine — 1/1/15
(11) Ni/Co/glycine — 1/1/22
(12) Ni/Co/glycine — 1/1/44

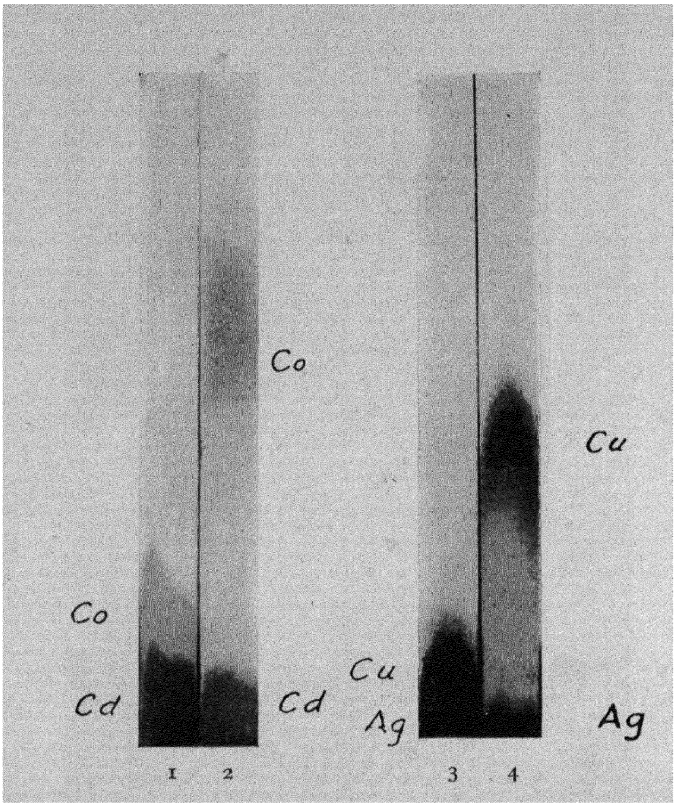


FIG. 6.—Chromatographic separation of Ag from Cu and Cd from Co by addition of glycinate (developed with H_2S).

(1) Cd/Co/glycinate = 1/1/0
(2) Cd/Co/glycinate = 1/1/4 (+ a little excess of glycine).
(3) Cu/Ag/glycinate = 1/1/0
(4) Cu/Ag/glycinate = 1/1/2

chromatograms are given). In the case of a Cu-Ni mixture, a mixed adsorption zone is formed, and the unadsorbed fraction also consists of both cations. In the other two cases only copper is found in the unadsorbed complex fraction. In the same way, addition of glycinate gives a good separation of Ni from Co, Ni from Cd, and Co from Cd, owing to the decreasing complex-forming tendency Ni, Co, Cd.

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SEPARATIONS ON 8-HYDROXYQUINOLINE COLUMNS

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This paper deals with the quantitative determination of small variations in zinc content of a cupro-nickel brazing alloy by a chromatographic technique. The original table of ions separable in this manner on 8-hydroxyquinoline columns is extended, and possible further quantitative separations are discussed Attention is also drawn to certain limitations of the method, though it is considered that it should have some popularity for certain specific quantitative applications and a wider range of appeal as a useful qualitative sorting test.

As previously noted by the present author, the technique proposed by Erlenmeyer and Dahn [1] of separating inorganic ions on a column composed of 8-hydroxyquinoline and starch was used to determine quantitatively small variations in zinc content of a cupro-nickel-zinc alloy.[2] The method consisted in adsorbing the metals (sample weight 2–6 mg.) from sulphate solution buffered to pH 5–6 with sodium acetate. Tubes of suitable size were found to be 10 cm. long by 5 mm. internal diam. Starch and 8-hydroxyquinoline in equal amounts by weight were well mixed and ground to pass a 60-mesh sieve and be retained on one of 80 meshes to the linear inch. Packing was effected by tapping the tube on the bench after each small addition of adsorbent ; tamping was found most unsatisfactory because of uneven pressures giving rise to slow filtration and unsatisfactory banding. Whilst dilution of the above sample weight to a concentration less than 2 mg./ml. proved to have no deleterious results, higher concentrations were found occasionally to give somewhat unsatisfactory banding. In this connection, the most frequent trouble was co-precipitation of the green fluorescing zinc complex along with the copper band (green in ordinary light) at the top of the tube. After adsorption of the coloured complexes, washing was most satisfactorily effected with 5 % sodium acetate solution. Dilute aqueous acetic acid as sometimes advocated has always seemed to promote undue channelling of the column before any effect on the banding could be seen. As in the chromatography of organic compounds using alumina columns, it was found to be important that the top of the column be never allowed to become free of liquid either during addition of the metal solution or the final washing. A suitable amount of washing liquid would seem to be three to four times the volume of the initial solution.

[1] Erlenmeyer and Dahn, *Helv. chim. Acta*, 1939, **22**, 1369 ; 1941, **24**, 878.
[2] Robinson, *Metallurgia*, 1947, **37**, 45, 107.

Quantitative measurements were obtained from the length of the appropriate band as compared with the lengths of corresponding bands obtained at the same time and under precisely the same conditions as the unknown sample, but using a solution containing accurately known amounts of the metals concerned. Where unusually small amounts of metallic ions had to be determined it was found advisable to reduce the diameter of the column of adsorbent in order to allow the formation of a measurable length of coloured band. The colours of the respective bands of the three metals were (in descending order down the tube): copper, dark green; nickel, yellow; zinc, yellow. Fortunately, only the zinc complex was fluorescent in ultraviolet light so that its zone length could easily be measured.

As foreshadowed in the previous report on the above work, it was hoped to utilize the 8-hydroxyquinoline technique as a means of identification and possible determination of the constituents of alloy steels. It was soon found, however, that the large amount of iron present in these alloys constituted a very serious difficulty because the whole of the column became stained with the ferric complex, so that virtually only one large black band existed. Various attempts were made to restrict the iron band to its correct position on the column (i.e., below the yellow zinc zone). The first line of attack was to find an optimum pH which would allow of a sharp separation, but no success was achieved within the range pH 3–8. This appeared the most likely range as below pH 3 the mobility of the iron might have been sufficient to enable it to be washed out of the column, but the other bands were far too imperfect, due to channelling, to allow of identification. Above pH 8, all that could be seen was a single heavy black zone of iron, which presumably contained everything else, even where complexing of the iron with tartrate was used to prevent its prior precipitation as the hydrate.

So far, the only conditions under which any other complexes than iron could be seen (and even then measurement was virtually impossible, so prohibiting quantitative work) have been achieved by reduction of the iron to the ferrous condition with hydroxylamine hydrochloride. In this circumstance the ferrous complex appeared as a faint greenish-grey band occupying almost the whole of the column, but above which could be seen a green band due to Mn, a yellow band due to Ni, and an orange band due to Mo in that order, when using nickel steels containing from 0·5 % to 3·5 % nickel and up to 0·5 % molybdenum. With a plain carbon steel containing 0·25 % vanadium, the grey vanadium zone was seen immediately above the manganese band.

Even these rather disappointing results were only obtained by packing the column for a distance of about 2 cm. above the 8-hydroxyquinoline-starch mixture with powdered hydroxylamine hydrochloride in order to maintain adequate reducing conditions: again, the most satisfactory pH range appeared to be about 5–6, though if one was prepared to sacrifice some definition of the manganese zone in ordinary low-alloy steels, and of the tungsten zone in high-speed steels, slightly better results were obtained at pH 3 (minimum). High-speed steels did not seem very satisfactory materials, owing to the need for either complexing the iron, to allow work at high pH values, or alternatively complexing the tungsten, for working at the other end of the scale. Both methods gave rise to indeterminate banding and doubtful colours.

8-Hydroxyquinoline columns have been used with far greater success in non-ferrous analysis and, provided one only attempts to use alloys of simple composition, the method has definite value for determining local variation in composition or rapid identification of extraneous metals, especially where one is limited as to amount of sample available. It has been possible to

determine the stability of silver in a brazing alloy on brazing, by a method similar to that reported elsewhere for zinc. The alloy concerned contained 89 % copper, 2 % silver and 9 % zinc. Silver produced a yellow band above the green copper zone when working at pH 3·5 to 4·5, which appeared the most suitable range, though to prevent channelling a 40 % oxine–60 % starch column had to be used instead of the normal 50/50 mixture. That the upper yellow band was free from possible contamination with co-precipitated zinc was verified by the fact that only a faint yellow fluorescence in ultra-violet light was observed whereas the lower zinc zone showed its familiar bright green fluorescence. At higher pH values than 5 some co-precipitation takes place and quantitative work is impossible. However, in no case where silver was absent has precipitation of the zinc above the copper been noticed, so it would seem that an upper yellow line or band may, in a cupro-zinc alloy, be taken as a suggestion of the presence of silver. One important observation noted in all experiments with brazing alloys after use was that contamination of the metal with iron during sampling was immediately evident by a comparatively broad band of the black ferric hydroxyquinolate complex appearing beneath the coloured non-ferrous zones. Most samples showed a thin black line at this position, rarely more than 0·5 mm. thick, and it was considered that this was due solely to diffusion of iron from the two metals being joined into the brazing alloy. The band referred to above as being due to contamination in sampling was always considerably greater than this and was seldom less than 2 mm. in length.

Results obtained for the two types of brazing alloy considered here, i.e., copper-nickel-zinc and copper-zinc-silver, in which only zinc and silver respectively were considered, were as follows, one typical case only for each type of alloy being quoted. Both cases were calculated by comparison with a solution in nitric acid and buffered to the appropriate pH with sodium acetate, containing 2 mg. of the " as received " alloy per ml. In some cases, better results for the cupro-nickel alloy have been obtained, however, by replacing the nitric acid by sulphuric before the addition of sodium acetate.

Copper-nickel-zinc alloy

Initial zinc content	21·75 %
Final ,, ,, 1st bar	16·50 %
,, ,, ,, 2nd ,, ...	14·50 %
,, ,, ,, 3rd ,, ...	14·00 %
,, ,, ,, 4th ,, ...	12·50 %

Copper-zinc-silver alloy

Initial silver content	2·18 %
Final ,, ,, 1st bar	2·00 %
,, ,, ,, 2nd ,, ...	1·75 %
,, ,, ,, 3rd ,, ...	1·9 %
,, ,, ,, 4th ,, ...	1·75 %
,, ,, ,, 5th ,, ...	2·00 %
,, ,, ,, 6th ,, ...	1·8 %

The use of a chromatographic adsorption technique on columns of 8-hydroxyquinoline would seem from the foregoing to be a useful method of dealing with the type of problem mentioned, where one is concerned with the sampling and ultimate analysis of the thin line of metal left at a brazing joint. In those cases quoted it rendered possible the required analysis without the necessity for destruction of the finished component. Even in those cases where the final product could be damaged completely, only a few milligrams of sample free from contamination with the surrounding ferrous material were available, and a micro or semi-micro technique would have been

necessary in any case. Nevertheless, the method has severe limitations, particularly in the field of analysis of ferrous materials. In the non-ferrous field the greatest problem would appear to be that of distinguishing between complexes, due to the fact that so many are yellow in colour, and none except zinc and magnesium have distinguishing fluorescence colours.

Other difficulties seem to be largely of a mechanical nature such as diffuse banding and channelling. In the opinion of the present author these faults would seem largely due to the solvent action of either the initial solution or the wash liquid on the oxine of the column. This would seem to prevent the use (so often suggested) of dilute acetic acid as a developing medium. The writer has attempted many times, especially with samples of ferrous materials, to use this method of development but without success owing to the production of comparatively large holes in the side of the column and the consequent mechanical displacement of the zones already produced. There is no doubt whatever that the black iron complex, so devastating in work with ferrous materials, could be washed through the column before the other complexes with about 5 % acetic acid were it not for the fact that the column itself is literally washed through as well.

The use of ammonia is complicated also by its solvent action on oxine and, since so many metallic oxine precipitates are remarkably stable at high pH values, it would seem hardly likely that this reagent would promote more satisfactory banding, except possibly with iron, tungsten and molybdenum. Thus, it appears fairly certain that the optimum pH value for work with columns of this reagent must be very close to the neutral point.

As a result of the previously mentioned experimental work it would now seem possible tentatively to extend the original order of separation on 8-hydroxyquinoline columns as suggested by Erlenmeyer and Dahn to include also manganese, silver and molybdenum, whose positions have been ascertained with some degree of certainty. Additionally, aluminium and magnesium can be added to this list in approximate positions as a result of attempts to use their distinctive fluorescence colours to separate molybdenum and cobalt, when fixing the position of the former metal, the order of separation now given being :

VO_3^{--}	grey
Mn^{++}	green
WO_4^{--}.. ..	yellow
Ag^{+}	yellow
Cu^{++}	green
Bi^{++}	yellow
Ni^{++}	yellow
MoO_4^{--} ..	orange
Al^{+++}	green (bright green fluorescence)
Co^{++}	reddish
Zn^{++}	yellow (bright green fluorescence)
Mg^{++}	green (light blue fluorescence)
Fe^{+++}	black
UO_2^{++}	orange

Further extension of this list appears improbable unless some satisfactory method of separating the yellow complexes can be devised.

The author is indebted to the directors of Messrs. W. Fearnehough Ltd. for permission to publish the work mentioned here.

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THEORY OF CHROMATOGRAPHY

VIII. The Separation of Lithium Isotopes by Ion Exchange and of Neon Isotopes by Low-temperature Adsorption Columns

BY E. GLUECKAUF, K. H. BARKER AND G. P. KITT

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An essential condition for the successful separation of isotopes (or of any substances of very similar sorption characteristics) is that the chromatographic boundaries between the species to be separated remain as sharp as possible. The effects of such experimental features as grain size, flow-rate, concentration, pore space, diffusion constants in the liquid and in the sorbent phase are discussed.

Preliminary experiments to test these conceptions have been made by separating (*a*) lithium isotopes by ion-exchange columns and (*b*) neon isotopes by low-temperature adsorption columns. Though in the case of lithium almost pure ^7Li has been obtained, the quantity of material enriched has been far less than expected. A similar observation was made by Taylor and Urey in 1938.

In the case of neon separations the effect of variations in experimental conditions on the enrichment is well represented by the theoretical equations, and the absolute values of the " theoretical plate " height calculated from the observations agree with the theory.

Symbols

a = numerical factor connected with the form of the grains ; for spherical grains = $1/4\pi^2$.
A = g. sorbent per cm. of column length = $\bar{x}/L$.
c = $\varphi(q)$ = the concentration of the solute, or in case of two solutes, of the following solute (mole/l.).
C = the total concentration of all solutes.
d = grain diameter (in cm.).
D_s and D_l = diffusion coefficients of the solute in the solid sorbent and in the liquid phase respectively.
D_{Ne} = self-diffusion coefficient of neon.
$f(c)$ = $f^*(c) + \alpha c$ = amount of solute per g. sorbent including the pore space.
F = eluate velocity in ml. per sec.
L = length of the whole column (in cm.).
q^* = amount of solute adsorbed when not in equilibrium with solution.
Q = total amount of all solutes adsorbed per g. sorbent.
s = density of sorbent material.
t = time (sec.).
v = volume of eluate from bottom of the column (in ml.).
V_t = threshold volume = $Q \cdot \bar{x}/C$.
x = distance from top of column (in g. sorbent).
$\bar{x}$ = length of column (for elution experiments) or otherwise : length of column passed by the frontal band, i.e., position of the front of solute 1 = Cv/Q (in g.).
$\bar{\bar{x}}$ = hypothetical position of the sharp front of solute 2, if no disturbances were active.
z = distance from top of column (in cm.).
α = pore space in ml. per g. sorbent.
δ = thickness of laminar solvent layer through which diffusion takes place.
Δ = disturbance factor, representing all disturbances : $\Delta/2A$ corresponds to the height of a " theoretical plate."

$\square$ = free cross-sectional area of the column through which the solvent flow passes.

η = relative enrichment $= \frac{c^\circ - c}{c^\circ}$.

The superscript $^\circ$ refers to the original concentration of a solute. The subscripts $_1$ and $_2$ refer to the less and more adsorbed solute respectively.

In the theory of chromatography it has become customary to introduce simplifying assumptions which render it possible to deal quantitatively with certain aspects of chromatographic procedure. In order to study the effects of the adsorption isotherm on the distribution of solutes in the column it has been necessary to assume that the grain size of the adsorbent is infinitely small and that no disturbing effects due to diffusion or non-equilibrium occur in the column. On the other hand, where such disturbing effects have been treated workers have usually dealt only with a linear isotherm, for which it is possible to solve the differential equation, although certain features occurring with the normal non-linear isotherms are lost.

The case of non-equilibrium due to grain-diffusion has been discussed for non-linear isotherms, but the discussion was restricted to the effect on self-sharpening front boundaries, such as occur in frontal analysis and displacement development (see Tiselius [1]). The reason for this was that these boundaries reach, after an initial period, a final shape and gradient, where the disturbing effects and the self-sharpening tendency due to the curved isotherm just balance each other. This final shape is accessible to calculations.

Table I gives a survey of these partial problems and of the authors who have dealt with them, and at the same time shows the gaps which still exist in our present knowledge.

The main gap in our knowledge is connected with the effect of disturbances on the shape of the rear boundary of chromatograms in the case of non-linear isotherms. This problem depends on the solution of differential equations of the type:

$$\left(\frac{\partial f(c)}{\partial v}\right)_x + \left(\frac{\partial c}{\partial x}\right)_v - \Delta \left(\frac{\partial^2 c}{\partial x^2}\right)_v = 0 \text{ (Disturbance type A, B), .} \qquad (1)$$

and

$$\left(\frac{\partial f(c)}{\partial v}\right)_x + \left(\frac{\partial c}{\partial x}\right)_v + \Delta' \left(\frac{\partial^2 c}{\partial x \partial v}\right) = 0 \text{ (Disturbance type C), .} \qquad (2)$$

which has so far not been possible, except in the case of linear isotherms.

The recent developments of the theory, however, make it possible to discuss with confidence the influence of some experimental factors on the efficiency of chromatographic separations. Such factors are grain size, flow rate, temperature, diameter of column, uniformity of packing and quantity of sorbent. The last two need not be discussed here. The need for uniform packing has been stressed from the very beginning of the chromatographic technique. The quantity of sorbent required for complete separation is a function of the quantity of the solutes to be separated and of the multiple adsorption isotherm (see [2 3 4]).

[1] Tiselius, *Arkiv. Kemi, Min. Geol. A*, 1943, 16, No. 5, paper 18.
[2] Glueckauf, *Proc. Roy. Soc. A*, 1946, **186**, 35.
[3] Coates and Glueckauf, *J. Chem. Soc.*, 1947, 1308.
[4] Glueckauf, *J. Chem. Soc.*, 1947, 1321.

The influence of the other four factors is somewhat complex and it is an advantage to deal rather with the fundamental phenomena of

(i) effects of finite grain size,
(ii) diffusive mixing in longitudinal direction,
(iii) non-equilibrium (due to slow diffusion in the particles),
(iv) non-equilibrium (due to slow diffusion in the liquid).

The best way to demonstrate the effect of these phenomena on the efficiency of separation is to discuss their effect on the gradient of self-sharpening front boundaries between two solutes for the case of displacement development.

TABLE I

SURVEY OF THEORETICAL LITERATURE

		Ideal Column	Disturbing Effects A Grain size B Diffusion C Non-equilibrium (grains) D Non-equilibrium (solution)
Linear Isotherm	Single Solute	(5)	A (6) (7) D (8)
	Two Solutes	(5)	A (7) this paper
Non-linear Isotherm	Single Solute	(9) (10) (11)	C. (12), A, B, C, D. this paper; discussion limited to front boundary. Diffuse rear boundaries: unsolved
	Two Solutes	(9) (2) (3) (4) (13)	A, B, C, D: this paper; discussion limited to frontal interboundary. Diffuse rear boundaries: unsolved
Calculation of non-linear isotherm from elution curves		(9) (14) (15) (16)	A, B: (17)

A. Boundary Considerations for the Case where a Frontal Band of Pure Solute 1 has been Formed.—It is apparent in displacement development where the separated bands do not move away from each other that the efficiency of the final separation depends entirely on the width of the separation zone, which itself is dependent on the gradient of the boundaries.

For the gradient dc/dx in the column and dc/dv of the elution curve of the slower of the two solutes we obtain the following equations.

In the case of the disturbance phenomena (i), (ii), (iii):

$$-\left(\frac{\partial x}{\partial c}\right)_v = \frac{c^\circ}{f(c^\circ)}\left(\frac{\partial v}{\partial c}\right)_x = \frac{(K_1 \text{ or } K_2) + K_3}{c^\circ f(c)/f(c^\circ) - c}, \quad . \quad . \quad (3)^*$$

[5] Wilson, *J. Amer. Chem. Soc.*, 1940, **62**, 1583.
[6] Martin and Synge, *Biochem. J.*, 1941, **35**, 1385.
[7] Mayer and Tompkins, *J. Amer. Chem. Soc.*, 1947, **69**, 2866.
[8] Boyd, Myers and Adamson, *J. Amer. Chem. Soc*, 1947, **69**, 2836, 2849.
[9] De Vault, *J. Amer. Chem. Soc.*, 1943, **65**, 532.
[10] Weiss, *J. Chem. Soc.*, 1943, 297.
[11] Glueckauf, *J. Chem. Soc.*, 1947, 1302.
[12] Glueckauf and Coates, *J. Chem. Soc.*, 1947, 1315.
[13] Glueckauf, This Discussion.
[14] Weil-Malherbe, *J. Chem. Soc.*, 1943, 303.
[15] Glueckauf, *Nature*, 1945, **156**, 748.
[16] Glueckauf, *Nature*, 1947, **160**, 301.
[17] Glueckauf, *J. Chem. Soc.*, 1949, 3280.

* Both K_1 and K_2 take account of mixing effects in more or less the same space so that the larger of the two ought to be used only.

and in the event of (iv):

$$= \frac{K_4}{c - \varphi\left(\frac{c}{c^\circ} \cdot f(c^\circ)\right)} \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

As is shown in the appendix in detail, one can derive for the value of the constants,

$$K_1 = \tfrac{1}{2}dA \, , \quad . \quad . \quad . \quad . \quad . \quad (3a)$$

$$K_2 = \frac{\alpha D_l A^2}{\sqrt{2}\,F} \, , \quad . \quad . \quad . \quad . \quad (3b)$$

$$K_3 = \frac{ad^2Ff^*(c^\circ)c^\circ}{D_s\,(f(c^\circ))^2} \, , \quad . \quad . \quad . \quad . \quad (3c)$$

$$K_4 = \frac{\delta dFs}{6\,D_l} \, , \quad . \quad . \quad . \quad . \quad (4a)$$

where for turbulent flow δ is proportional to $1/F$ and for laminar flow (and small grain sizes) $\delta \simeq ad$.

In the case of isotope separation the variable boundary functions (in the denominator) become simplified and identical, and we obtain

$$-\left(\frac{\partial c}{\partial x}\right)_v = \frac{f(c^\circ)}{c^\circ} \cdot \left(\frac{\partial c}{\partial v}\right)_x = \frac{(K-1)}{\Delta} \cdot \frac{c_2}{C} \cdot (c^\circ_2 - c_2), \quad . \quad (5)$$

where C is the total concentration of both isotopes and c_2 that of the slower isotope, K is the separation factor and $\Delta = (K_1 \text{ or } K_2) + K_3 + K_4$. In the case of ion exchange K is the equilibrium constant, and for adsorbents it is the ratio of the adsorption coefficients of the two isotopic solutes.

Eqn. (5) shows that, while boundary phenomena may be of secondary importance in the separation of compounds with large separation factors, they attain a first-rate importance, when K is small. Except for H, He and Li isotopes, separation factors are always less than 1·01.

If numerical values are inserted into eqn. (3) and (4) one can see that for different types of chromatographic technique different terms will be predominant. In *ion exchange*, the predominant term is usually due to *non-equilibrium* (K_3 or K_4), though we have been able to reduce grain size and flow speed to such an extent that the diffusion term K_2 becomes dominant, which results in a not very pronounced optimum as regards sharpness of the boundaries. An increase of temperature, increasing the values of D_s (and D_l), is, as a rule, beneficial in the case of dominant non-equilibrium, and this is borne out by experiments (see [1 7 12]).

In the chromatography of *organic substances* on impermeable adsorbents, where adsorption equilibrium is almost instantaneous, the predominant term is due to the effect of finite grain size K_1. Thus the separation of substances which are very difficult to separate should therefore be considerably improved by reduction of the grain diameter.[13] Accordingly one would expect here little effect (within limits) from variations of flow velocity; this corresponds to general experience. The effect of the diameter of the column and of the particle size, in this case, was well shown by Weil-Malherbe [14] (Fig. 3 and 4), and whilst his experiments (non-linear isotherm + disturbance + complete band development) cannot be interpreted quantitatively, the effect observed corresponds to what one would expect from eqn. (3*a*).

Apart from excessively low flow velocities it is only in *gas chromatography* and possibly in partition chromatography on a paper adsorbent that the

diffusion term attains major importance and here the flow velocity cannot be reduced beyond certain limits. In the first case a reduction of temperature should be of double benefit, since not only will the separation factor be increased, but D_l (and D_s) will also be reduced.

DIAMETER OF COLUMN.—This is defined by the factor A (g. adsorbent per cm. of column) which enters K_1 and K_2. It is therefore important in those cases where the finite grain size effect is predominant, e.g., in the separation of organic substances by surface-active sorbents. A reduction of the tube diameter combined with increased length will therefore be an advantage. This corresponds to the general practice when difficultly separable substances are concerned.

B. Frontal Phenomena in the Case of Partial Enrichment only. —In most cases of isotope separation a frontal band of a pure species does not usually appear but only smaller or larger enrichments of the less adsorbed isotope are achieved. In this case the form of the boundary is not given by eqn. (5) which applies only for the " final " state, i.e., after appearance of an S-shaped boundary and of a pure frontal band If there is only moderate enrichment, eqn. (1) for the more strongly adsorbed isotope c_2, if this is only as a small proportion of the total concentration C_1, becomes

$$\frac{QK}{C} \cdot \left(\frac{\partial c_2}{\partial v}\right)_x + \left(\frac{\partial c_2}{\partial x}\right)_v - \Delta \left(\frac{\partial^2 c_2}{\partial x^2}\right)_v = 0, \quad . \quad . \quad (6)$$

as

$$f(c_2) = \frac{QK}{C} \cdot c_2/(1 + (K-1) \cdot c_2/C), \quad . \quad . \quad (7)$$

where $(K-1)c_2/C \ll 1$ and can be neglected for $c_2 \ll C$.

Here Q is the total amount adsorbed per g. adsorbent in equilibrium with C; C is the total concentration of both isotopes; K is the separation factor.

$$K' = \frac{f(c_2)\ (C - c_2)}{(Q - f(c_2)) \cdot c_2} \text{ and } \bar{x} = v\ Q/C.$$

In the following the subscript 2 is dropped. The boundary conditions are $c = c^\circ$ for $v = 0$, $x < 0$,

and

$$\frac{QK}{C} \int_0^{\bar{\bar{x}}} (c_0 - c) \cdot dx = v \cdot c_0 \cdot (K-1),$$

which is a condition for the conservation of mass of solute 2 within the boundaries of the total solute band. Instead of $\int_0^{\bar{\bar{x}}} \cdot$ we can write with an almost infinitesimal error $\int_{-\infty}^{\bar{\bar{x}}}$. The solution for eqn. (6) under these conditions has been obtained by Dr. B. Davison (A.E.R.E.):

$$\frac{c}{c^\circ} = \tfrac{1}{2}\left[1 - \operatorname{erf}\frac{(x - \bar{\bar{x}})}{2\sqrt{\bar{\bar{x}}\Delta}}\right] - (K-1)\sqrt{\frac{\bar{\bar{x}}}{\Delta\pi}} \cdot \exp\left\{-\frac{(x-\bar{\bar{x}})^2}{4\bar{\bar{x}}\Delta}\right\} +$$

$$\tfrac{1}{2}\left[1 - \frac{(K-1)(x-\bar{\bar{x}})}{\Delta} + 2(K-1)^2\frac{x}{\Delta}\right]\left[\exp\left\{(K-1)^2\frac{\bar{\bar{x}}}{\Delta} - (K-1)\frac{(x-\bar{\bar{x}})}{\Delta}\right\}\right]$$

$$\times \left[1 + \operatorname{erf}\left\{\frac{(x-\bar{\bar{x}}) - 2(K-1)\bar{\bar{x}}}{2\sqrt{\bar{\bar{x}}\Delta}}\right\}\right] \quad . \quad . \quad . \quad (7)$$

where $\bar{\bar{x}} = \bar{x}/K$, which is the point where the front boundary of solute 2 would be in the case of $\Delta = 0$, i.e., if there were no disturbance. For

values in the vicinity of the front boundary in the case of small enrichment, i.e., for $\bar{x} < \Delta/(K-1)^2$ this equation simplifies to

$$\frac{c^\circ - c}{c^\circ} = 2(K-1)\sqrt{\bar{\bar{x}}/\Delta\pi} - (K-1)^2\bar{\bar{x}}/2\Delta - (K-1)(\bar{x}-x)/\Delta \,. \quad (8)$$

Eqn. (8) gives the possibility of calculating both K and Δ from the experimentally observed slope and the enrichment at the front boundary. Using the symbols η for the relative change of the isotope ratio and S for the gradient of the latter with respect to x, both referring to the front of the band, we obtain

$$\bar{\eta} = \left[\frac{c^\circ - c}{c^\circ}\right]_{\bar{\bar{x}}} = 2(K-1)\sqrt{\bar{\bar{x}}/\Delta\pi} - (K-1)^2\bar{\bar{x}}/2\Delta, \quad . \quad (9)$$

$$S = \left[\frac{\partial\left(\frac{c^\circ - c}{c^\circ}\right)}{\partial x}\right]_{V_t,\bar{\bar{x}}} = \frac{K-1}{\Delta},$$

or in the case of eluted solutes

$$S' = \left[\frac{\partial\left(\frac{c^\circ - c}{c^\circ}\right)}{\partial v}\right]_{V_t,\bar{\bar{x}}} = \frac{C}{Q}\cdot\frac{(K-1)}{\Delta} \,. \quad . \quad . \quad (10)$$

Solving for $(K-1)$ and for Δ (whereby $\bar{\bar{x}}$ in eqn. (9) can be considered equal to $\bar{x}$) gives

$$(K-1) = \frac{\pi\bar{\eta}^2}{4vS'}\left(1 + \frac{\pi\bar{\eta}}{4} + \frac{5}{64}\pi^2\bar{\eta}^2 \ldots\right), \quad . \quad . \quad (11)$$

$$\Delta = \bar{x}\pi\left(\frac{\bar{\eta}}{2vS'}\right)^2\left(1 + \frac{\pi\bar{\eta}}{4} + \frac{5}{64}\pi^2\bar{\eta}^2 \ldots\right), \quad . \quad . \quad (12)$$

where the last term in the bracket is no longer of importance.

If K is known, it is possible to calculate Δ from any observation near the front boundary by transforming eqn. (8) into

$$\Delta = \left[\frac{4(K-1)^2\bar{x}}{\eta^2\pi}\right] - \left[(K-1)^2\frac{\bar{x}}{\eta}\right] - \frac{2(K-1)\bar{x}(v - V_t)}{\eta V} \quad . \quad (13)$$

C. Application to the Separation of Isotopes.—It is observed from eqn. (5) that, in the case of isotopes, due to the small factor $(K-1)$, the enrichment zone extends greatly in length and becomes the most important part of the chromatogram; moreover in the case of isotopes a frontal band of pure species is not usually obtained. For a better understanding, it is useful to draw a comparison between this enrichment zone and a distillation column.

Both are based on the equilibrium distribution of two or more substances between two phases. In distillation these phases are liquid and gas, in chromatography they are liquid and solid. In the first case we have two mobile fluids, which easily lend themselves to a counter-current process owing to their physical characteristics. This counter-current leads to a cascading of the single-stage equilibrium enrichment.

In chromatography we have only one fluid. In order to produce a counter-current, it is necessary, as the solid phase cannot move, that the "fractionation column" is shifted with respect to the solid phase, i.e., the bands of solutes pass through the column. The parallelism, between distillation and chromatography becomes complete, if we consider the

enrichment-zones of the chromatographic band as "mobile fractionation columns." If the observer travels along with the enrichment zone in such a way that he always stands at the sharp front boundary, then the situation is exactly analogous to a distillation column with the observer standing at the reflux head. There are many similarities in the equations and $\Delta/2A$ in eqn. (1) corresponds to the height of a "theoretical plate" in a distillation column.

Experimental

Experiment on the Separation of the Lithium Isotopes.—In order to obtain the smallest possible value of Δ experiments were made using :

(*a*) an ion-exchange resin, Zeo-Karb H.I., for which a diffusion coefficient of about 10^{-6} cm.^{2}/sec. was obtained (faster ion exchangers are now available) ;

(*b*) a fairly uniform grain size of about $1{\cdot}5 \times 10^{-3}$ cm. diam., which was obtained by grinding and subsequent fractionation by air elutriation ;

(*c*) a flow-rate of only 4×10^{-4} ml./sec.

Other experimental data were :

Amount of exchanger, 45 g. $= \bar{x}$.
Original solution, N lithium acetate.
Threshold volume, 162 ml. $= \left(\frac{Q}{C} \cdot \bar{x}\right)$.
Pore space ratio (α), 0·3 ml./g. exchanger.
Length of column, 90 cm. $= \bar{x}/A$.

Samples of $\frac{1}{3}$ ml. (= 3 drops) were collected and were analyzed for isotopic composition.

Several runs were made with a number of identical tubes, and it could be observed that by far the most important factor influencing the efficiency of separation was any distortion of the horizontal boundary. Inclined and serrated boundaries give rise to lateral diffusion, the effect of which cannot be assessed quantitatively. As the column of the Zeo-Karb changes to a paler hue at the front boundary of the Li acetate solution, this feature could be checked visually. Only one run gave a perfectly straight and horizontal boundary and the data of this run are discussed below in detail. As would be expected the other runs gave much lower changes of the isotopic ratio in the eluted solution.

Determination of the Isotopic Ratio of Lithium.—A nuclear reaction was used for the isotopic analysis. When ^{6}Li is bombarded with slow neutrons, heavy ionizing particles are emitted according to the reaction :

$$^6_3\mathrm{Li} + ^1_0\mathrm{n} \rightarrow ^7_3\mathrm{Li}^* \rightarrow ^4_2\mathrm{He} + ^3_1\mathrm{H} + 4{\cdot}6 \text{ MeV},$$

while no comparable reaction occurs with $^7_3$Li.

The ranges for the disintegration products in air are 1·5 cm. for the ^{4}He and 5·5 cm. for ^{3}H particles. As the two particles are emitted in opposite directions the chances that one of them enters the ionizing chamber and produces an ionization sufficient for triggering the counting device are very large. Thus the number of disintegration particles counted under neutron irradiation gives a convenient measure of the relative amount of the lighter isotope. By reference to a standard of unseparated lithium salt with 7·5 % ^{6}Li, these observations can be expressed as percentage enrichments or depletions.

After some preliminary experiments it was decided to employ an air-filled ionization chamber in preference to either a fast, argon-filled chamber or a proportional counter. This method has the advantage of simplicity and reliability of operation and greatly facilitates the changing of specimens. The efficiency of detection of the disintegration particles can be made greater than in the other method by actually incorporating the specimens in the chamber. As shown in Fig. 1 the target disc D constituted one plate of the ion chamber.

To minimize the background due to γ-rays from the Ra-Be neutron source, the volume of the chamber was kept as small as possible compatible with a good signal to noise ratio. The chamber was of the parallel plate type and

had a cylindrical guard ring G surrounding the collecting electrode E'. Polythene insulation was used and the outer cylindrical cover E was connected to the H.T. electrode. This cylindrical shape facilitated the insertion of the chamber in a paraffin block employed to slow down the fast neutrons. The electrode spacing was 4 mm. and the applied H.T. voltage 2000 V, giving a collecting field of about 5000 V/cm. throughout the series of experiments. The effective volume of the chamber was about 1·5 cm.³.

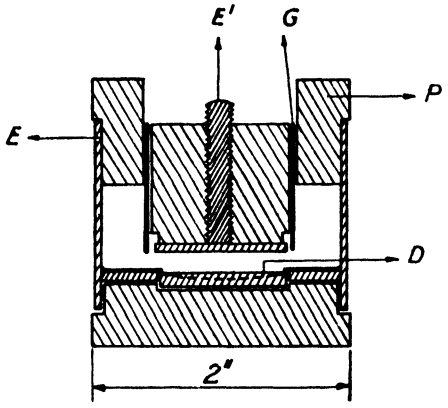


FIG. 1.—Ion chamber for observing the particle emission from ^{6}Li during neutron irradiation.

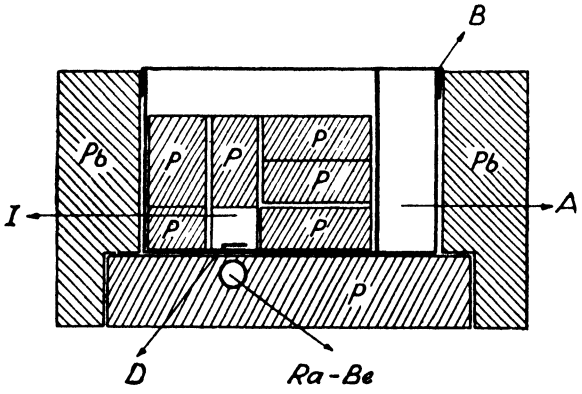


FIG. 2.—Irradiation arrangement.
I = Ion chamber.

Fig. 2 shows the dispositions of Ra-Be source, paraffin moderator P and lead shielding Pb. At least 10 cm. paraffin surrounded the source in every direction. The radium was pushed into a lead tunnel when the specimens were changed by hand. No lead was required between the source and chamber to cut down γ-radiation, which were about 2–3 cm. apart.

For convenience the pre-amplifier A was contained in the same screened box S as the ion chamber, to keep the effective collecting capacity at a low value. This was followed by a five-stage resistance-capacity coupled amplifier of conventional design, with a negative feedback. The filaments of the amplifier were supplied in series from a stabilized source of D.C. in an attempt to improve the gain stability.

The characteristics of amplifiers were :

Noise level (root mean square), 4μV.
Equivalent number ion pairs at input, ~1000.
Time constant of differentiating stage, 200 μ sec.
Pulse growth time of amplifier, 5 μ sec.

The amplifier was followed by a discriminator, a scale-of-100 counting unit and a cathode ray oscilloscope for visual monitoring of the amplifier output. The introduction of the neutron source increased the noise level by roughly a factor 2. The target discs were made circular with a slightly concave surface to counteract surface tensional forces which tended to cause a heavier deposit around the circumference of the discs. The reverse side of the target was flat and rested in a recess bored in a block of polythene.

The lithium acetate samples were converted into sulphate and a solution of approximately 5 mg. of the latter was evaporated on the circular target disc of 1 in. diam. All specimens were measured several times in alternation with blanks, with natural lithium sulphate samples and with no source present; corrections were made for background, contamination of the sample by natural radioactive substances, differences in weight, etc.

Results and Discussion

In the most successful run, the front boundary of the "total lithium" was exceedingly sharp. Seven drops after the first spectroscopically noticeable trace of Li the concentration had risen to 88 % of the final value.

The result of the isotopic analysis is shown in Table II.

TABLE II

DATA FROM THE CHROMATOGRAPHIC SEPARATION OF LITHIUM ISOTOPES

Sample No.	v-162	C	$\frac{^6Li}{^6Li + ^7Li} = \frac{c_2}{C}$	Milli-equivalent ^{7}Li enriched
	< 0	Spectr. pure		
1	0–0·33	0·12	0·005 ⎫	0·04
2	0·33–0·67	0·59	0·014 ⎬ ± 0·01	0·16
3	0·67–1·00	0·88	0·050 ⎭	0·10
4	1·00–1·33	0·92	0·067	0·03
5	1·33–1·67	0·96 ⎫		Est. 0·01
6	1·67–2·00	1·0 ⎬	0·075 = c/C	Total 0·34
		1·0 ⎭		

In Table II Col. 2 gives the volume of eluate after the lithium threshold (162 ml.), Col. 3 the concentration C of total lithium in the eluate sample, Col. 4 the ratio $^6Li/(^7Li + ^6Li)$ analyzed by the described nuclear reaction method, and Col. 5 the amount $\Delta\mu_1$ of less adsorbed isotope (^{7}Li) by which the frontal region has been enriched. This is given by

$$\Delta\mu_1 = \Delta v\,(c_1 - c_1^\circ\, c_2/c_2^\circ) = \Delta v\, C(c_2^\circ - c_2)/c_2^\circ.$$

These data disagree considerably with what one would expect from the theory.

First of all, the total amount μ_1 of ^{7}Li separated is far too small. One would expect this to be

$$\mu_1 = (v - \alpha\bar{x})\,.\,c_1^\circ(K - 1)/K, \text{ (see [2,5])}$$

i.e., 2·8 milli-equivalents instead of 0·34 found.

Secondly, the gradient of the eluted boundary (at the centre, where $c_2 = c_2^\circ/2$) is acc. to eqn. (5),

$$-\left[\frac{\partial c_2}{\partial x}\right]_{c_2^\circ/2} = \frac{(K-1)}{\Delta} \cdot \frac{c_2(c_2^\circ - c_2)}{C} = \frac{(K-1)\cdot(c_2^\circ)^2}{4\Delta}.$$

Using $K = 1{\cdot}022$ (see [18]), $c_2^\circ = 0{\cdot}075$ and the experimentally found $(\partial c_2/\partial x)_{\text{max}} = 0{\cdot}30$ then $\Delta = 1{\cdot}0 \times 10^{-4}$ g. (or 2×10^{-4} cm. length).

An estimate for the value of $\Delta = (K_1 \text{ or } K_2) + K_3 + K_4$ using the values

$$\alpha = 0{\cdot}3 \text{ ml./g.},$$
$$D_l = 5 \times 10^{-6} \text{ cm.}^2\text{/sec.},$$
$$D_s = 10^{-6} \text{ cm.}^2\text{/sec.},$$

leads to

$$K_1 = 4 \times 10^{-4} \text{ g.} \qquad K_2 = 7 \times 10^{-4} \text{ g.}$$
$$K_3 = 3 \times 10^{-6} \text{ g.} \qquad K_4 = \ < 10^{-6} \text{ g.}$$
$$\Delta \simeq 7 \times 10^{-4} \text{ g.}$$

It would appear from this that the assumptions made for K_1 and K_2 were too conservative. But it would be premature to draw conclusions until further experiments have been made. A similar reserve must also apply to remarks on the deficiency of ^{7}Li at the enriched front.

One might suspect that this result is due to a small proportion of an irreversible adsorption or exchange taking place at the front boundary, when the Li ions first contact the H^+ exchanger. In this way a small proportion of the enriched front would constantly be stripped off. Some support for this assumption may be gained from the (single) observation that no enrichment of ^{6}Li was observed at the rear of the Li band when the latter was eluted with HCl. If anything, a slight deficiency of ^{6}Li was observed, which would be the case if irreversibly adsorbed matter from the frontal region were eventually released by the stronger adsorbed H^+ ions.

It may be of interest to recall the early experiments of Taylor and Urey [18] on the chromatographic separation of the lithium isotopes in columns up to 30 m. in length. The large grain size ($\sim$0·05 cm.) and flow-rate ($\sim$0·05 ml./sec.) used precluded any spectacular enrichments, as, in spite of its length, the column cannot have possessed more than 1000–2000 " theoretical plates." But both the enrichment obtained and the actual amount of enriched material fell far short of what one would calculate from this figure and from the value of $K = 1{\cdot}022$. In the present experiment, too, the amount of isotope separated was by one order too small.

There is an alternative to the irreversible adsorption hypothesis which also might explain the failure of these separations. It might be that the separation factor of the Li isotopes on a Li^+ saturated exchanger might be ten times smaller than the separation factor on an exchanger which is substantially in the Na^+ or H^+ form. It is the former which is effective in the chromatographic separations as carried out, but it is the latter which was independently determined by Taylor and Urey.

Some preliminary experiments showed that such factors can be operative. Thus a mixture of $CuSO_4$ and $MnSO_4$ did not separate when passed through a column of Zeo-Karb H.I., but gave some separation when 10 % of H_2SO_4 was added to the mixture. Experiments to study these phenomena are planned.

[18] Taylor and Urey, *J. Chem. Physics*, 1938, **6**, 429.

D. Separation of Neon Isotopes by Chromatographic Separation on Charcoal at − 196° C

Experimental.—The essential part of the apparatus is shown in Fig. 3 (*a*), (*b*). It consisted of a tube filled with charcoal, into one end of which neon of atmospheric pressure was fed. The other end led to a distributing system supplying a number of sample tubes which were analyzed with a mass spectrograph. The small dead space in the capillary leading from the charcoal to the stopcock was further reduced by filling it with an almost fitting glass rod. The charcoal column was filled with nitrogen and left in communication with a container of atmospheric neon. A Dewar vessel with liquid nitrogen was then slowly raised (by a motor), thus progressively cooling the charcoal tube from the rear end. This caused a constant inflow of neon into the charcoal up to the end of the low-temperature region, and an adsorption boundary forms which in every respect can be compared with a chromatographic boundary. The small amount of nitrogen originally added is more strongly adsorbed and disappears progressively as the charcoal tube is immersed. When the liquid N_2 level had reached the top of the charcoal tube samples were taken.

A number of experiments were made varying the experimental conditions as described in Table III. Run 6 and the preliminary runs were made with a column shown in Fig. 3 (*a*), while subsequent experiments were made on the type of Fig. 3 (*b*), using thinner and longer tubes. In run 10 and 11 a different technique was used. The charcoal column was immersed from the start in liquid nitrogen and then filled with helium. Then through the column was passed a stream of neon which replaces the less adsorbed helium, and advances with a strongly self-sharpening front boundary. The arrival of the neon at the end of the charcoal column was noticed by the colour of a high-frequency discharge, and the front sample of the neon band was analyzed.

TABLE III

EXPERIMENTS ON NEON ISOTOPE SEPARATION

o.	$\bar{x}$	L	φ diam. of column	d diam. of grains	V_t	Time of experiment t	Sample 1		Sample 2		Sample 3	
							$v - V_t$ ml.	$\frac{c_2^\circ - c_2}{c_2^\circ}$	$v - V_t$ ml	$\frac{c_2^\circ - c_2}{c_2^\circ}$	$v - V_t$ ml.	$\frac{c_2^\circ - c_2}{c_2^\circ}$
	g.	cm.	cm.	cm.	ml.	sec.		× 100		× 100		× 100
5	4·5	25	0·7	0·012	234	840	0–0·4	5·9	0·4–1·0	4·8	1·6–2·2	4·1
7	3·0	52	0·3	,,	156	1320	lost		0·3–0·6	6·9	Sample 4 of No. 6	
8	,,	,,	,,	,,	,,	,,	0–2	7·1	2·0–2·1	5·0	3·2–3·8	3·9
9	3·4	47	,,	0·017	197	1200	0–0·3	8·4	0·3–0·6	8·0		
0	,,	,,	,,	,,	,,	780	0–0·5	14·9				
1	,,	,,	,,	,,	,,	4900	0–0·6	5·9				

Discussion

The combined data of run 7, 8 and 9 render possible a calculation of $(K - 1)$ by means of eqn. (11) and (12). With a lesser accuracy (on account of the scatter of the points) the data of run 6 can also be used. From run 7, 8 and 9 we obtain $K = 1{\cdot}0021$; from run 6, $K = 1{\cdot}0019$.

Using the value of $K = 1{\cdot}0021$ we can calculate by means of eqn. (13) the values of Δ for the various runs from the change observed in the isotopic ratio and obtain the figures in Col. 2 of Table IV. $\Delta = K_2$ can also be calculated from eqn. (3*b*). (As mentioned before, in gas chromatography

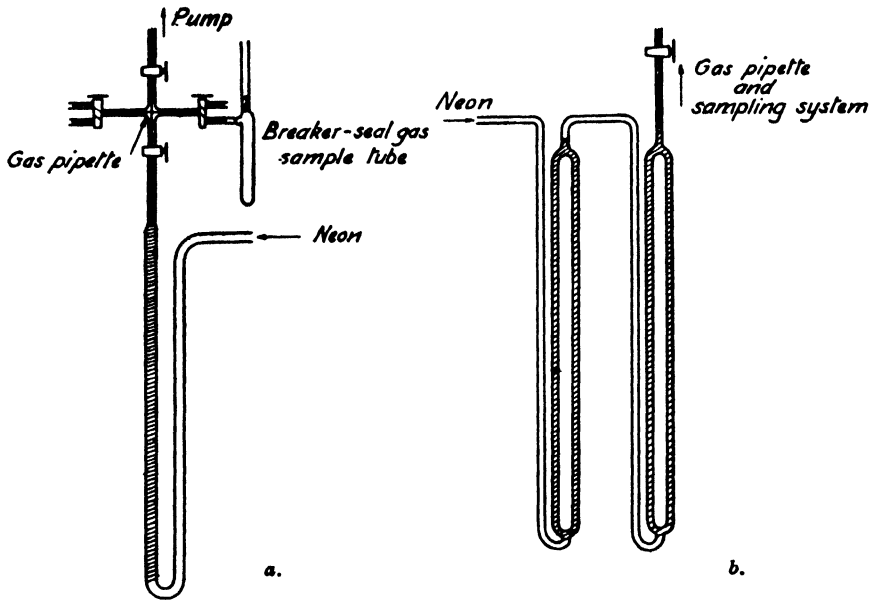
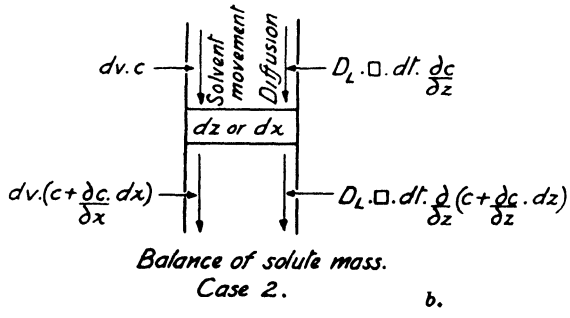


FIG. 3.—(*a*) Single charcoal tube, int. diam. 0·7 cm., length ~25 cm.; (*b*) two double charcoal columns, each 25 cm. in length, about 0·3 cm. int. diam., for separation of neon isotopes at liquid nitrogen temperature.



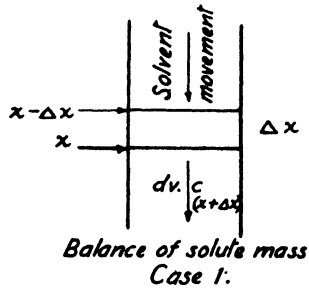


FIG. 4.—Scheme for balance of solute mass: (*a*) In case (i) (disturbance caused by finite grain size); (*b*) in case (ii) (disturbance caused by longitudinal diffusion).

longitudinal diffusion is the dominant disturbance.) The values used (at 77° K) are

$$A = \frac{\bar{x}}{L}, \quad F = \frac{V_t}{t} \cdot \frac{77}{293}, \quad D_{Ne} = 0{\cdot}445 \times \frac{273 + 252}{77 + 252} \cdot \left(\frac{77}{273}\right)^{5/2} = 0{\cdot}0298$$

(the Sutherland constant for neon being 252°).

TABLE IV

"THEORETICAL PLATE" VALUES CALCULATED FROM THE OBSERVATIONS AND FROM EQN. (3b)

No.	10^3 Δ calc from observations with eqn. (13)	$10^3 \times K_2$ calc from eqn. (3b)	Δ/K_2
6	6·7, 9·4, 10·8, 8·5 ; av. 8·8	7·4 (3·9)	1·2
7	2·8	1·8 (0·95)	1·5
8	2·0, 3·2 ; av. 2·6	1·8 (0·95)	1·4 } av. 1·4
9	2·4, 2·4	2·0 (1·05)	1·2
10	0·64	1·3 (0·68)	0·49 } 0·54
11	4·9	8·3 (4·4)	0·59

It can be noted that the variations of flow-rate and diameter of the tube are well represented by eqn. (3b) for both types of experiment. Also, no effect of the change of grain size is observable, as would be expected for a diffusion-controlled disturbance. As in the case of the Li separation, the best experimental set-up (10 and 11) gives a boundary which is sharper (i.e., Δ is smaller) than expected, though the discrepancy is only by a factor of 1·8. The reason for this is probably that only a fraction of the pore space α is used in the longitudinal transport of the gas and consequently the linear flow-rate is faster than anticipated. If this is confirmed by further experiments it will be proposed to change the $\sqrt{2}$ in eqn. (3b) against an empirical factor around 2·7. The bracketed figures in Col. 3 of Table IV correspond to this change.

No reason has so far been found why the first set of runs, using the movement of the liquid nitrogen bath as the speed-determining factor, should give a less favourable separation (i.e., a larger Δ/K_2). But the absence of any influence of the tube diameter and of the time seems to indicate that this is not due to any lack of temperature equilibrium.

In conclusion, it may be said that the experiments support the theoretical deductions as well as may be expected, so that some reliance may be placed on predictions made on this basis. This enables one to draw some conclusions concerning the value of the chromatographic technique for the purpose of isotope separation. It is quite apparent that it cannot be easily adapted to isotope separation in bulk, when the separation factor is small. Both in the case of Li and of neon the amounts of enriched isotope have been of the order of a milligram, and while the scaling-up to gram quantities is conceivable, the difficulties of maintaining sharp boundaries under such conditions should not be underestimated. There is only one field in which the chromatographic method is likely to be superior and this applies to cases where the absolute quantities involved are exceedingly small and where the separation factor is comparatively large. Such a case, for example, may be the separation of ^{3}He from enriched helium. With a theoretical plate height of 0·2 mm. which has been achieved in the neon experiments and a separation factor which, at the temperature of liquid hydrogen, should not be less than $K = 1{\cdot}1$, it ought to be possible to enrich ^{3}He by a factor of 10 in a column of 1 m. length.

Appendix

Derivation of the Constants of the Disturbance Term

CASE (I) (FINITE GRAIN SIZE)

We consider (see Fig. 4 (*a*)) the balance of solute in a small section Δx of the column, which corresponds to a "theoretical plate." Δx may be assumed to be identical with the grain diameter d. The content of the section changes from

$$\Delta x\, f(c) \text{ to } \Delta x \left(f(c) + \frac{\partial f(c)}{\partial v} \cdot \mathrm{d}v \right).$$

Total balance of mass requires that

$$\left(\frac{\partial f(c)}{\partial v}\right)_x \mathrm{d}v\, \Delta x + \mathrm{d}v(c_{(x)} - c_{(x-\Delta x)}) = 0. \quad . \quad . \quad . \quad (14)$$

By means of a Taylor expansion we can write

$$c_{(x-\Delta x)} = c_x - \Delta x \cdot \frac{\partial c}{\partial x} + \tfrac{1}{2} (\Delta x)^2 \cdot \frac{\partial^2 c}{\partial x^2} \cdot \ldots\ldots, \quad . \quad . \quad (15)$$

which leads to

$$\left(\frac{\partial f(c)}{\partial v}\right)_x + \left(\frac{\partial c}{\partial x}\right)_v - \frac{\Delta x}{2} \cdot \left(\frac{\partial^2 c}{\partial x^2}\right)_v = 0. \quad . \quad . \quad . \quad (16)$$

From this is obtained, for the final state of a self-sharpening boundary, the concentration gradient in the effluent as given by eqn. (3), (3*a*).

CASE (II) (DIFFUSION)

We consider the balance of solute in a small section of the column where the content of the section changes from

$$\mathrm{d}x.\, f(c) \text{ to } \mathrm{d}x \left(f(c) + \frac{\partial f(c)}{\partial v} \cdot \mathrm{d}v \right).$$

The balance from solvent and diffusion movements plus change of content of section (see Fig. 4 (*b*)) equals zero; thus:

$$\frac{\partial c}{\partial x}\, \mathrm{d}v.\, \mathrm{d}x - D_l \square\, \mathrm{d}x \cdot \mathrm{d}t \cdot \frac{\partial^2 c}{\partial x^2} + \frac{\partial f(c)}{\partial v} \cdot \mathrm{d}v \cdot \mathrm{d}x = 0. \quad . \quad (17)$$

We replace

$$\mathrm{d}t \text{ by } \mathrm{d}v/F,$$
$$\mathrm{d}x \text{ by } \mathrm{d}x/A,$$
$$\square \text{ by } A \cdot \alpha/\sqrt{2},$$

(the $\sqrt{2}$ should account for the increased length of the diffusion path between the grains) and obtain

$$\left(\frac{\partial f(c)}{\partial v}\right)_x + \left(\frac{\partial c}{\partial x}\right)_v - \frac{D_l \alpha A^2}{F} \cdot \left(\frac{\partial^2 c}{\partial x^2}\right)_v = 0. \quad . \quad . \quad (18)$$

For the final state of a self-sharpening boundary this leads to eqn. (3), (3*b*).

CASE (III) (NON-EQUILIBRIUM DUE TO GRAIN DIFFUSION)

Mass conservation requires that

$$\left(\frac{\partial c}{\partial x}\right)_v + \left(\frac{\partial (q^* + \alpha c)}{\partial v}\right)_x = 0. \quad . \quad . \quad . \quad (19)$$

This is eqn. (17) without the diffusion term, with the equilibrium term replaced by $q^* + \alpha c$. The change of absorbate at a given point can be represented with good approximation by

$$\frac{\partial q^*}{\partial t} = \frac{D_s}{a d^2} (f^*(c) - q^*), \quad . \quad . \quad . \quad . \quad (20)$$

which means that the rate of diffusion into the grains is essentially proportional to the amount still required to produce equilibrium. Replacing t by v/F,

$$\frac{\partial q^*}{\partial v} = \frac{D_s}{a d^2 F} (f^*(c) - q^*), \quad . \quad . \quad . \quad . \quad (21)$$

and differentiating by ∂v

$$\frac{\alpha d^2 F}{D_s} \cdot \frac{\partial^2 q^*}{\partial v^2} = \frac{\partial f^*(c)}{\partial v} - \frac{\partial q^*}{\partial v}.$$

Elimination of $\partial q^*/\partial v$ in eqn. (19) then results in

$$\frac{\partial c}{\partial x} + \frac{\partial f(c)}{\partial v} - \frac{a d^2 F}{D_s} \cdot \frac{\partial^2 q^*}{\partial v^2} = 0. \quad . \quad . \quad . \quad (22)$$

Differentiation of (19) by ∂v gives

$$\frac{\partial}{\partial v}\left(\frac{\partial(q^* + \alpha c)}{\partial v}\right) = -\frac{\partial}{\partial v}\left(\frac{\partial c}{\partial x}\right) \simeq \frac{\partial^2 q^*}{\partial v^2}, \quad . \quad . \quad . \quad (23)$$

so that (22) can be written :

$$\left(\frac{\partial f(c)}{\partial v}\right)_x + \left(\frac{\partial c}{\partial x}\right)_v + \frac{a d^2 F}{D_s} \cdot \frac{\partial^2 c}{\partial x \cdot \partial v} = 0. \quad . \quad . \quad . \quad (24)$$

For a front boundary which moves without change of form,

$$\left(\frac{\partial v}{\partial x}\right)_c - \alpha = \frac{dq^*}{dc} = \frac{f^*(c^0)}{c^0}.$$

As $q^* = 0$ for $c = 0$, we obtain

$$q^* = c \cdot \frac{f^*(c^0)}{c^0}. \quad . \quad . \quad . \quad . \quad . \quad (25)$$

This permits the integration of eqn. (24) leading to eqn. (3), (3*c*).

CASE (IV) (NON-EQUILIBRIUM DUE TO DIFFUSION THROUGH A THIN LIQUID FILM SURROUNDING THE GRAIN)

The balance of solute mass again leads to eqn. (19). The change of q^* by diffusion through a layer of thickness δ cm. during the time $dt = dv/F$ for 1 g. sorbent, the surface O of which is

$$O = \frac{\pi d^2}{1/6\pi d^3 s} = \frac{6}{ds} \quad . \quad . \quad . \quad . \quad (26)$$

is approximately given by

$$dq^* = \frac{D_l O(c - c_s) \cdot dt}{\delta} = \frac{6 D_l (c - c_s) dt}{\delta ds F}, \quad . \quad . \quad . \quad (27)$$

where c_s is the concentration on the sorbent side of the liquid film, in equilibrium with $q_0{}^*$.

Thus $$c_s = \varphi(q^*).$$

The change of concentration at the point x is therefore given by

$$\left(\frac{\partial q^*}{\partial v}\right)_x = \frac{6 D_l}{\delta ds \cdot F}(c - \varphi(q^*)). \quad . \quad . \quad . \quad (28)$$

As in the final state of the self-sharpening boundary q^* can be replaced by $\frac{c}{c^0} \cdot f(c^0)$ (see eqn. (25)) ; this leads to eqn. (4), (4*a*).

The experiments with lithium isotopes were carried out for the Department of Atomic Energy, M.O.S., at the University Science Laboratories at Durham (E. G.). The method for isotopic analysis of the lithium was developed and the measurements done in the laboratory of Dr. B. Rotblat, Physics Department, Liverpool University (K. H. B.). The work with neon isotopes was done at the Atomic Energy Research Establishment, Harwell (E. G. and G. P. K.). Our thanks are due to the Director, A.E.R.E., for permission to publish this paper, to the Durham Colleges and Prof. F. A. Paneth for laboratory facilities and to Dr. B. Rotblat (University of Liverpool), Dr. H. London (A.E.R.E.) and Dr. B. Davison (A.E.R.E.) for valuable discussions.

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LARGE-SCALE SEPARATION OF RARE-EARTH SALTS AND THE PREPARATION OF THE PURE METALS

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For many years one of the most difficult processes in the field of chemistry has been the separation of the rare earths from each other into their pure states. Their chemical and physical properties are so similar that in general a single operation leads only to a partial separation or enrichment.

Ever since the beginning of the Manhattan Project there has been a constant demand for samples of rare earths of exceptional purity in gram amounts or greater. This demand arose for numerous reasons, but mainly because some of the rare earths are formed as fission fragments during fission of the heavy elements. It was highly desirable, therefore, to have a means of preparing pure rare earths so that their nuclear properties could be studied and also to allow a more thorough consideration of their chemical behaviour.

In general, the best means of separating these elements has been the well-known, but laborious, method of fractional crystallization, etc., as used by James, Hopkins and others. Cerium with its quadrivalent state and samarium, europium and ytterbium with their divalent states which do permit a means of separation from the normal trivalent rare earth ions are exceptions.

Starting in 1945, the Ames Laboratory of the United States Atomic Energy Commission developed a series of ion-exchange processes for separating adjacent rare earths on the macro scale These all involved the adsorption of the mixed rare-earth ions on the top of resinous ion-exchange columns such as Amberlite IR-100 and the later elution of these rare earths down the columns by means of citrate solutions of various concentrations and pH values The conditions for the good separation of the mixed rare-earth into pure rare-earth components are discussed in detail in the paper

The Ames Laboratory has also developed processes for producing pure rare-earth metals. Lanthanum, cerium, praseodymium, neodymium and yttrium have been prepared as massive metal to date.

The group of elements known as the rare earths and the similar elements scandium, yttrium and lanthanum usually associated with them have fascinated me ever since my graduate school-days. I believe that a study of these elements offers one of the best keys to a better understanding of the relationships between atomic structure and the various physical and chemical properties which metals and compounds possess. The rare earths are a group of some 14 elements which start with atomic number 58 and extend through atomic number 71. They have almost identical physical and chemical properties. These properties are so similar to those of lanthanum, element 57, that in the condensed periodic table these elements are always grouped together in the square with lanthanum. Almost all the physical and chemical properties of a substance are primarily determined by the outermost electrons of the atoms of the substance since it is these outermost electrons which are involved in chemical bonds and which are shifted about when chemical processes take place. In this group of elements there are three electrons which principally determine their chemical behaviour so that they all have a valency of 3 and, therefore, form very similar compounds which are extremely difficult to separate from one another The electronic structures of the elements as they exist in solid salts are shown in Table I. Starting with cerium, an incomplete inner shell, the $4f$ begins to take up electrons and since this inner sub-shell is protected by the completed $5s5p$ sub-shells which project farther out from the centre of the atom, these inner

electrons are very little affected by the bonding with the neighbouring atoms. These inner electrons are, therefore, frequently unpaired electrons and give rise to the paramagnetic properties of the rare-earth salts. Even in the solid state, these inner electrons are so well protected from external bonding and coupling by the completed sub-shells outside them that, as a first crude approximation, the energy states arising from them can be treated theoretically [1] in the same manner as if the rare-earth atoms were in a gaseous form. In a more precise calculation, however, we have to take into consideration the fact that these states are affected by the surrounding atoms in the solid since these atoms subject the rare-earth atom to strong electric fields. It has been found that if the solid salts are cooled to low temperatures, the transitions between energy states arising from the inner electrons give rise to extremely sharp absorption spectra and that the fine structure of these absorption spectra varies from compound to compound.

TABLE I

ELECTRONIC ARRANGEMENTS OF THE RARE-EARTH ELEMENTS IN THEIR SALTS

	At. No.	1_1	2_1 2_2	3_1 3_2 3_3	4_1 4_2 4_3 4_4	5_1 5_2 5_3 5_4 5_5	6_1
Sc	21	2	2 6	2 6 1	2		
Y	39	2	2 6	2 6 10	2 6 1 0	2	
Ba	56	2	2 6	2 6 10	2 6 10 0	2 6 0 0 0	(2)
La	57	2	2 6	2 6 10	2 6 10 0	2 6 (1) 0 0	(2)
Ce	58	2	2 6	2 6 10	2 6 10 1	2 6 (1) 0 0	(2)
Pr	59	2	2 6	2 6 10	2 6 10 2	2 6 (1) 0 0	(2)
Nd	60	2	2 6	2 6 10	2 6 10 3	2 6 (1) 0 0	(2)
61	61	2	2 6	2 6 10	2 6 10 4	2 6 (1) 0 0	(2)
Sm	62	2	2 6	2 6 10	2 6 10 5	2 6 (1) 0 0	(2)
Eu	63	2	2 6	2 6 10	2 6 10 6	2 6 (1) 0 0	(2)
Gd	64	2	2 6	2 6 10	2 6 10 7	2 6 (1) 0 0	(2)
Tb	65	2	2 6	2 6 10	2 6 10 8	2 6 (1) 0 0	(2)
Dy	66	2	2 6	2 6 10	2 6 10 9	2 6 (1) 0 0	(2)
Ho	67	2	2 6	2 6 10	2 6 10 10	2 6 (1) 0 0	(2)
Er	68	2	2 6	2 6 10	2 6 10 11	2 6 (1) 0 0	(2)
Tm	69	2	2 6	2 6 10	2 6 10 12	2 6 (1) 0 0	(2)
Yb	70	2	2 6	2 6 10	2 6 10 13	2 6 (1) 0 0	(2)
Lu	71	2	2 6	2 6 10	2 6 10 14	2 6 (1) 0 0	(2)
Hf	72	2	2 6	2 6 10	2 6 10 14	2 6 (2) 0 0	(2)

( ) Involved in valency bonds.

Using quantum mechanics it is possible to calculate theoretically where these sharp lines should appear in the spectra and how the groupings of the fine structure should vary as the symmetry and strength of the fields vary due to the surrounding atoms. We have, therefore, within this group of elements, a chance to study directly the short-range forces which surround an atom in a solid and to correlate the many physical and chemical properties of the solid with the absorption spectra. This then enables us to correlate the properties of the solid directly with such fundamental data as the charge on the electron, the energy states of the atoms and the distances and positions of the surrounding atoms in the solid. Further, since the chemical and physical properties are primarily determined by the three outermost electrons these elements form a series of very similar compounds having the same valency forces and, therefore, similar crystal structures. However, since the charge on the nucleus increases as the atomic number

[1] Hund, *Linienspektren und periodisches System der Elemente* (J. Springer, Berlin, 1927).

increases, the inner shells are pulled in closer so that the lattice parameters change in a predictable way. Occasionally in these series, when this shrinking has reached the point where the atoms can no longer be grouped together without strain due to their atomic radii, there will result a change in the crystal structure which should also be predictable. Since these factors can be taken into account in almost any theory put forward concerning the structure of solids or metals, or the solubility of salts, etc., the compounds of these elements, their alloys or even the pure metals themselves, offer the ideal group for checking the present theories concerning the solid or liquid state and the various theories of solution. It should be pointed out that most of these theories contain a number of arbitrary constants. If an attempt is made to check these theories using other elements in the periodic table, so many factors vary from element to element that it is almost always possible to find arbitrary constants which will fit. However, in the rare-earth group it is possible to hold many of these variables constant, and the variables that do change should do so in a predictable manner. Accordingly, in any theory where the constants are made to fit one member of the rare-earth series, they should, after suitable corrections have been made, fit all of the members. If they do not, something must be wrong with the theory. Once the theories have been made to fit the entire rare-earth group, then it should be possible to deal separately with the changes which will take place when the valencies and crystal structures change in going from one element to another in various parts of the periodic table.

It should be pointed out that when a sub-group is completed or half-completed a very stable configuration is formed. In these cases the energy required to remove an electron from the $4f$ shell or from one of the outer valency shells should not differ greatly. This accounts for the fact that certain of the rare-earth elements can exist in other valency states than the trivalent state. For example, in cerium the single $4f$ electron can go to the outside and give rise to a 4-valent cerium. There is a slight tendency for the same thing to happen with terbium where the half-completed shell of 7 tends to permit the 8th electron to go to the outside. Terbium in its oxide form is known to possess a higher valency state, but in solution no higher valency state has been reported. It appears that the stabilizing action of the half-shell is not sufficient to make the higher state stable in the presence of water. On the other hand, when one more electron is needed to complete or half-complete the shell, an outer electron can go to the inside, thus giving rise to the divalent state, as it does in europium and ytterbium and to some extent in samarium. In general, these anomalous valency states are less stable than the trivalent state, but in separating these particular rare earths from the others advantage can be taken of the fact that such valencies do exist. Considerable success has been obtained, in this regard, by workers such as Marsh,[2] Hopkins,[3] Yntema,[4] McCoy,[5] etc.

For many years one of the most difficult processes in the field of chemistry has been the separation of rare-earth mixtures into their pure salts. The chemical and physical properties, aside from the exceptions mentioned

[2] Marsh, *J. Chem. Soc.*, 1937, 1367; 1942, 398; 1942, 523; 1943, 8; 1943, 531.

[3] Andrieth, Jukkola, Meints and Hopkins, *J. Amer. Chem. Soc.*, 1931, **53**, 1805. Meints, Hopkins and Andrieth, *Z. anorg. Chem.*, 1933, **211**, 237. Jukkola, Andrieth and Hopkins, *J. Amer. Chem. Soc.*, 1934, **56**, 303. West and Hopkins, *J. Amer. Chem. Soc.*, 1935, **57**, 2185. Pearce, Naeser and Hopkins, *Trans. Electrochem. Soc.*, 1936, **69**, 8.

[4] Yntema, *J. Amer. Chem. Soc.*, 1930, **52**, 2782. Yntema and Ball, *J. Amer. Chem. Soc.*, 1930, **52**, 4264.

[5] McCoy, *J. Amer. Chem. Soc.*, 1935, **57**, 1756; 1936, **58**, 1577; 1936, **58**, 2279; 1937, **59**, 1131; 1941, **63**, 1622; 1941, **63**, 3432. McCoy and Hammond, *J. Amer. Chem. Soc.*, 1942, **64**, 1009.

above, are so similar that usually a single operation leads only to a partial separation or enrichment. In the past, the best means of separating these elements have been the well-known but laborious methods of fractional crystallization, fractional decomposition, etc., as developed by workers such as Hopkins,[6] James,[7] Urbain [8] and many others. These processes were tedious and required a great deal of drive, patience and determination on the part of the research workers in order to obtain small amounts of the pure rare earths. The same operations had to be repeated many times and in some of the rarer and more difficult to separate rare earths it has required up to 20,000 operations to accomplish purification. Therefore, except to the few stout souls who were willing to devote a lifetime to this sort of study, the rare earths were not generally available. While these pioneers were very generous in lending samples to other workers, the quantities they had were strictly limited. Also, it is understandable that when these scientists had devoted many years to obtaining a few grams of the rare earths they themselves wanted to study the properties of the salts. For these reasons the rare earths have not been nearly as extensively studied as the other elements of the periodic table and many of their properties, particularly the properties of the metals and their alloys, are almost unknown even to the present day.

Ever since the beginning of the Manhattan Project there has been a constant demand for samples of rare earths of exceptional purity in gram amounts or greater. This demand arose for numerous reasons, but mainly because some of the rare earths are formed as fission fragments during the fission of the heavy elements. In other words, they are among the ashes of the atomic reaction. It was highly desirable, therefore, to have a more satisfactory means of preparing pure rare earths so that their nuclear properties could be studied, and also to allow a more thorough determination of their chemical behaviour.

A number of workers have reported, in the literature, studies on the application of chromatographic and ion-exchange methods to the separation of rare earths. While they obtained some enrichment, their results were not sufficiently promising to lead to further intensive investigations or to the production of pure rare earths in quantity. Shortly after the Plutonium Project was organized at Chicago, Dr. George Boyd [9] introduced the use of Amberlite as a research tool in studying the complicated mixtures which occur when uranium fissions. Dr. Tompkins, Dr. Khym and Dr. Cohn [10] used Amberlite rather extensively in their studies of fission products. At that time all sections of the Manhattan Project worked closely together and at a meeting of section chiefs in December, 1944, Dr. Cohn stated that they had succeeded fairly well in separating the cerium fission fragments from the yttrium fission fragments. They accomplished this by using citric acid solutions of controlled pH for eluting the various ions from Amberlite columns upon which the fission fragment ions had been adsorbed. He also mentioned that in the eluant between the rich yttrium fraction and the cerium fraction there was a concentration of the other rare earths. It

[6] Hopkins, numerous publications in the literature.
[7] James, numerous publications in the literature.
[8] Urbain, numerous publications in the literature.
[9] Boyd, Schubert and Adamson, *J. Amer. Chem. Soc.*, 1947, **68**, 2818. Boyd, Adamson and Myers, *J. Amer. Chem. Soc.*, 1947, **69**, 2836. Boyd, Myers and Adamson, *J. Amer. Chem. Soc.*, 1947, **69**, 2849. Ketelle and Boyd, *J. Amer. Chem. Soc.*, 1947, **69**, 2800.
[10] Tompkins, Khym and Cohn, *J. Amer. Chem. Soc.*, 1947, **69**, 2769. Harris and Tompkins, *J. Amer. Chem. Soc.*, 1947, **69**, 2792. Tompkins and Mayer, *J. Amer. Chem. Soc.*, 1947, **69**, 2859.

occurred to the author that this offered a possibility of actually separating adjacent rare earths in macro quantities if the right conditions could be found. The Ames Laboratory set to work and during the next few months succeeded in separating gram quantities of praseodymium from neodymium. When this work was reported before the section chiefs' council in April, Dr. Hume [11] reported that the men at Oak Ridge had also succeeded in separating the rare earths on the micro and tracer scale. The history of how this development took place has been described in some detail in a paper by Johnson, Quill and Daniels,[12] so I shall not go into it further here, but shall confine the rest of my paper to the work at Ames where we continued to develop the macro separation of non-radioactive rare-earth atoms.

Macro Separation of Non-radioactive Rare-earth Atoms

Since, at best, any single chemical operation gives only a slight enrichment of one adjacent rare earth over another, it is obvious that to separate successfully the rare earths into their pure fractions it will be necessary to have a process which automatically repeats the same operation many times. These conditions can be fulfilled in an ion-exchange column. In the work which we carried out at Ames we took advantage of the ion-exchange type column, using a synthetic resin either Amberlite IR–1 or –100 or the high-capacity resins such as the Dow Chemical Company and others put out. Most of our work was done with the Amberlite IR–100 which is a synthetic resin built up from a condensation of phenol, formaldehyde and sulphuric acid. The apparatus consisted essentially of a long tube into which the resin had been packed; the resin was supported at the bottom by a metal screen or a sintered glass plate and a suitable pinch clamp was located at the bottom so that the flow rate through the column could be controlled (Fig. 1). As will be shown later, the particle size can be very important in the separation, so generally the resin was classified either by sieving, or in the earlier stage by back-washing, so that the fine material was washed out the top and the coarser particles were segregated according to size down the column. The size of the column is immaterial since a column of any size will work providing certain conditions are fulfilled. The columns can vary all the way from capillary tubes a few centimetres in length, which might be used when tracer activities are separated from each other, up to columns several feet in length and many inches in diameter. In most of our work for experimental purposes we use column beds from 30 to 120 cm. length and about 22 mm. diam. Once the Amberlite had been packed and classified in the column, it was put in the acid cycle by pouring a 5 % hydrochloric acid solution through the resin, thus putting hydrogen ions on the active exchange points of the resin. Next, a slightly acid solution of the mixed rare-earth chlorides was poured in the top of the column and allowed to adsorb in a band in the upper section. Usually the width of the adsorbed band of rare earths in the column occupied from one-tenth to one-third of the column length. The column was then washed out with distilled water to remove the chloride ions. It was then in condition to start a rare-earth separation run. Previously a solution of citric acid of fixed concentration had been prepared in a large reservoir and brought to the desired pH by adding ammonium hydroxide until a pH meter gave the selected reading.

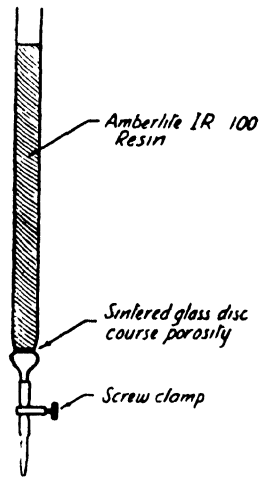

FIG. 1.—Schematic diagram of an Amberlite column with a sintered glass support.

[11] Swartout and Hume, *Metallurgical Projects Report*, CN–1839 (July 10, 1944).
[12] Johnson, Quill and Daniels, *A.C.S. Chem. Eng. News*, 1947, **25**, 2494.

To face page 219]

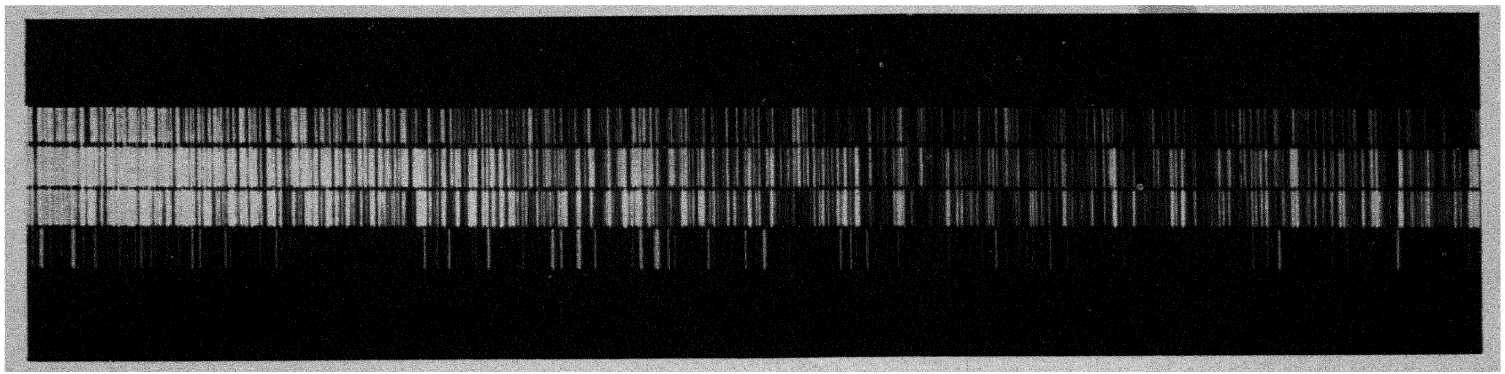

FIG. 2.—Typical second order rare-earth spectra from a 21-ft. grating spectrograph having a dispersion of 2·5 A°/mm. exposures from top to bottom; pure neodymium, a mixture of neodymium and samarium, pure samarium and the relatively simple iron spectrum.

This solution was started flowing slowly through the column and samples were taken from the bottom periodically.

While the rare-earth ions are tightly adsorbed on the Amberlite in the low pH range, at the pH where the citric acid solution is flowing through the column an equilibrium is set up between the Amberlite–rare-earth complex and the citrate complex. The equilibrium constants differ slightly for the different rare earths and this causes the heavier rare earths to move down the column more rapidly than the lighter rare earths. Since the shifting back and forth between the resin and the complex ion must occur thousands of times as the rare earths move down the column, this ultimately results in the separation of the rare earths which come out at the bottom. The exact mechanism by which this occurs is very complex, so until we have more data we do not intend to say very much about the nature of the mechanism involved. Each time we complete a set of experiments, we believe we understand the mechanism and then invariably find that with further data this mechanism has to be modified in the light of the additional results. I think we can say, however, that there is no doubt that there is an equilibrium competition set up between the various ions and that in the pH range which we have so far investigated there are at least several rare-earth citrate complexes which are involved. We have spectrophotometric evidence from studying the adsorption lines of the neodymium ion and the neodymium complexes in solution that there are at least four citrate complexes involved in the equilibrium in the pH range studied. In addition, there is the possibility that the solubility of the hydrated salts, the formation of Werner-type [13] complexes, etc., may be involved in a complete explanation of all the phenomena encountered. Further, in some ranges the rate of establishing the complex equilibrium is the rate-determining steps but in others the diffusion rate in the liquid or the diffusion rate in the Amberlite may be the predominating rate-controlling step ; we are convinced that the best way to attack this problem is to obtain adequate data and to leave the theoretical explanation until we know the facts. Also, we have been seriously handicapped in our work in that we have not had available a number of the rarer rare earths in pure form so that we could investigate their physical constants and the analytical procedures for determining them quantitatively. We have had to prepare these rare-earth salts the best we could by taking the richest fraction from the various runs. We then used these salts to determine the constants and procedures for subsequent runs. In determining such constants as extinction coefficients, and such optimum conditions as the best pH range, etc., we have had to, in a manner of speaking, lift ourselves by our boot straps. It is only recently that we have obtained some of the rarer rare earths in high concentrations in reasonable amounts. It should also be pointed out that the analytical methods for determining the impurities of the rare earths in each other are not at all satisfactory. The spectrophotometric methods are good for only the paramagnetic salts and at best can determine the concentrations to only 1 %–2 %. The emission spectra of the rare earths are so rich in lines that even in the second-order of a 21-ft. grating (Fig 2) the lines are frequently broader than the distances between the lines. It is, therefore, difficult to obtain the purities spectrographically although it can be done and is being done in our laboratory. However, this method of analysis requires that calibration curves be run on the pure rare earths for the impurities of the other rare earths and if you do not have supplies of all the pure rare earths in sufficient quantity to set up these standards, the analyses may subsequently prove to be in error as purer sources of the major rare-earth constituents become available. I might say in passing that the so-called spectrographically pure rare earths obtained from commercial companies in four out of five cases turn out to be nowhere near as spectrographically pure as claimed. In one case we determined the extinction coefficient of an adsorption band from a so-called spectrographically pure standard. Later we found in using this value that our salt was analyzing 120 % purity. The atomic-weight method of determining purities is not very sensitive since the atomic weights of all the

[13] Werner, *New Ideas on Inorganic Chemistry*, Tran. by Hedley (Longmans, Green & Co., London, 1911).

rare earths are so close together. Perhaps the most sensitive method of determining purity is to send a sample to one of the atomic reactors and have it bombarded with neutrons; the impurities will then give activities which can be followed by counters and some idea of the absolute purity can be determined. However, it is not usually convenient to send a sample to Oak Ridge for analysis and in most cases the spectrographic methods are sufficiently accurate to detect impurities which occur in significant amounts.

Fig. 3 shows the separations which can be obtained between neodymium and praseodymium using a 5 %-citric acid solution at a pH of about 2·55. This represents the separation of a mixture of about equal amounts of praseodymium and neodymium on a 70-in. column operating at near optimum conditions. It will be noticed that about 30 % of the neodymium comes out spectrographically pure and that about 85 % or 90 % of it comes out better than 95 % pure. However, with such high concentrations of citric acid, the cost of this acid became very great when scaled up to large operations. It was very desirable to see whether a more dilute solution of citric acid could be used.

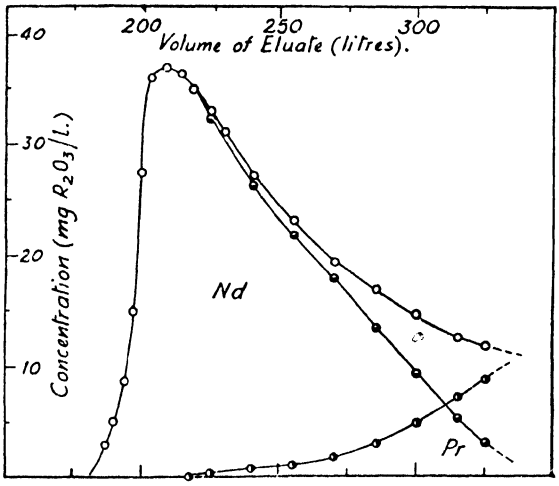

FIG. 3.—The elution of a sample, 0·25 g./sq. cm., analyzing 51·7 % Pr_6O_{11} and 48·3 % Nd_2O_3, from a bed of Amberlite IR-1 resin, 6·4 × 175 cm., using 5·0 % citrate solution at a pH value of 2·55 and a flow-rate of 1·5 cm./min.

Fig. 4 shows one of our pilot plants for separating rare earths. Here we are using 8-ft. columns of 4 in. diam. and are adsorbing on top of each column from 50 to 100 g. mixed rare-earth oxides after they have been converted to the chlorides. The samples are collected in 45-l. bottles and these are changed every 12 hr. The citric acid elution solution is mixed in the large tank shown in the background. In our more recent work we are using the same columns with 4-ft. beds. At this point it should be emphasized that in obtaining rare earths on the macro scale the important thing is not how much rare earth a given adsorbent will hold or how pure an infinitesimal fraction can be obtained from the bottom of the columns, but rather how many grams of a rare-earth salt of a given purity can be obtained per man-month of labour involved. Therefore, since it takes very little more energy to watch 20 columns than it does to watch one, it pays to make the set-up on a multiple scale. When operating on such a large scale as this the cost of chemicals has to be seriously considered and if a more dilute solution can be made to work, it may be desirable to use the more dilute solution even though the separations are slightly inferior.

Table II shows the composition of the crude didymium which we adsorbed on the columns in our early production runs where we were separating praseodymium, neodymium and samarium. This material can be obtained from the Lindsay Light and Chemical Company and is the residue from the monazite

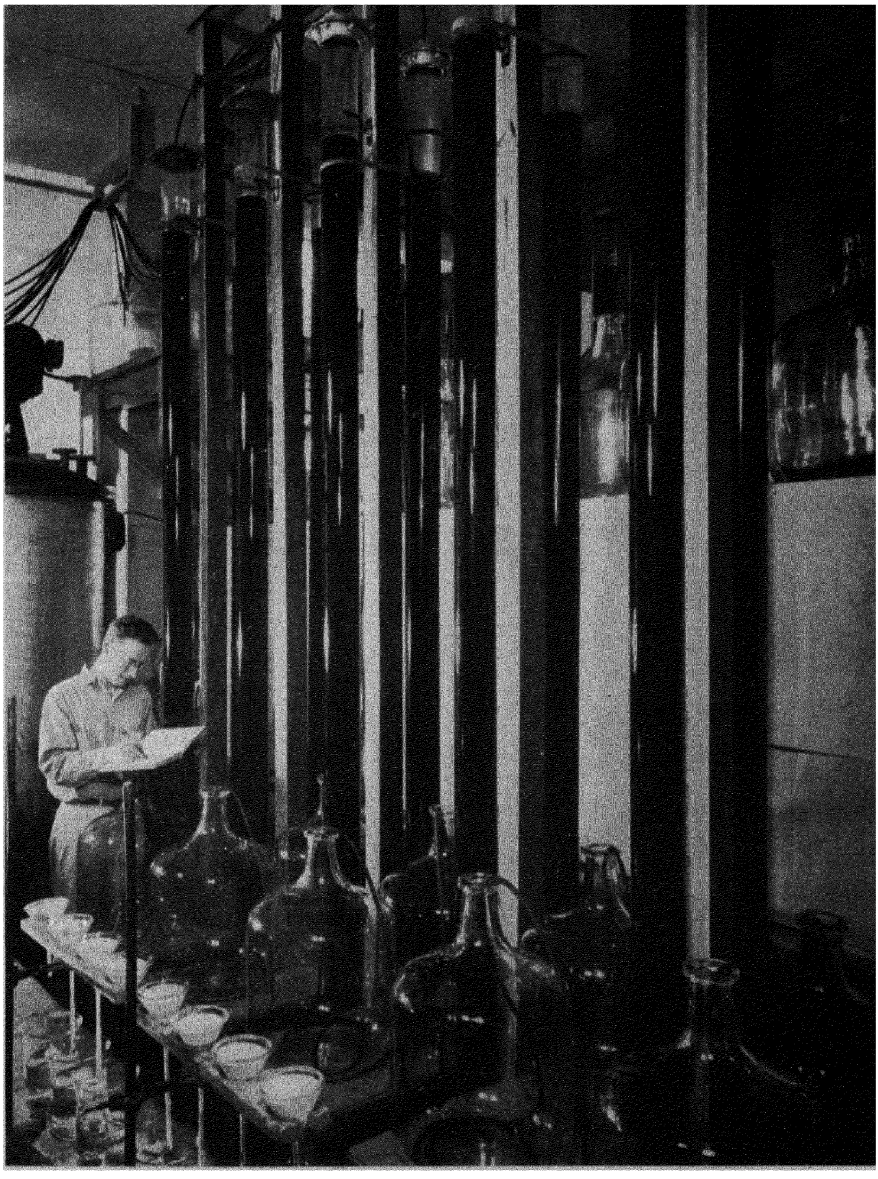

FIG. 4.—Pilot plant for the separation of rare earths by ion-exchange columns.

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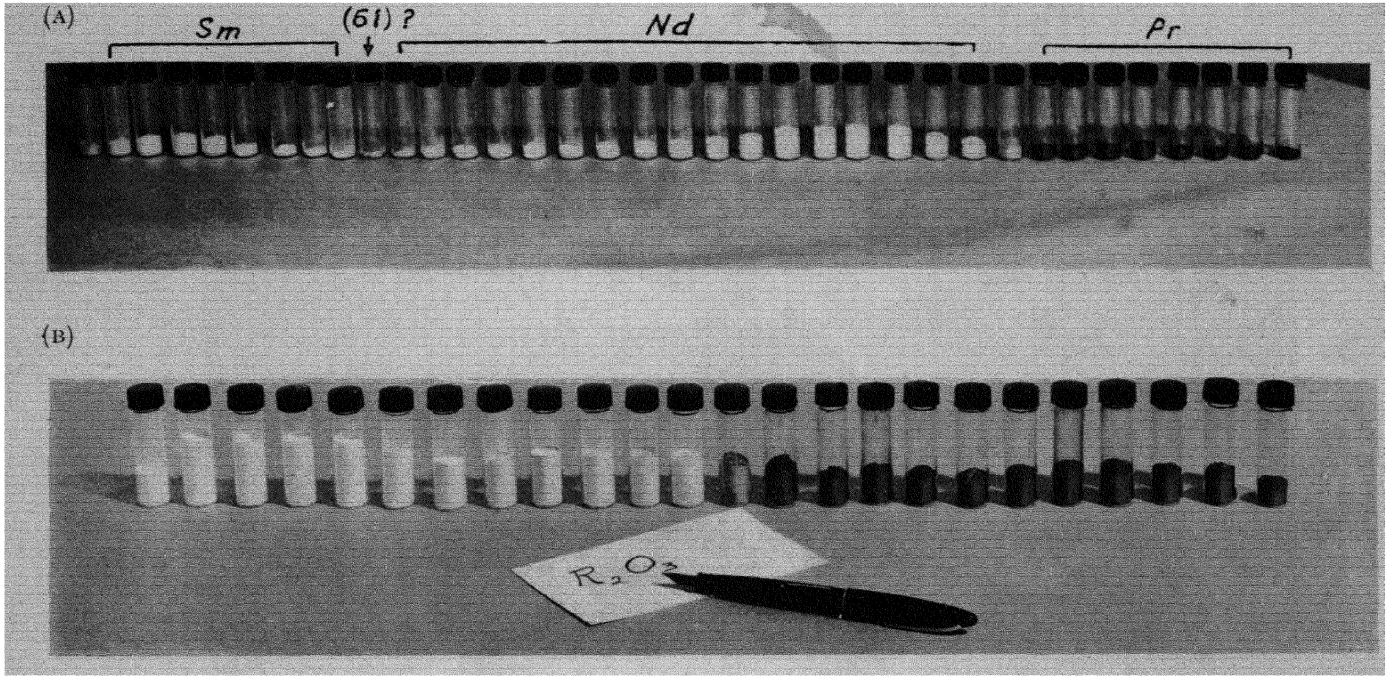

FIG. 5.—Typical separations of rare-earth elements by ion-exchange columns: upper series of vials (A) using 0·5 % citrate solution at a pH of 3·9; lower series (B) using 0·1 % citrate at a pH of 6·0.

sand after they have removed the thorium, cerium and lanthanum for which they have commercial markets.

Fig. 5 shows how well this mixture is separated by one pass through the column. The upper row of vials shows an early separation where 0·5 % citric acid at a pH of 3·9 was used. The lower row of vials represents a run using 0·1 % citric acid at a pH of 6·0. Each of the bottles which were taken off at 12-hr. intervals had its rare-earth fraction precipitated by adding oxalic acid. The insoluble oxalate was filtered out and a prorated fraction of the precipitate was collected in each of the little vials. Therefore, each vial can be considered a 12-hr. period. Of course, there was a considerable time element before any rare earth broke through the bottom of the column. The gadolinium and terbium fractions came off first, then the samarium fraction. The 61 fraction would have come off next if any appreciable amount had been present. Next came the neodymium fraction and finally the praseodymium fraction.

TABLE II

TYPICAL ANALYSIS OF LINDSAY "NEODYMIUM CARBONATE" INDIVIDUAL RARE EARTHS CALCULATED ON BASIS OF TOTAL R_2O_3 CONTENT OF 73·5 %

Constituent	Percentage
Nd_2O_3	80·1
Sm_2O_3	10·4
Pr_6O_{11}	8·4
Gd_2O_3	1·0

Fig. 6 shows the results of near optimum conditions for the separation of Lindsay's "neodymium carbonate" and is a graphical representation of Fig. 5 (A). It will be noticed that the quantities of pure salts which can be obtained using a pH of 3·9 and a citric acid concentration of one-tenth of 5 % are considerably better than those obtained when using a 5 %-citrate solution at a pH of 2·55.

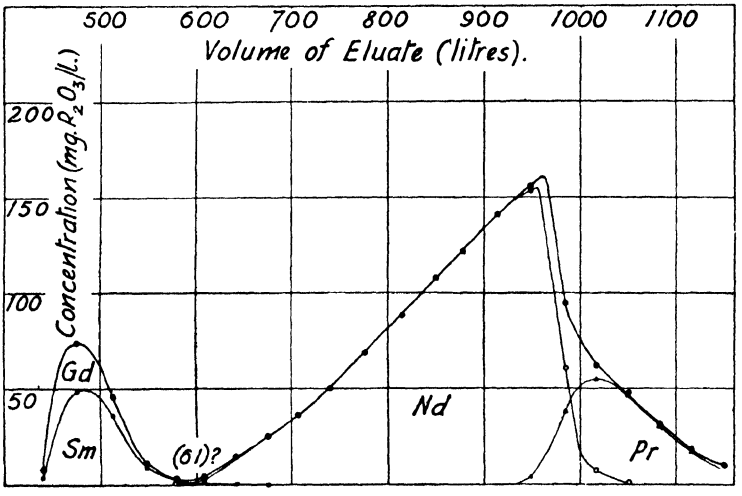

FIG. 6.—Large scale separation of a crude rare-earth mixture by ion exchange; typical elution curve obtained at a pH of 3·9 with 0·5 % citrate solution: ●, total R_2O_3; □, Sm_2O_3; O, Nd_2O_3; Δ, Pr_6O_{11}; difference between total R_2O_3 and Sm_2O_3 curves is due to Gd_2O_3, Eu_2O_3, etc., which do not separate cleanly from Sm_2O_3 under these conditions.

Fig. 7 shows the effect of pH on the shape of the elution curves using 0·5 % citric acid and the degree of separation which can be obtained at various pH's. It will be noticed that at a pH of 4·2, while the separation is not as good as at a lower pH, it takes only a total of about 17 l. to remove the rare earths completely from the column while at a pH of 3·8 it takes as many as 47 l. It is obvious from this and other data that different complexes are involved in elution at the pH of 3·8 as against a pH of 4·2. It should also be pointed out that in general

an intermediate pH range between these two extremes may be more desirable than either of them. While the degree of separation may not be so great, the man-hours involved in making a run are much less, and the grams of pure rare earth obtained per man-month may be greater. All factors have to be considered and it may be possible to make two passes through the column at a higher pH value in the same time it takes to run one pass at the lower pH. While the separation is not as good for a single pass, at the higher pH the double pass will give better over-all separation.

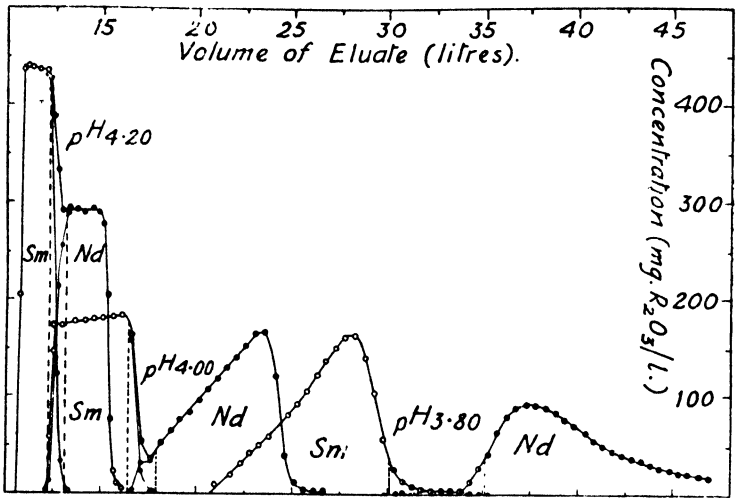

FIG. 7.—The effect of pH on the elution of 1·713-g. samples of equimolar Sm_2O_3–Nd_2O_3 mixtures from 2·2 × 120 cm. beds of − 30 + 40 mesh size Amberlite IR-100 using 0·5 % citrate solutions at a linear flow-rate of 0·5 cm./min.: ○, Sm_2O_3; ◑, mixed fractions; ●, Nd_2O_3.

Fig. 8 shows similar data for the praseodymium-neodymium bands under the same conditions and indicates similar conclusions.

Fig. 9 shows the effect of particle size and flow-rate on the shape of the elution curves. In general the breakthrough and the maximum of the peak occur at lower volumes of eluant with finer resins and for slower flow-rates. This is about what might be expected, since at the slower flow-rates more complete equilibrium can be obtained within the column and the diffusion rate in the solid which tends to blur the separation will be less important for finer resins.

Fig. 10 shows the effect of temperature on the shape of the elution curves. In general the breakthrough and the peak are shifted to greater volumes of eluant with increasing temperature in much the same way that a decrease in pH would shift the peaks, and part of this shift is undoubtedly due to the shifts in the equilibrium constants which occur as the temperatures change. This figure also substantiates the conclusions regarding the effect of flow-rate on the elution curves and separation factors as pointed out in Fig. 9.

Fig. 11 shows the effect of pH on the shape of the elution curves when the strength of the citric acid is dropped to 0·1 % or in other words to 1 g. citric acid monohydrate per litre of distilled water. It will be noticed that in this case sharp breakthroughs and sharp cut-offs are obtained and that there is very little trailing of the leading rare-earth elution band over the succeeding band. Also, the bands are flat-topped as might be predicted from certain types of chromatographic adsorption theory. In general, the degree of separation is better the lower the pH. On the other hand, it takes so many litres of eluant to elute the rare earths that the factors of time and cost become important. While a pH of 5·0 would probably give a better separation, we use a pH of 6·0 in most of our runs as the man-hours of labour to obtain a gram of pure rare earth are much less where a pH of 6·0 is used.

Fig. 12 shows the effect of length of column on the shape of the elution curve and the degree of separation obtained. It will be noticed that the 30-cm. column has been overloaded because we get the typical humping-up of the elution curve which is characteristic of an overloaded column. The widths of the elution bands are the same for the 60, 90 and 120 cm. columns although the amount of the eluant for the breakthrough increases with the length of the column. Therefore in this pH range there is no advantage in longer columns once the bands have attained equilibrium on the column. It also appears in this

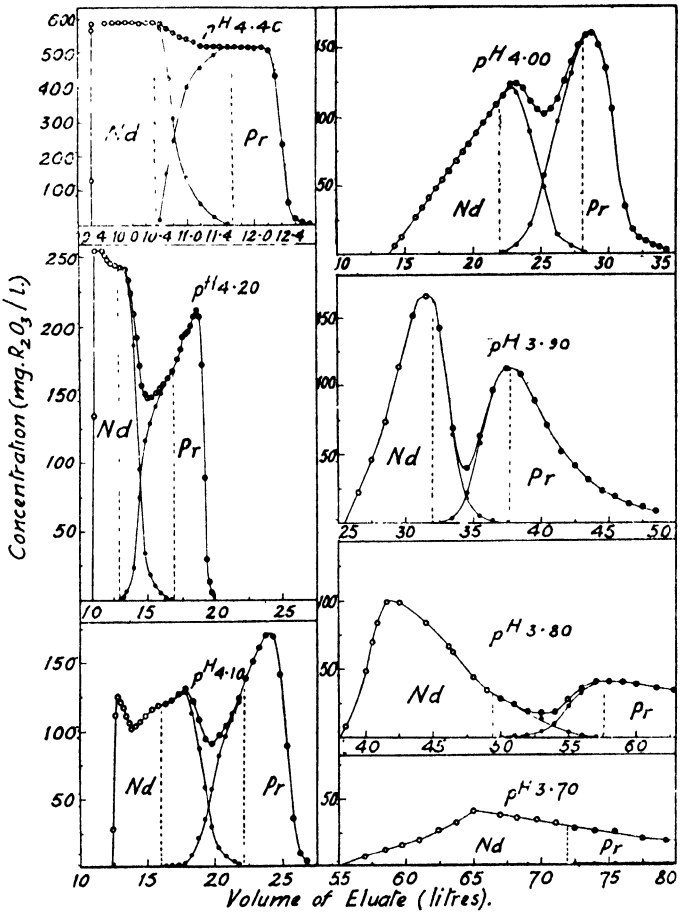

FIG 8.—The effect of pH on the elution of 1·695-g. samples of a mixture containing equal amounts of Nd_2O_3 and Pr_6O_{11} by weight from 2·2 × 120 cm. beds of − 30 + 40 mesh size Amberlite IR-100 resin, using 0·5 % citrate solutions at a linear flow-rate of 0·5 cm /min : O, Nd_2O_3 ; ◑, mixed fractions ; ●, Pr_6O_{11}.

pH range that one rare earth replaces the other on the column and pushes it along. In other words, the bands do not travel independently of each other. These conclusions are not borne out at lower pH's and higher citrate concentrations. On the right-hand side of the graphs, we have plotted the pH values of the solutions coming off and it will be noticed that there is a break in the pH at the point where the rare-earth bands change from one species to another. We hope ultimately to take advantage of this pH break in setting up automatic controls on the columns.

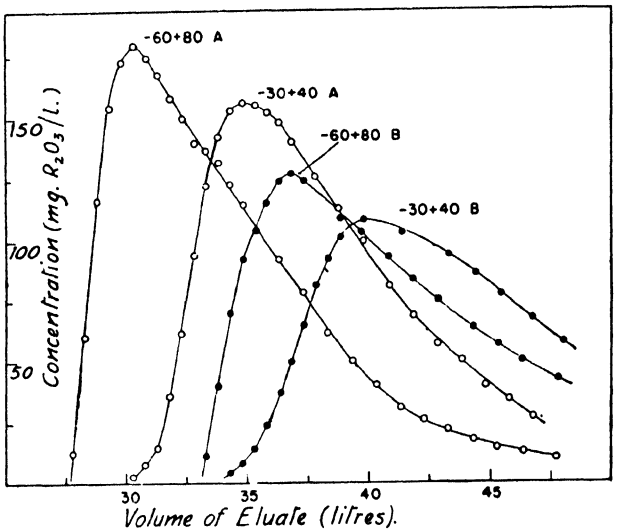

FIG. 9.—The effect of particle size of resin on the elution of 1·683-g. samples of pure Nd_2O_3 from 2·2 × 120 cm. beds of Amberlite IR-100 resin, using 0·5 % citrate solution at a pH of 3·80 and linear flow-rates of 0·5 cm./min. and 2·0 cm./min.: O, 0·5 cm./min.; ●, 2·0 cm./min.

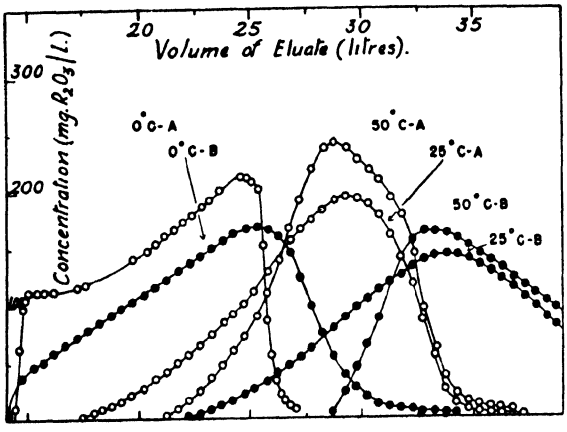

FIG. 10.—The effect of temperature on the elution of 1·714-g. samples of pure Sm_2O_3 from 2·2 × 120 cm. beds of − 30 + 40 mesh size Amberlite IR-100 resin, using 0·5 % citrate solution at a pH value of 3·80 (measured at room temperature) and linear flow-rates of 0·5 cm./min. and 2·0 cm./min.: O, 0·5 cm./min.; ●, 2·0 cm /min.

Fig. 13 shows the separation of a mixture of three rare earths and indicates that an appreciable fraction of each of the rare earths can be obtained in a pure state with one pass through the column.

Fig. 14 shows the effect of increasing the load on the column at the same time the column is increased proportionately in length. It will be noticed that the overlap range remains about constant in all three cases. It would appear that the more heavily the columns are loaded, when the lengths of the columns are made proportionately longer, the higher the percentage of the spectrographically pure rare earths which could be obtained from a single pass through the column. Unfortunately this process cannot go on indefinitely because there seems to be an upper limit to the amount of salt which can be loaded upon a given size column.

This appears to be determined by the time it takes the bands to progress down the column. If the columns are overloaded, after a given time interval a precipitate will appear which invalidates the separation. We believe this precipitate is a citrate complex containing water of hydration which has a very slow rate of crystallization, and at the present time it is giving us considerable trouble in our separations. We have not yet tied down all the variables which determine the nature and appearance of this precipitate. However, if the loads are not too heavy it can be avoided. In our early production runs we were not much troubled with it, but after our resins became older and perhaps became seeded on the inside with this precipitate, it made its appearance more and more frequently and we are at present trying to find the cause of this precipitate and the methods by which its formation can be prevented.

At one time our columns became ineffective and we spent several non-productive months trying to find the cause. At that time we traced the difficulty to the growth of a mould in the column which prevented the separation occurring either by blocking the exchange points or by poisoning the cyclic processes due to the formation of oxalic acid or other by-products of the mould growth. This

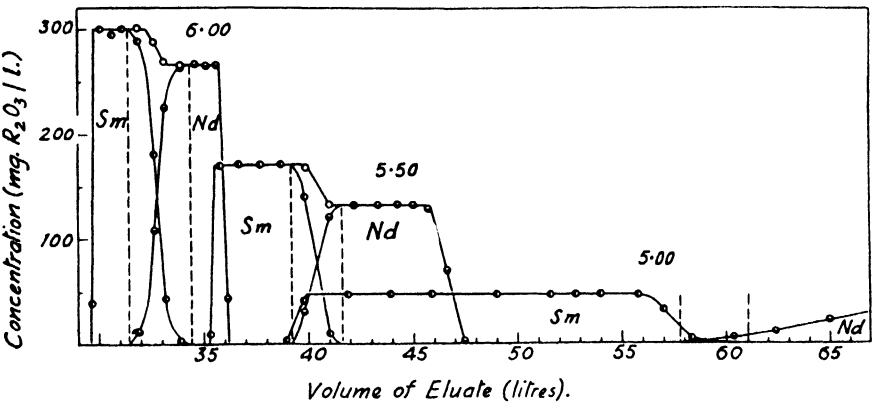

FIG. 11.—The elution of 1·713 g. equimolar mixtures of Sm_2O_3 and Nd_2O_3 from 2·2 × 120 cm. beds of − 30 + 40 mesh size Amberlite IR-100 resin, using 0·1 % citrate solutions at pH values of 5·0, 5·5 and 6·0 at a linear flow-rate of 0·5 cm./min.: O, total R_2O_3; ◐, Sm_2O_3; ◒, Nd_2O_3; broken vertical lines indicate amount of overlap between Sm and Nd bands.

difficulty was overcome by adding 0·1 % phenol to our citric acid solution. This prevented the mould from being troublesome for about two years. The moulds are again giving us trouble, however, for during this time we seem to have developed a strain of mould which can grow in a 0·1 % phenol solution. We are now faced either with the proposition of increasing the phenol concentration or of sterilizing our columns and replacing the Amberlite with fresh material.

Fig. 15 shows the effect of particle size on the separation of the rare earths at a 0·1 % citrate concentration and at appropriate pH ranges. In general, the smaller the particle size the better the separation and this might be expected because the rare earths exchanging near the surface of the particles would move down the column faster than the rare earths which exchange in the centre of the particles where the liquid has to diffuse into the centre and then out again before it can proceed down the column. Another factor which determines to a marked extent the degree of separation is how effectively channelling can be prevented in the columns. In other words, it is important that the diffusion front proceed down the column on a parallel face. If the liquid in one part of the column can flow more rapidly than in another part then this will tend to blur the separations. One might ask why we do not use fine resins exclusively and the answer

is that it is extremely difficult to get them. The resins do not grind up readily since they are semi-plastic and tend to smear into flat platelets rather than grind when put through a mill.

The pure heavy rare earths have proved much more difficult to separate than the light rare earths by the column method, just as they were more difficult to separate by the old fractional crystallization methods. In general, the equilibrium constants are closer together for adjacent rare earths in this range than they are for the lighter rare earths. Further, yttrium which is always

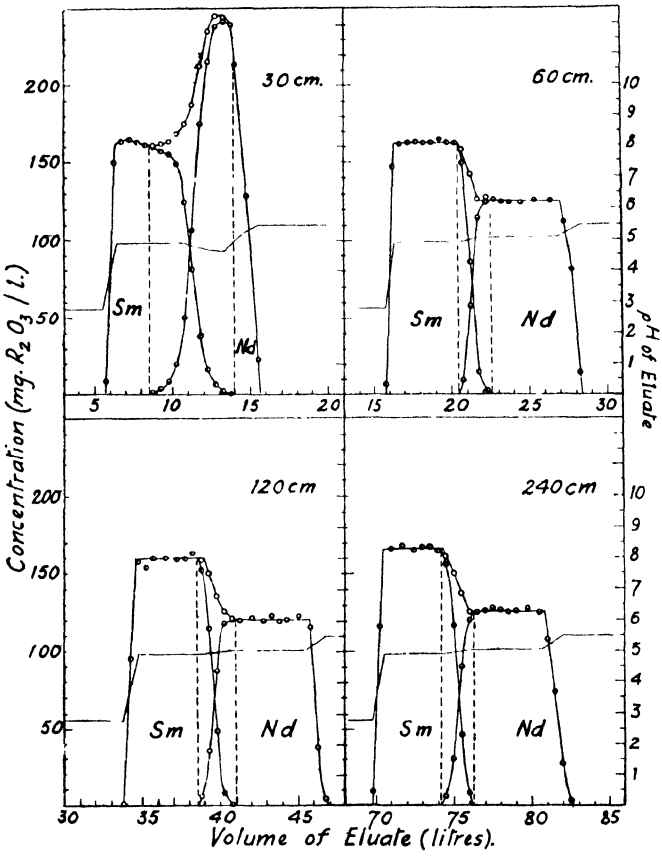

FIG. 12.—The elution of 1·713 g. equimolar Sm_2O_3–Nd_2O_3 mixtures from − 30 + 40 mesh size Amberlite IR-100 resin beds, 2·2 cm. diam. and 30, 60, 120 and 240 cm. long, respectively, using 0·1 % citrate solution at a pH of 5·50 and a linear flow-rate of 0·5 cm./min.: O, total R_2O_3; ◐, Sm_2O_3; ◒, Nd_2O_3; broken vertical lines indicate amount of overlap between Sm and Nd bands; solid line across figures gives the pH of the eluate, reading on the right-hand scale.

present in large excess has an equilibrium constant almost identical with dysprosium. Another difficulty in this range is that the rare earths which can exist in the divalent state seem to have their equilibrium constants shifted in the direction of the next heavier rare earth. While praseodymium, neodymium and samarium separate very readily, it is extremely difficult to separate samarium from gadolinium. The same is true with regard to ytterbium and lutecium. Here, however, nature is kind to us in that it is possible to combine with the column technique the amalgam techniques developed by Marsh, McCoy and Yntema which take advantage of the divalent state so that separations can be obtained. Another difficulty in obtaining the heavier rare earths is that they

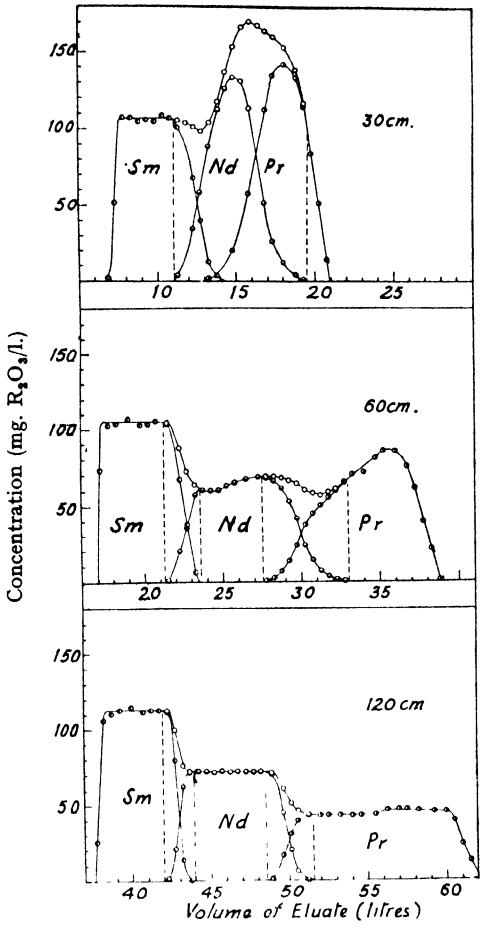

FIG. 13.—The elution of mixtures of Sm, Nd and Pr from − 30 + 40 mesh size Amberlite IR-100 beds, 2·2 cm diam and 30, 60 and 120 cm long, with 0·1 % citrate solution at a pH of 5·30 and a linear flow-rate of 0·5 cm /min : O, total R_2O_3, ◐, Sm_2O_3; ◒, Nd_2O_3, ◑, Pr_6O_{11}, broken vertical lines indicate overlap between bands

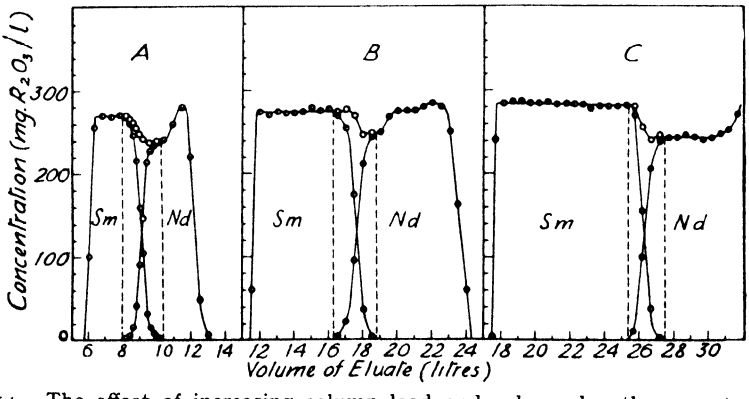

FIG. 14.—The effect of increasing column load and column length proportionately (A) 1·65 g. of equimolar Sm_2O_3–Nd_2O_3 mixture on a 2·2 × 30 cm bed of Amberlite IR-100 resin, (B) 3·30 g. on a 2·2 × 60 cm. bed and (C) 4·95 g. on a 2·2 × 90 cm. bed : O, total R_2O_3 ; ◐, Sm_2O_3 ; ◒Nd_2O_3 ; broken vertical lines indicate amount of overlap between bands.

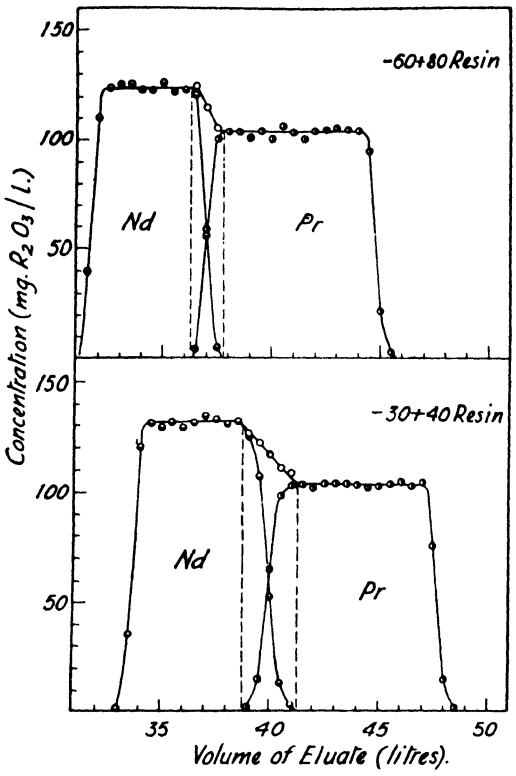

FIG. 15 —The effect of particle size of the resin on the separation of equimolar mixtures of Nd and Pr using 0·1 % citrate at a pH of 5·50 and a linear flow-rate of 0·5 cm./min. : O, total R_2O_3 ; ◒, Nd_2O_3 ; ◑, Pr_6O_{11}, broken vertical lines indicate amount of overlap between the Nd and Pr bands.

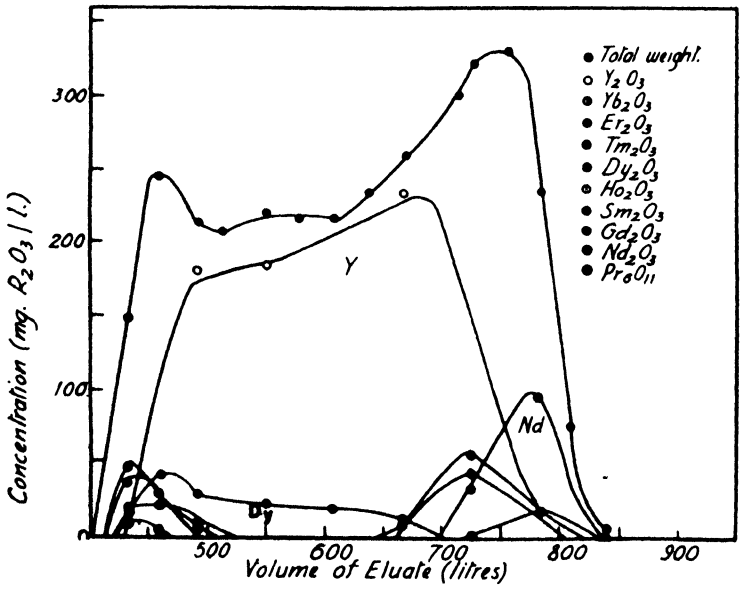

FIG. 16.—Elution of 100 g. rare-earth oxides obtained from gadolinite ore using 0·1 % citrate solution at a pH of 6·00 and a linear flow-rate of 0·5 cm./min. The column consisted of a − 30 + 40 mesh size Amberlite IR-100 bed, 4 in. diam. and 4 ft. long.

exist in nature in much smaller quantities than the lighter rare earths and considerable amounts of ore or heavy rare-earth concentrates have to be worked over in order to get very small quantities of these rarer rare earths. Our sources for obtaining the heavy rare earths have been either the ores gadolinite, blomstrandine or the crude yttrium oxalate which Lindsay has on the market.

Table III shows an approximate analysis of Lindsay's concentrates and it will be noticed that after the yttrium, cerium, neodymium and samarium have been removed only a small fraction of the concentrate remains containing the heavy rare earths.

TABLE III

TYPICAL ANALYSIS OF THE LINDSAY "CRUDE YTTRIUM OXALATE"

Constituent	% by weight
Loss on ignition (800° C)	40
CeO_2	10
Nd_2O_3	7
Sm_2O_3	5
Gd_2O_3	6
Y_2O_3	15
Dy_2O_3	3
Er_2O_3	0·2–0·5
Ho_2O_3	0·2–0·5
SiO_2	10

Fig. 16 shows a typical production run on 100 g. concentrate from gadolinite ore. It will be noticed that the yttrium tends to split the rare earths roughly into two groups, the heavy and light fraction, and that the dysprosium drags across the entire yttrium band. It will also be noticed that there is a minimum in the elution curve where the yttrium occurs. This might be expected since the ordinate is plotted in mg./l. and an equal molar quantity of yttrium will contain far less grams than a similar mixture of the rare earths proper.

Fig. 17 shows a subsequent pass of the column of the heavy rare-earth fraction from the previous run. It will be noticed that there is some separation of the bands but it is not as pronounced as with the light rare earths. It is, therefore, necessary to make from four to ten passes through the columns to obtain appreciable fractions of some of these rare earths in greater than 99·5 % purity. Fewer passes would accomplish this if only two component systems were involved,

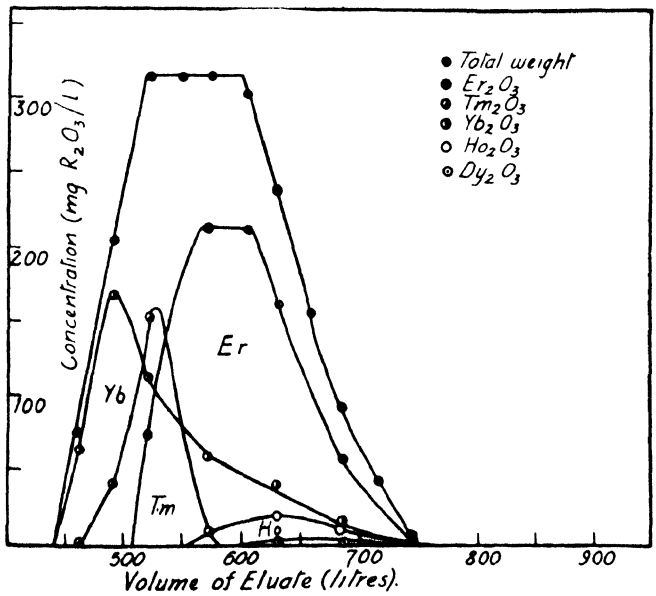

FIG. 17.—Elution of 67 g. of rare-earth oxides originally obtained from gadolinite ore using 0·1 % citrate solution at pH 6·00 at a flow-rate of 0·5 cm./min.: original composition was 20 % Yb_2O_3, 13 % Tm_2O_3, 60 % Er_2O_3, 5 % Ho_2O_3 and 2 % Dy_2O_3.

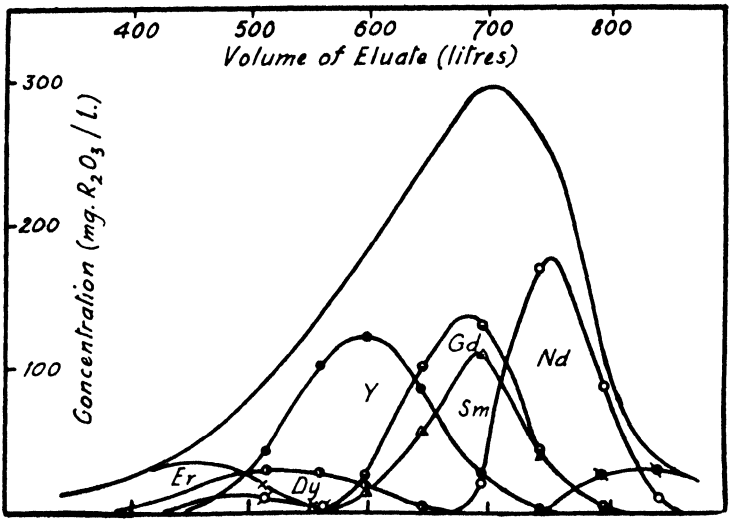

FIG. 18.—Elution curve for 100 g. R_2O_3 from acid-soluble fraction of Lindsay Light and Chemical Company "yttrium oxalate" from an Amberlite IR-100 bed, 4 in. diam. and 8 ft. long, using 0·5 % citrate solution at a pH of 3·9 and a linear flow-rate of 0·5 cm./min.

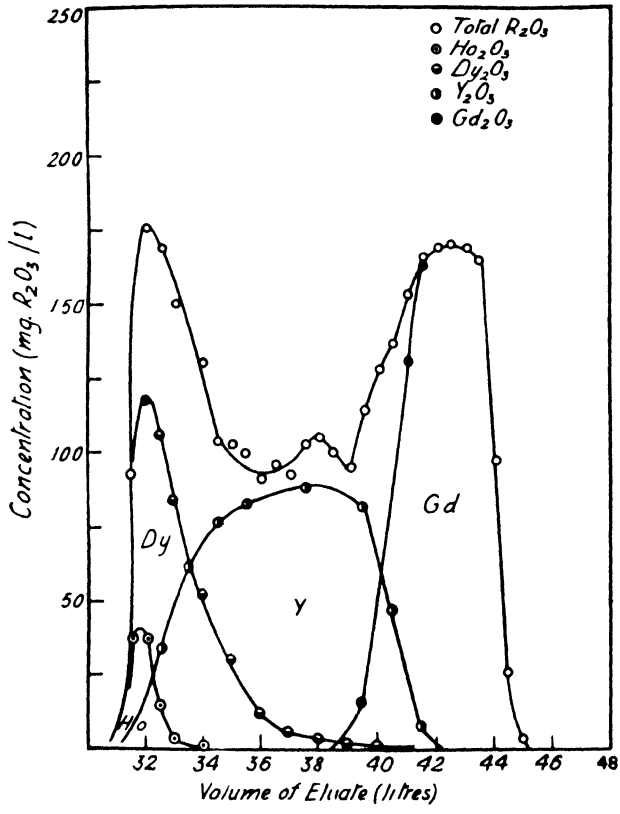

FIG. 19.—Elution of 1·5 g. mixture of Ho, Dy, Y and Gd oxides from an Amberlite IR-100 resin bed, 2·5 cm. diam. and 90 cm. long, using 0·1 % citrate solution at a pH of 5·4.

but where mixtures of 8 or 10 rare earths are to be separated more passes are necessary to separate them into their pure components.

Fig. 18 shows the positions of the elution bands for the various components when the concentrates from Lindsay's yttrium fraction were used. Fig. 19 shows the separation of holmium, dysprosium, yttrium and gadolinium when a pH of 5·4 is used for the eluant.

As an example of the success of these procedures, it might be stated that we now have about 300 g. pure ytterbium, 15 g. spectrographically pure lutecium and almost an equal amount of very rich thulium.

As mentioned earlier in the paper, we have been anxious to obtain the metals of these rare earths and we have been able to develop methods for producing them pure and in appreciable quantities. The process used is essentially the thermal reduction of the halides by means of calcium and the recasting of the metal into cylinders. This process was in the main part developed under our contracts with the Atomic Energy Commission and has not yet been declassified. At present we have a paper about ready for declassification. In the meantime it will not be possible to give further details regarding the method of reduction.

At the time of writing this paper I expect to be able to attend the symposium in England, and if this turns out to be possible I shall exhibit metal bars of pure lanthanum, cerium, praseodymium, neodymium and yttrium as well as some salts of the rarer rare earths. These metals have about the density of iron and are soft like calcium. They react readily with air and we keep them under mineral oil to protect them from corrosion. In a massive bar, while the surface corrodes readily, the rate of corrosion slows down as it penetrates the bar and it is possible, though not desirable, to keep a cylinder exposed to air for several years. It will, however, be encrusted with a thick coating of oxide which can be brushed off readily.

The work described in this paper was carried out in the Ames Laboratory of the United States Atomic Energy Commission during the past four years and the author wishes to acknowledge that it was done in collaboration with the following colleagues and graduate students: E. I. Fulmer, A. F. Voigt, B. O. Ayres, T. A. Butler, P. Figard, E. M. Gladrow, M. Gobush, P. E. Porter, J. E. Powell, N. R. Sleight, A. D. Tevebaugh, R. Q. Thompson, J. M. Wright, V. A. Fassel, D. H. Ahmann, V. Bulgrin, A. H. Daane and C. F. Miller.[14]

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[14] Spedding, Voigt, Gladrow and Sleight, *J. Amer. Chem. Soc.*, 1947, **69**, 2777. Spedding, Voigt, Gladrow, Sleight, Powell, Wright, Butler and Figard, *J. Amer. Chem. Soc.*, 1947, **69**, 2786. Spedding, Fulmer, Butler, Gladrow, Gobush, Porter, Powell and Wright, *J. Amer. Chem. Soc.*, 1947, **69**, 2812. Spedding, Fulmer, Ayres, Butler, Powell, Tevebaugh and Thompson, *J. Amer. Chem. Soc.*, 1948, **70**, 1671. Spedding, Fulmer, Butler and Powell (two papers submitted to *J. Amer. Chem. Soc.* for publication). Spedding, Daane, Ahmann, Miller and Bulgrin (unpublished work).

APPLICATION OF ION EXCHANGE TO THE SEPARATION OF SUBSTANCES IN LOW CONCENTRATIONS

By Edward R. Tompkins

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Ion exchange was investigated as a means of separating substances in low concentration. The programme, which began in an attempt to separate the cationic fission species without addition of isotopic carriers, was so successful that it was continued. A method and an apparatus for separating multi-Curie quantities of fission products by remote control was developed for use in producing pure radio-isotopes. This apparatus has proved useful for a number of other separations in the radio-isotope production programmes of the United States Atomic Energy Commission.

The separation of rare earths was studied by both equilibrium and column methods. Conditions which were satisfactory to separate rare earths, scandium and alkaline earths in very high purities were established by studying the effect of a number of variables on the distribution coefficient and the shape of the elution curves.

The " plate " theory was applied to ascertain the degree of separation of two or more solutes eluted from a column. By determining the column constants and the equilibrium distribution coefficients of two solutes, it was possible to calculate elution curves which closely approximated the curves determined experimentally. The theoretical implications and the general applicability of ion exchange for difficult separations are discussed

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Introduction.—In attempting to devise methods for separating the fission elements without the addition of isotopic carriers, the ion-exchange technique was investigated. Several groups connected with the United States Atomic Energy programme were active in this programme in various capacities. This summary will be limited to the contributions of the group consisting of W. E. Cohn, D. H. Harris, J. X. Khym, G. W. Parker, S. W. Mayer and E. R. Tompkins.[1-8]

In the early experiments the mixture of fission products was adsorbed in a narrow band at the top of a column of Amberlite IR-1 exchanger, and subsequently, the fission products were eluted from the column by replacement with an alkali salt or a strong acid of 0·1 to 1 M concentration. Although the early analytical methods were inadequate, it was obvious from the differential radioactive measurements on aliquots of the eluate that the separations were incomplete.

At about this time, E. R. Russell [9] demonstrated that the fission zirconium and columbium could be desorbed selectively from an Amberlite IR-1 bed upon which a fission product mixture had been adsorbed by passing a dilute solution of oxalic acid through the column. These observations suggested the possibility of using other reagents which would form soluble

[1] Tompkins, Khym and Cohn, *J. Amer. Chem. Soc.*, 1947, **69**, 2769.
[2] Harris and Tompkins, *J. Amer. Chem. Soc.*, 1947, **69**, 2792.
[3] Tompkins and Mayer, *J. Amer. Chem. Soc*, 1947, **69**, 2859.
[4] Mayer and Tompkins, *J. Amer. Chem. Soc.*, 1947, **69**, 2866.
[5] Tompkins, *J. Amer. Chem. Soc.*, 1948, **70**, 3520.
[6] Cohn, Parker and Tompkins, *Nucleonics*, 1948, **3**, 22.
[7] Tompkins, *J. Chem. Educ.*, 1949, **26**, 32, 92.
[8] Tompkins, Harris and Khym, *J. Amer. Chem. Soc.*, 1949, **71**, 2504.
[9] Russell (unpublished work).

co-ordination compounds with the various fission elements. It appeared that it might be possible to select reagents which would selectively elute the fission products in groups (i.e., columbium and zirconium, alkaline earths, rare earths, alkali metals, etc.). Because of the ability of citrate and tartrate to form soluble alkaline earth complexes, they were chosen for the initial investigations. Citrate was employed more extensively than tartrate since the greater solubility of its salts permitted studies over a wider range of concentrations.

Preliminary experiments showed that citric acid in a concentration of 0·23 M would not remove a measurable fraction of any of the fission elements from Amberlite IR-1 after columbium and zirconium had been eluted with oxalic acid. Tri-ammonium citrate was tried next and it was found to remove not only the alkaline earths but also the other fission elements as nearly quantitatively as could be determined by preliminary radioactive measurements. Investigations employing citrate solutions of pH's between that of citric acid (about 1·8) and that of tri-ammonium citrate (about 7·5) showed that a fair degree of separation of all the cationic fission products, including the rare-earth elements, could be effected with this one reagent.[1]

In attempting to improve the separations, particularly of the rare earths, further studies were carried on employing both the column procedure and equilibrium methods. The results of most of these investigations have been reported elsewhere.[2-8] This account will summarize them and give the more important conclusions which have been drawn from these studies, particularly as they apply to the separations of rare-earth and alkaline-earth elements.

Factors Affecting Separation.—The degree of separation of similar solute ions, such as adjacent rare earths, when they are eluted from a column is determined by (i) the separation for each exchange (i.e., the equilibrium separation factor) and (ii) the average number of exchanges that the ions make as they travel down the column.[4] Variables affecting the separation factors may be studied most readily by equilibrium experiments while those that determine the number of exchanges must be studied by column experiments.

Factors Affecting the Equilibrium Separation of Solutes.—A number of factors which affect the separation of solutes were studied by equilibrium experiments.[3] In most of these experiments, a known mass of exchange resin in the ammonium form was shaken with a measured volume of solution of known composition. Radio-isotopes of the solutes being investigated (usually fission products) were employed for facilitating the analyses. Radioactive measurements to determine the changes in the solute concentrations in the solution phase permitted the calculation of the distribution coefficients. There was no appreciable error in the use of this technique since the total solute concentration was usually very low.

A comparison of several exchangers as to their effectiveness in separating adjacent rare-earth elements showed considerable variation.[3] Synthetic resins with methylene sulphonic acid groups were less effective than those with nuclear sulphonic acid groups. Of the latter group, the sulphonated hydrocarbons were superior to sulphonated phenol-formaldehydes.

There is ample evidence from both the equilibrium and column experiments to show that separation is most efficient when the concentrations of the solutes are low.[1 2 3 8] For ionic concentrations below a certain critical value, which varies with the type of exchange resin employed (e.g., $\sim 10^{-7}$ M rare earths for Amberlite IR-1 and $\sim 10^{-4}$ M rare earths for Dowex 50), changes in the solute concentration do not affect the distribution coefficients of the solutes between the solid and liquid phases appreciably, and thus do not alter their separation.[3] At higher ionic concentrations, the distribution

coefficient of each solute is reduced, the net effect being a decrease in their separation. In this respect, the ion-exchange and adsorption methods of separating substances are unique since, unlike most other methods, they are more effective at very low ionic concentrations than for macroscopic quantities of substances.

Complexing agents serve a double purpose in increasing the separation of similar solutes, particularly when they are present in macroscopic quantities. Such substances not only contribute to the separation due to the differences in the dissociation constants of their co-ordination compounds with the solutes, but they also reduce the fractions of the solutes in the ionic form and thus their ionic concentrations. This permits larger quantities of solutes to be separated without exceeding the ionic concentrations above which the separation is affected adversely.

Because of the initial success in separating similar elements by use of citrates, it has been studied more extensively than other complexing agents. Preliminary studies indicated that several other compounds such as tartrates, lactates, sulphosalicylates and acetylacetonates were equally effective or superior to citrates for separating rare earths.[3]

The ideal situation would be to have a specific complexing agent for each substance which would form a very strong complex with it and not form a complex with any other substance. This would permit any mixture of ions to be separated by adsorbing them on an exchanger and subsequently equilibrating the exchanger successively with solutions each containing the complexing agents specific for a single substance.[7] Although this is applicable for some mixtures of dissimilar substances, it cannot be used for separating very similar ions such as the rare earths. A complexing agent for this application should form a water-soluble co-ordination compound with a dissociation constant in the range [3 4] of 10^{-4} to 10^{-6}.

Complexing agents which must be ionized in order to form co-ordination compounds with solutes are affected by changes in pH which determine the fraction in the ionized form. If they have more than one dissociable hydrogen (e.g., citric and tartaric acids), several complexes with the solutes are possible. For separating rare earths the complex with the di-hydrogen citrate ion proved most effective, the separation factor decreasing as the pH was increased.[1 2 3] The alkaline earths were separated most readily at pH 5 to 8, indicating that the completely dissociated citrate ion was functioning.[1 5 6]

The relative strengths of the bonds between an exchanger and similar solutes depend upon the hydrated radii of the solute ions, while the relative strengths of the complexes usually depend upon their non-hydrated radii. The hydrated radius of the rare-earth series increases and the non-hydrated radius decreases with increasing atomic number. Thus the separation effects of the exchanger and the complexing agent supplement each other, the former acting through variations in the relative bond strengths and the latter by varying the relative ionic concentrations of the rare earths due to different degrees of complex formation. Studies in which a sulphonated hydrocarbon resin (Dowex 50) was equilibrated with solutions of rare-earth salts with and without citrate indicated that the separation due to the exchanger and to the complexing agent were of about equal importance.[8] As would be expected, studies with alkaline earths showed the reverse relationship between the atomic numbers and the distribution coefficients of solutes between the citrate solution and the exchanger, radium having the highest distribution coefficient.[1 5 6]

Factors Affecting Column Efficiency.—An exchange resin consists of a highly cross-linked organic matrix with attached sulphonic acid groups, distributed uniformly. However, since the resins are in the form of discrete

particles, the column bed is not homogeneous. To exchange with another ion attached at the surface of a particle an ion in a solution flowing through the column needs only to diffuse through the immobile liquid layer surrounding it. Exchange of an ion within a resin particle is dependent upon ionic diffusion through the resin.

If the flow-rate of solution through a column is sufficiently slow and the particles of exchanger are very small, the differences between the behaviour of the ions at various distances from the surface of the particles will be minimized.[4 7 8] Increasing the rate of diffusion by raising the temperature likewise will result in a closer approach to equilibrium.[10]

If a very small quantity of solute ions are first adsorbed in a narrow band at the top of a column and then a solution containing other ions passed through the column, a portion of the solute ions will be replaced from their original resin attachments and be carried down the column by the solution so that they come into contact with fresh exchanger. As each solute ion in solution passes an exchange position, there is a certain probability that it will exchange.[4 7]

When a column is operated in such a manner that equilibrium conditions are approached, the solute ions will travel a certain average distance through the column before exchanging. For each exchange, the separation between solutes will approach the equilibrium separation factor.[4 7] Therefore, for purposes of calculating separations of solutes, a column may be considered as being made up of a number of " batches " of exchanger, each successively equilibrating with portions of solution of volumes sufficient to occupy the void spaces around the particles of exchanger. To adopt more commonly used terminology, these elements of the column may be referred to as " plates."

The application of the " plate " theory to ion-exchange column separations [4] is very similar to its application to partition chromatography.[11] For this reason it will not be discussed here. Its applicability for analyzing individual elution curves and for calculating the degree of separation of solutes with overlapping elution curves has been demonstrated amply.[4 7] By determining the equilibrium distribution coefficients of any two solutes and their separate elution curves from a short column, it is possible to predict accurately how much elutrient must be passed through a column of any other size before solute first appears in a measurable concentration in the eluate. Furthermore, the calculations are simple and the method is easy to apply, especially if the analyses are made by radioactive techniques.[7]

Any factor which changes the average distance a solute ion travels down a column between exchanges will affect the column efficiency. Thus the flow-rate is of prime importance since, as it is increased, the ions are carried at a faster rate down the column and so have less time to diffuse to an exchange position. The size of the resin particles determines the size of the liquid interstices between them and thus the average distance an ion must diffuse to reach the particle. Temperature which increases the diffusion rate increases the number of exchanges in a given column length.[10] Likewise, the exchange capacity of the resin (i.e., the number of exchange positions per unit weight) affects the efficiency.[4] The sulphonated hydrocarbons with exchange capacities of about 6 m.-equiv./g. are far superior to the sulphonated phenol-formaldehydes with capacities of one-third to one-half of this amount.[2 3 5 8 10]

In addition to these factors, the distribution coefficient affects the column efficiency since it determines the fraction of time the ions are in solution

[10] Ketelle and Boyd, *J. Amer. Chem. Soc.*, 1947, **69**, 2800.
[11] Martin and Synge, *Biochem. J.*, 1941, **35**, 1385.

and thus the distance they will be carried down the column between exchanges. The use of 0·23 M citrate solutions at pH above 3·0 (or higher citrate concentrations at somewhat lower pH) tends to give poor separations of rare earths.[1 2 3 8] Likewise, the use of high concentrations of strong acids and solutions of their salts give poor separations of similar solutes.

All of the factors discussed above, which affect column efficiency, are interdependent.[7] For example, decreasing the flow-rate will compensate for increasing the resin particle size, the use of an elutrient which will give a low distribution coefficient, etc.[4 8] For this reason, it is difficult to study the effect of column variables on efficiency. A large quantity of data was accumulated without adequate control of the different variables before this fact became apparent.[1 2 6 8] Although they were helpful in establishing optimum operating conditions for some separations, they are of doubtful value for theoretical interpretations.

Factors which Affect the Shape of Elution Curves.—The theoretical elution curves for solutes in low concentrations approach the normal curve of error.[4 11] Deviations in form from the theoretical curves usually indicate decreases in separation.[1 2 4 6 8] The most common form of elution curve is that which is asymmetrical with a steeper slope on the leading than the trailing edge.

The following are a few of many causes for asymmetry in elution curves:

(i) Too rapid a flow-rate and/or too large particles of exchanger will cause a graded behaviour of ions depending on their positions in the resin particles. This results in a long trailing edge of the elution curves and thus in an increased overlap of curves for similar solutes.

(ii) A quantity of solutes so high or an elutrient which will give a low enough distribution coefficient that the concentration of solute exceeds that value above which the distribution coefficient is affected will produce asymmetry of elution curves. This condition usually prevails in only that portion of the column having the highest concentration of solutes. This causes all similar solutes to move more rapidly in this zone, thus giving asymmetrical curves with steep leading edges and decreased distances between them.[8]

(iii) When a complexing agent such as ammonium di-hydrogen citrate is used to elute a rare earth from an exchanger which is principally in the hydrogen form, a pH boundary will be formed in the column.[6 8] This is caused by the conversion of the hydrogen-form resin to the more stable ammonium form. But the citrate is thereby converted to citric acid which has a very low concentration of complex-forming citrate ions. Thus the solute ions are re-adsorbed from the citrate solution at this boundary. If the pH of the elutrient is sufficiently high to cause the solutes to move faster than the pH boundary, they will concentrate at that boundary and be eluted in high concentration, when this boundary reaches the bottom of the column, giving curves whose degrees of asymmetry depend upon the relative rates of the boundary and the solutes. If, on the other hand, the pH boundary moves down the column faster than any of the solutes, their elution curves will be of the same form as though they had been eluted from a column of exchanger originally in the ammonium form.[8]

In addition to decreasing the separation, conditions causing asymmetrical elution curves do not lend themselves readily to calculations of the probable degree of separation. For this reason, it usually is preferable to choose operating conditions which will give symmetrical elution curves, particularly in exploratory work.

Conclusions.—Applications of the method developed for separating fission elements by members of the group named above have resulted in the following:

(i) The separation, concentration, purification and metathesis of multi-Curie quantities of each cationic-fission product, including the rare-earth elements, with specific activities as high as several Curies per milligram of total solids and in radiochemical purities greater than 99·9 %.

(ii) Separation and purification of gram to kilogram quantities of each rare earth in purities greater than 99·9 % and in some cases with estimated purities greater than 99·99 % (upper limits of impurities determined by activation analysis). Also, it was demonstrated that adjacent rare earths differing in concentration by a factor of 10^9 could be separated with approximately the same efficiency as when they were both present in comparable concentrations.

(iii) Purification of several hundred grams of scandium to such a degree that the levels of impurities were below detection even by means of activation analysis.

(iv) Separation of macroscopic quantities of strontium, barium and radium in high purities.

(v) Demonstration of the applicability of this method for separating similar substances of many kinds both organic and inorganic.

The experimental work, the results of which are summarized here, was performed at Oak Ridge National Laboratory, Oak Ridge, Tennessee.

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GENERAL DISCUSSION

Dr. B. A. J. Lister (*A.E.R.E., Harwell*) said : The mechanism of adsorption of cations on alumina described by Dr. Sacconi is interesting to me in view of some tests I carried out to study the effect on the adsorption series of replacing water as solvent by a non-ionizing solvent, dioxan. I examined the adsorption of nine cations : Fe^{3+}, Ni^{2+}, Ag^{+}, Co^{2+}, Cr^{3+}, Zn^{2+}, Cu^{2+}, Cd^{2+} and Ce^{3+} and found the order of preference considerably different from that found in aqueous solution or with any of the tried complexing agents

Dioxan	Water	Ammonia	Tartrate
Fe^{3+}, Ni^{2+}	Fe^{3+}, Cr^{3+}	Co^{2+}	Cd^{2+}
Ag^{+}, Co^{2+}, Cr^{3+}	Cu^{2+}	Zn^{2+}	Cu^{2+}, Zn^{2+}
Zn^{2+}	Ag^{+}	Cd^{2+}, Cu^{2+}	Fe^{3+}, Cr^{3+}
Cu^{2+}	Zn^{2+}	Ni^{2+}	Co^{2+}
Cd^{2+}, Ce^{3+}	Cd^{2+}	Ag^{+}	Ni^{2+}
	Ni^{2+}		
	Co^{2+}		

For example, the order of adsorption of Cu^{2+} and Co^{2+} ions was reversed and only reverted to the normal order for aqueous solution when there was about 5 % water present. Similarly in dioxan solution, silver is more strongly held than copper whereas the reverse is the case in aqueous solution. In the dilute salt solutions used there was generally a trace of water present from the hydrated salt but this was never $> 0{\cdot}5$ %. From the colour of the solutions of the nitrates of copper, cobalt, nickel, etc., in dioxan and from the colour of the adsorbed bands it seems probable that the metals are present not in ionized form but as neutral molecules both in solution and in the adsorbed state.

In this case the hypothesis of the water providing the adsorption link is perhaps modified. The cupric ion, for example, has 4 water molecules in the

inner hydration shell, but possibly in a non-ionizing solvent where the copper nitrate may occur as neutral molecules there are fewer water molecules per copper atom. Incidentally I may add that, contrary to Dr. Flood's experience, we have never found silver to be more strongly adsorbed than copper from aqueous solution.

Dr. L. Sacconi (*Florence*) (*communicated*) : The hypothesis of a hydrolytic adsorption of electrolytes on alumina holds, of course, only for aqueous solutions of aquo-ions. The adsorption of inorganic salts from solutions in non-ionizing solvents is probably molecular. Anhydrous $FeCl_3$ dissolved in benzene or toluene, e.g , is firmly adsorbed on an alumina column forming a yellowish-brown band. Development with KCNS or $K_4[Fe(CN)_6]$ solution has no effect. The red or blue colour produced by Fe^{***} ions takes place only after washing with HCl solution, which evidently splits off Fe^{***} ions from the adsorbed undissociated $FeCl_3$.

The different order of preference of many cations from the different solutions, according to the different solvents and complexing agents employed, confirms that atomic groups co-ordinated around cations play a part in chromatographic adsorption. This is also confirmed by the results reported by Dr. Lister about the influence of water mixed with dioxan. It is evident that 5 % water is enough to form aquo-ions from the dissolved Cu and Co salts

Mr. R. A. Wells (*Teddington*) said : In answer to Dr. Angell's question regarding the lower limit of concentration of nickel which may be estimated by the cellulose column method, we have made no accurate measurements of the limits of the process, but from our experience there appears to be no difficulty in separating very large amounts of cobalt and copper from small amounts of nickel. Dr. Davies informs me that he has estimated, semi-quantitatively on a paper strip, 0·03 % of nickel in cobalt metal.

Dr. Pollard mentions the difficulty of detecting the alkali metals on paper strips. The following method may be of interest : we have found when using a neutral chloride solution for the separation of lithium, sodium and potassium that the metals move as their salts ; thus, by testing the strip for chloride, by spraying with silver nitrate and warming it has been possible to locate the position of the cations.

Dr. F. H. Pollard (*Bristol*) (*communicated*) : It should be pointed out that the kojic acid oxine spraying reagent which we describe in our paper is satisfactory for the identification of the position of the alkali-metal ions.

With regard to the test described by Mr. Wells, we should like to observe that in our experience and that of Westall [1] the anions may under some conditions travel independently of the cations present on the filter paper. Thus it would seem that any test for a cation which depends on the presence of the anion at the same spot must be used with caution.

Dr. E. Glueckauf (*A.E.R.E., Harwell*) said : Mr. G. Robinson mentions that the 8-hydroxyquinoline columns cannot be applied to steel analysis on account of the large amount of iron present. This difficulty can be obviated by removal of the iron by partition chromatography on Highflow Super-cel saturated with 6·5 M HCl (aqueous). The steel, dissolved in HCl, is concentrated as much as possible and the HCl content is brought up to 6·5 M. This solution is put on top of the column, and then developed with ether previously shaken with 6·5 M HCl (aqueous). (This figure is fairly critical.) Instead of ether, *iso*propyl ether may be used with advantage. Under these circumstances only iron will be found in the ether eluate, while all the other metals remain in the column. They can subsequently be eluted with aqueous solutions and subjected to the 8-hydroxyquinoline treatment.

Mr. G. Robinson (*Sheffield*) said : Dr. Glueckauf's transfer of the familiar Rothé ether separation to the chromatographic column is a masterly adaptation of an accepted technique to modern methods. At first sight it appears to be an ideal method of eliminating iron from steel solutions and I shall be happy to try out the method prior to adsorption on an 8-hydroxyquinoline column.

[1] Westall, *Biochem. J.*, 1948, **42**, 249.

Prof. L. Zechmeister (*California*) said : It is proposed that the brush method as applied in collaboration with Dr. Frehden [2] to the detection of certain metals on the alumina column could be further developed. By making use of a special brush (dipped in a specific colour reagent) for each metal and by drawing parallel streaks along the column, the respective metals can be easily detected, even if no clean geometrical separation had taken place.

Mr. G. Robinson (*Sheffield*) said : I have used a technique which involves the application of dimethylglyoxime as a specific reagent for nickel in a manner analogous in some respect to Prof. Zechmeister's brush method. If one pre-coats alumina with dimethylglyoxime from alcoholic solution and then prepares a column from the dried material it is possible to pass an alkaline solution of a steel (with the iron complexed as tartrate) through the column and obtain a pink band due to nickel at the top. The length of band is proportional to the nickel content and by use of suitable standards one can arrive at an approximation of the nickel percentage

Similar results for copper in non-ferrous alloys can be obtained on a column of alumina pre-treated with sodium di-ethyldithiocarbomate.

Prof. A. Tiselius (*Uppsala*) said : It ought to be quite interesting to try the application of displacement development in the separation of ions on the ion-exchange resins, on alumina (Schwab) or on alumina-impregnated filter paper (Flood). The experiments of Partridge [3] demonstrate the application of this procedure to some organic bases and amino acids, and it is obvious from the results of Flood and Schwab that a strong mutual displacement often takes place, and that addition of a displacement agent in a suitable concentration is likely to make the separation more complete Thus it may be possible to utilize the capacity of the columns (i e., the filter paper) to its full extent

Also, I would like to ask what is considered to be the most favourable dimensions of columns, both for ion-exchange and other types of chromatography

Dr. E. Glueckauf (*A.E.R.E , Harwell*) said . The important question : what is the optimum length/diameter ratio for a column is to some extent answered by eqn (3*a*, *b*, *c*) and (4*a*) * which contain the variable A, giving the amount of

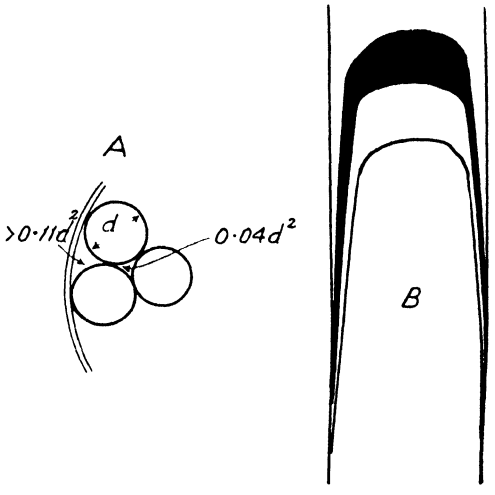

FIG. 1.– Wall-channelling due to difference in pore areas.

adsorbent per cm. length of column. We see that A enters only in those cases where finite grain size and longitudinal diffusion are the disturbing factors, and not in the case of non-equilibrium. In the latter case the length/diameter ratio is

[2] *Bull. Soc. chim. Biol.*, 1940, **22**, 458.
[3] This Discussion.

* See p. 202

therefore immaterial, while in the former case decreased diameter offers advantages provided that the total amount of sorbent in the column is constant. But some reservations must be made due to features which are not considered in these equations. If the column is too short and fat, disturbances due to channelling and tilting of the boundaries become predominant, effects which cannot be assessed numerically.

If the column chosen is too thin and long then the area of the larger pores at the wall (see Fig. A) becomes an appreciable fraction of the total cross-sectional area of the column and severe wall-channelling arises leading to a boundary form in the column of the type (Fig. B) which causes mixing of the different separated solutes in the eluate.

This effect disappears again when extremely thin micro-columns, with diameters which are a small multiple of the grain diameter only, are used In this case, lateral diffusion and convection are sufficiently effective in turning the sharp but tilted boundaries into diffuse horizontal ones, which can be sharpened up again by the chromatographic process. We thus see that there must be a range of column diameter somewhere between, say, 5 and 50 grain diameters which is less favourable for chromatographic separations, but it does not seem possible to define this region precisely.

For substances difficult to separate ($(K - 1) < 0{\cdot}1$) extra allowance must be made for the fact that the boundaries spread over a considerable length depending on the values of $(K - 1)$ and on the height of the theoretical unit Δ/A. In order to obtain a pure product (> 99 %) the length of the column must exceed the value $\left(\frac{K}{K-1}\right)^2 \frac{\Delta}{A}$ by a considerable factor (> 30) depending on the ratio C_1°/C_2°.

Thus we find that whilst there are quite a number of considerations which have to be weighed against each other, it is usually possible to make reasonable estimates of the required column conditions beforehand.

Dr. W. M. Smit (*Amsterdam*) said: In connection with this it is noteworthy that we found experimentally that in development the separation efficiency of a column is not affected if the weight ratio sample/adsorbent is kept constant and the column is properly packed.

Dr. R. Lessing (*London*) said: There is a close analogy between the problem of dimensioning adsorption columns and the chemical engineer's problem in designing packed towers and selecting the best combination of diameter, height, type and size of packing elements. The complexity of the disturbing factors militates against the achievement of the perfect design, and one cannot hold out much hope that the desire of Prof. Tiselius to find the ideal dimensions of an adsorption column will find a satisfactory answer in the foreseeable future.

Dr. E. Glueckauf (*A.E.R.E., Harwell*) said: Dr. Synge asked why in Fig. 9 in Spedding's paper the increase of grain size results, in addition to the usual broadening of the bands, also in an increase of the breakthrough volume. The reason for this is that here we have solute in bulk and not in tracer quantities. Consequently we have a curved isotherm which is also shown by the asymmetrical form of the bands. The self-sharpening front of such a band moves with a velocity which is the greater, the higher the frontal concentration. Larger grain size results in less complete equilibrium which leads to a greater spreading of the band. This involves decrease of the frontal concentration, and hence, for curved isotherms, a reduced travel rate of the front. This effect cannot occur at tracer concentrations because there we have a linear isotherm.

Mr. C. D. Cook (*Widnes*) said: Using a *n*-hexane-nitromethane-silica system and solutes of linear isotherms, I have found that, in addition to the factors influencing the asymmetry of the elution curves enumerated by Dr. Tompkins, one of the major factors was in the silica itself.

Having examined a large number of commercial silicas, and silicas prepared in the laboratory under differing conditions, it was found that the silicas could be divided into two groups:

(*a*) silicas in which the elution curves were approximately symmetrical;

(*b*) silicas in which the elution curves were asymmetrical. The elution curves usually exhibited a right-hand skew.

It has been found possible to predict whether or not the elution curves will exhibit asymmetry from the appearance of the silica under the electron microscope. I hope to have more to say on the characterization of silicas and the dependence of reproducibility of the elution curves on the type of silica, using the electron microscope, in the near future.

Impure nitromethane, used as the immobile phase, containing higher nitroparaffins, resulted in asymmetrical elution curves Loosely packed columns, in which the gel volume decreases during elution, usually results in asymmetrical elution curves.

It is essential that rigid gel packing techniques should be followed when preparing a column for elution purposes. We have found that it is possible to reproduce gel volumes to within ± 5 ml, and for some silicas even within ± 3 ml., on 200–300 ml. columns when silicas from the same batch are used.

Prof. C. W. Davies (*Aberystwyth*) said: Mr. Nancollas at Aberystwyth has studied carbonate-hydroxide equilibria on the strong base resin Amberlite IRA-400. As would be expected, the divalent ion is preferentially held by the resin. This can be turned to good account in the preparation of CO_2-free alkali, as the whole of the carbonate can be removed when a caustic soda solution, prepared without special precautions, is run through a suitable column of the resin

II(B). ORGANIC AND BIOCHEMICAL

THE CHROMATOGRAPHY OF GASES AND VAPOURS

By C. S. G. Phillips

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A simple self-recording apparatus is described for the analysis of gas and vapour mixtures by displacement from an adsorbent. The influence of factors, such as particle size, flow-rate, column design, type of adsorbent, temperature and nature of the mixture, upon the accuracy of the analysis is discussed. Illustrations are given of the application of the method.

The separation of gas and vapour mixtures by fractional desorption from an adsorbent has been investigated by a number of workers. A list of references is given by Claesson.[1] Two general methods have been employed. In the first the gases are removed from the adsorbent by driving a heater along the column. This method has recently been applied by Turner,[2] and an instrument employing the principle is now available in America for the analysis of gas mixtures containing light hydrocarbons.[3] In the second method, an inert carrier gas is passed through the column of adsorbent, and plays a part similar to that played by the solvent in liquid chromatography. The various components of the mixture are desorbed one by one, to produce an "elution" curve as in Fig. 1A. This method has been considerably improved by Claesson,[1] who employed a carrier gas containing a constant concentra-

[1] Claesson, *Arkiv. Kemi, Min. Geol. A*, 1946, **23**, No. 1.
[2] Turner, *Oil Gas J.*, 29th April, 1943; *Petroleum Refiner*, May, 1943.
[3] *Bull No. 205, Burrell Technical Supply Co.* (Pittsburgh, Pennsylvania).

tion of a vapour more strongly adsorbed than any of the components of the mixture. The displacement curve thus produced is illustrated in Fig. 1B. Each step corresponds to one component, and Claesson was able to demonstrate that, for a wide range of hydrocarbons, the step height was constant and characteristic for any particular component, and the step length was proportional to the amount of the component present in the mixture.

We have been applying Claesson's method to the analysis of the products obtained from experiments on gas kinetics, and, while the work is still very incomplete, it was felt that it might be valuable to indicate some of the main points that have so far emerged, if only to call attention to the simplicity and efficiency of the method. A full report of the work will be published later.

Experimental

The apparatus (Fig. 2) consists of a device A for the production of a constant flow of nitrogen (carrier gas), means for passing the mixture on to the column of adsorbent B, and for the introduction of a constant concentration of displacer into the nitrogen stream C, the column D and the analyzer E.

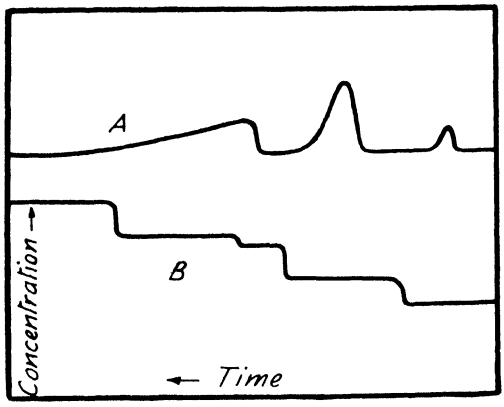

FIG. 1.—A. Elution analysis. B. Displacement analysis.

Nitrogen, obtained from a cylinder through a large buffering volume and a needle-valve, is passed through a simple compensating flowmeter, designed by Clark,[4] and illustrated in Fig. 3 In this device, the rate of gas flow, in the direction of the arrows, is fixed by the pressure of water BC across the capillary A. B is constant as long as the stream of bubbles is maintained, but C will alter with any change of the pressure inside the system, the difference in pressure between the system and the atmosphere being given by the height CD (+ EF). However, if the diameter of the inner tube is considerably less than that of the outer tube, the change in the internal pressure has normally only a negligible effect upon C. Any very marked alteration of pressure can be counteracted by adjusting the level of the water in the side tube EF.

The mixtures are conveniently brought on to the column by condensing them in a liquid-air trap, through which the nitrogen can subsequently be passed. The constant concentration of desorbent is obtained by passing the nitrogen through a saturator. We have used ethyl acetate in a bath at 0° C for most of the work hitherto. The column, a glass tube built in sections (see below), is surrounded by a jacket, which enables a vapour to be distilled around the column, so that experiments can be conducted at a series of temperatures. The column can readily be removed from the jacket for filling and cleaning.

[4] Clark (British Oxygen Co.) (private communication)

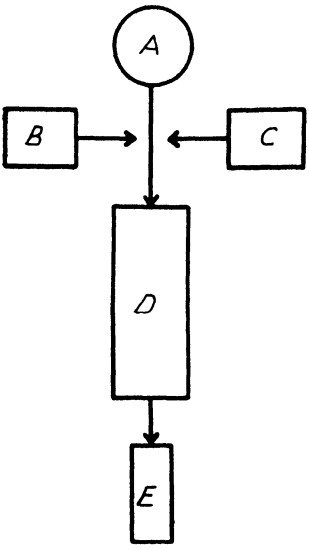

FIG. 2.—The apparatus.

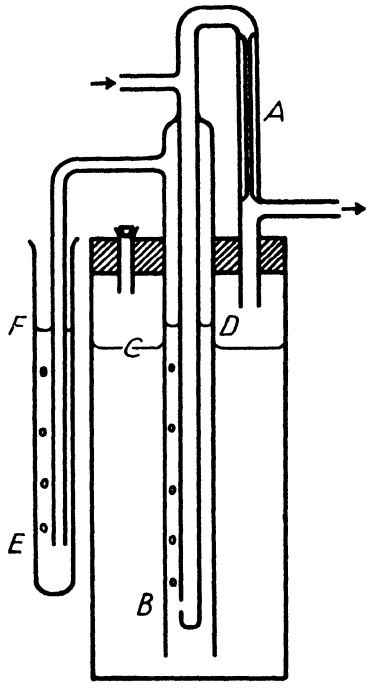

FIG. 3.—Compensating flowmeter (Clark)

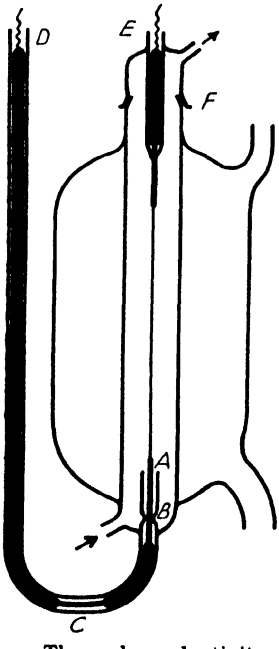

FIG. 4.—Thermal conductivity cell.

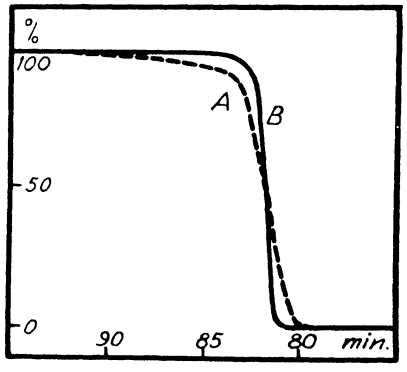

FIG. 5.—Fronts for ethyl acetate (in N_2, 80 ml./min.).

A. Through 9 mm. diam. column (4 cm^3 activated coal charcoal).

B. Through the same followed by $2\frac{1}{2}$ mm. column ($\frac{1}{2}$ $cm.^3$ charcoal).

A thermal conductivity cell constitutes the analyzer. This consists (Fig. 4) of a Pt wire (14 cm long, 0·05 mm. diam.), heated electrically and passing centrally down a glass tube (1·0 cm. diam.), which is again surrounded by a jacket so that the cell can be operated at a variety of temperatures. The wire is held in position by a Pt weight A, which passes through a narrow constriction B into a Hg pool. C is a Pt wire sealed into the glass. Electrical contact is made at D and E. The ground joint F enables the wire to be removed and replaced with ease. The wire forms one arm of a Wheatstone bridge, the other three arms being similar wires kept in sealed tubes immersed in a thermostat. The voltage for the bridge and for heating the wire is supplied by a high-capacity 2-volt battery. A recorder (Ether Indicorder, full-scale deflection 1·8 mV, chart speed 1 in. in 5 min.) is placed across the centre of the bridge.

Provision has also been made for measuring the densities of the various components with a quartz buoyancy microbalance, previously used for atomic weight determinations. This, however, has proved unnecessary.

The accuracy of the analysis will be related to the degree of overlap between one component and the next, i.e., to the sharpness of the steps in the displacement curve. This has been found to depend upon a number of factors.

(i) PARTICLE SIZE.—In general, it is found that the sharpness of the step fronts is increased by diminishing the size of the particles of adsorbent and by increasing their homogeneity. In the work hitherto, we have found no marked advantage in reducing the size of the particles below B.S.S. 40 mesh, while smaller particles are inconvenient as they impede the flow of gas. Dust must be carefully avoided as it deposits on the tubes away from the rest of the adsorbent, and thus produces irregular and diffuse fronts.

(ii) FLOW-RATE.—The fronts are improved by slowing down the rate to about 50 ml /min., though below this rate the improved sharpness has been insufficient to merit further reduction. Flow-rates greater than 100 ml /min tend to produce irregularities in the record of the analyzer.

(iii) COLUMN SIZE.—The sharpness of the fronts increases markedly with a lowering of tube diameter. For a given diameter the maximum separation is achieved in a remarkably small length of tube. In order to obtain sharp fronts, and yet to be able to work with fairly large quantities of material without resort to very long tubes, the column is built in sections, each section smaller than the one above and preceding it. The effect of this is shown in Fig. 5. The column in use at the time of writing is made of three 10 cm. lengths of tube, with internal diameters of 15 mm., 8 mm. and 2 mm. The adsorbent in each section is supported on a disc of wire-gauze. The same type of divided column has been used by Claesson for work with liquid chromatography.[5]

(iv) ADSORBENTS.—Activated charcoals have been used in most of the work hitherto. These are carefully sieved and dried at 120° C. Slightly improved fronts are obtained if the charcoal is first saturated with the desorbent (ethyl acetate), which is then removed by distilling, e.g., with boiling nitrobenzene round the column. For work with the more volatile gases (e.g., propane) a highly activated charcoal is recommended to prevent elution. An increased sensitivity is obtained by use of weakly adsorptive charcoals, owing to the increase in the specific length (step length per unit of component). This is particularly important with the higher molecular-weight components which have the smaller specific lengths. The same effect can, however, be obtained by using a more strongly adsorbed desorbent or a lower concentration of desorbent, by saturating the charcoal at an elevated temperature with a substance which will not be displaced or appreciably eluted, or by raising the temperature of the adsorbent.

(v) TEMPERATURE.—The effect of temperature is illustrated by the figures in Table I. The temperature of the thermal conductivity cell was the same (20° C) throughout. It will be noted that for each gas the product of step height and specific length is virtually constant, the thermal conductivity being approximately a linear function of the concentration.[1]

Sharp steps have been obtained with a range of organic vapours including hydrocarbons (saturated, unsaturated and aromatic), halogenated hydrocarbons,

[5] Claesson, *Arkiv. Kemi, Min. Geol. A*, 1947, **24**, No. 16.

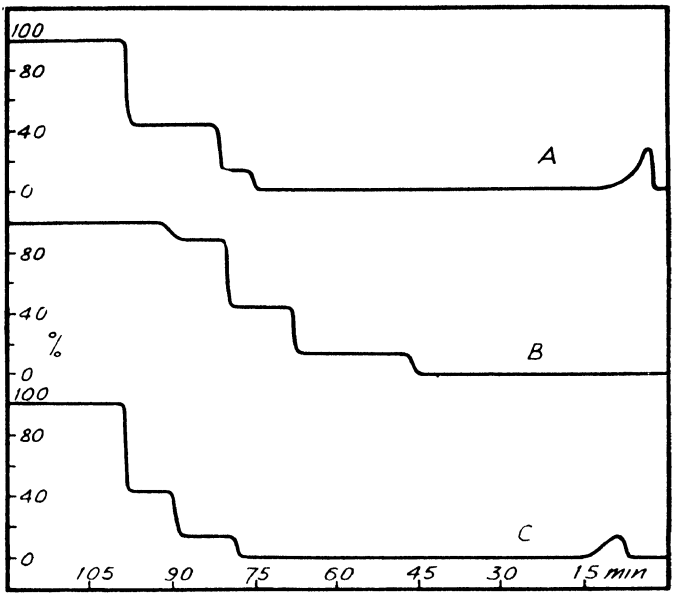

FIG 6.—Displacement analyses of hydrocarbon mixtures.

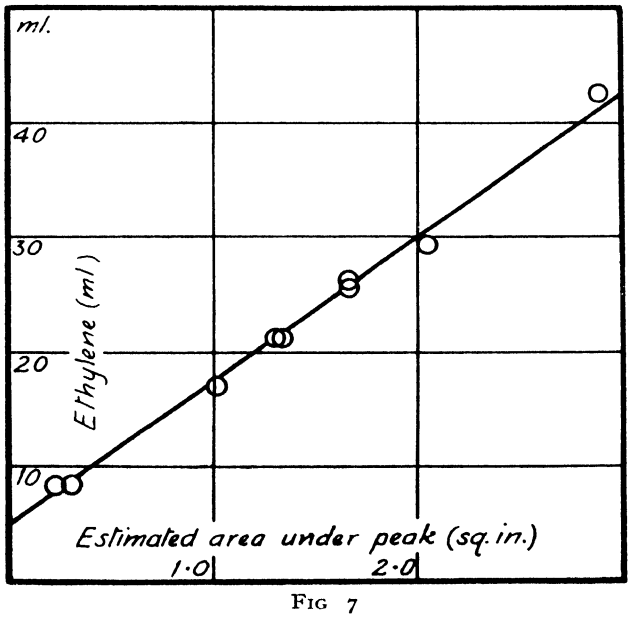

FIG 7

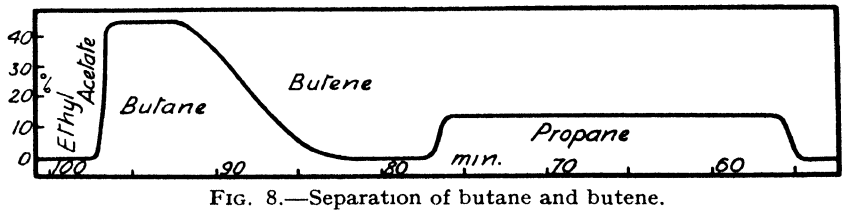

FIG. 8.—Separation of butane and butene.
Adsorbent: Activated coal charcoal, flow-rate: 80 ml./min.

nitroparaffins, ethers, ketones, alcohols and esters. Some typical curves are reproduced in Fig. 6, and the corresponding analyses in Table II.

Ethylene is not displaced as a step, and the calculations have to be made by measuring the area under the peak It is impossible to do this completely owing to the fact that the tail soon becomes indistinguishable from the record for N_2. However, if a plot is made of ml. ethylene (1 ml. = 0·00125 g.) against estimated area under peak, a straight line is obtained (Fig. 7), and from this the concentration of ethylene can be calculated. It will be noticed that the line does not pass through the origin, thus indicating the amount of material unobserved in the tail.

TABLE I

Gas	Propane		Butane	
Temperature (° C)	Step height (% of Ethyl Acetate)	Specific length (in /g)	Step height (% of Ethyl Acetate)	Specific length (in /g)
126·5	No step	—	12·0	135
57	7·0	232	30·5	53·0
20	17·5	95·5	44·0	36·3
6·5	23·5	71·7	47·0	34·4

Adsorbent · Dorsite (coconut charcoal) ; flow-rate : 80 ml /min.

TABLE II

Analysis	Component	Step height (% of Ethyl Acetate)	Found value (g)	Correct value (g.)
A	Ethylene	—	0·0215	0·0213
	Propane	14·0	0·0038	0·0034
	n-Butane	43·5	0·0408	0·0413
B	Propane	14·0	0·0171	0·0167
	n-Butane	43·5	0·0334	0·0331
	n-Hexane	86·5	0·0265	0·0261
C	Ethylene	—	0·0108	0·0107
	Propane	13·5	0·0085	0·0083
	n-Butane	43·5	0·0246	0·0246

Adsorbent . 2 cm.³ activated coconut charcoal (Sutcliffe, Speakman No. 208C) , flow-rate . 40 ml /min.

The diffuse region between two components corresponds in almost all cases to flow period of about two minutes This region is slightly increased between alcohols, a fact presumably to be related to their high association. The separation is particularly marked along an homologous series but with substances of similar boiling point the separation is less perfect and, moreover, is masked by the fact that two such substances will often have almost the same step height. It is, therefore, often convenient to combine the column separation with some other means. For example, butane and butene appear to come off together as one step, but if the gases, before entering the thermal conductivity cell, are passed through a tower, containing pumice soaked in conc. sulphuric acid, the butene is removed and a curve as in Fig. 8 is obtained. From this the percentage of each component is readily calculated. In this case the diffuse region corresponds to 10-min. flow period (i.e., 0·050 g. of the butane-butene mixture).

An alternative method can be devised using silica gel, for we have observed that, with the exception of ethylene, the unsaturated hydrocarbons are not displaced from silica gel by ethyl acetate, while the saturated hydrocarbons are removed readily.

A rather surprising feature of the method is that the accuracy with which the amount of one component is determined seems to be independent of the amounts of the other constituents present. The method is, therefore, very suitable for the estimation of a small percentage of one component in a relatively large amount of a mixture. Thus, in one series of experiments to determine the possible ether content of a sample of ethylene, no step corresponding to ether could be produced from 15 g. ethylene. 0·0714 g. ether was then mixed with the same amount of ethylene, and a step of 3·25 in. was obtained for the ether. The same quantity of ether, in the absence of the ethylene, gave a step length of 3·22 in. Fig. 9

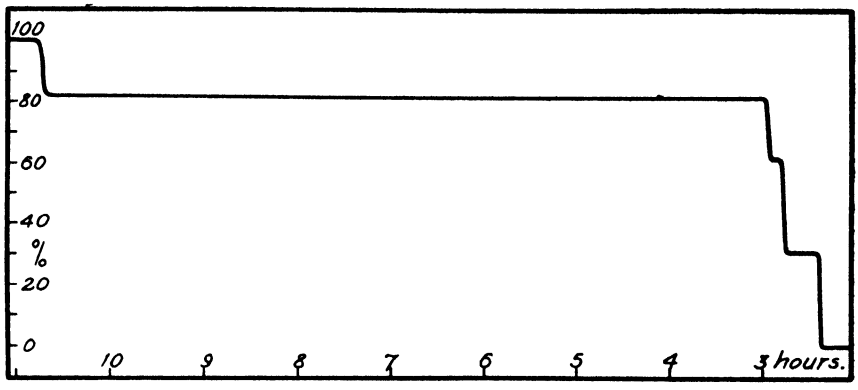

Fig. 9.—Analysis of residue from fractional distillation column
Adsorbent: 19 cm.³ activated coal charcoal; flow-rate · 40 ml./min.

illustrates an analysis of a residue (approximately 1·5 ml.) from a fractional distillation column, in which 2·1 (± 0·1) mole-% of C_5 hydrocarbons and 8·6 (± 0·2) mole-% of C_4 hydrocarbons were separated from the main component, *n*-hexane.

Self-sharpening fronts and, in the case of elution, diffuse tails are to be expected, on the simple theory, for substances whose adsorption isotherms are convex towards the pressure axis. This has been generally observed. Conversely for substances, whose adsorption isotherms are concave towards the pressure axis, diffuse fronts and sharp tails should be observed. This effect is demonstrated in Fig. 10, which illustrates the elution of water from charcoal.

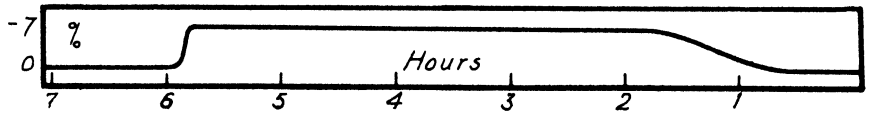

Fig. 10.—Elution of water from charcoal

As a result of this behaviour water is not displaced as a single step by a desorbent such as ethyl acetate, but, if present in any but very small amounts, it produces a number of steps in company with other components. The length of the step, e.g., containing ethyl acetate and water, can be used as a measure of the water present, but in general much confusion is avoided if the mixture can be kept moisture-free.

The behaviour of aldehydes is curious. They are not displaced from charcoal by ethyl acetate or other similar desorbents. If, however, the charcoal is wet or the aldehyde contains water, then a normal step is produced. Further, while an aldehyde will tenaciously resist elution in N_2, it is readily removed by water vapour, or by the vapours of either methyl or ethyl alcohol. In each case the

aldehyde is desorbed in company with the vapour in question. The aldehyde, under such circumstances, can be used to displace other materials from the column.

The author wishes to thank Dr. S. Claesson, Prof. Sir Cyril Hinshelwood and Dr. B. Lambert for their valued advice and encouragement.

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Oxford.

CHROMATOGRAPHY OF PETROLEUM HYDROCARBONS

By W. M. Smit

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A review of applications of the adsorptive percolation method using petroleum hydrocarbons is given. The development method with a series of developers is recommended if a more detailed separation of petroleum fractions is the aim. It is shown that, at least on silica gel, the hydrocarbons of a certain group arrange themselves during development according to their refractive indices. The results of adsorptive percolation through a silica gel column have been compared with a treatment in a thermal diffusion column, both applied to a completely hydrogenated lubricating oil-fraction. In the case studied, the result of adsorptive percolation is somewhat superior. The results of percolation of two other lubricating oils are discussed.

The use of the word chromatography in connection with hydrocarbons is mainly based on historical considerations. Other expressions less stressing the colour element for indicating the adsorptive separation method of Tswett, such as adsorptive percolation, might be more suitable. For, excepting aromatics which show fluorescence when exposed to ultra-violet light, hydrocarbons are colourless. Consequently, with hydrocarbons the process of adsorptive percolation is nearly always continued until all the constituents of the mixture have left the adsorption column and the effluents are analyzed separately, mainly by optical means (liquid chromatography). Besides this special feature, it is more important to realize that petroleum fractions are usually very complex mixtures of various groups of closely related substances. This accounts for the moderate success of adsorptive percolation when applied to petroleum fractions. Furthermore, the questions arising with adsorptive percolation of hydrocarbons are quite comparable with those arising with other compounds. These questions are : which method of adsorptive percolation is most suitable for the purpose aimed at, which adsorbent and which solvents may give the best results, and how is the separating efficiency as compared to other separation methods.

As to the methods, both theoretical and experimental work have now led to the general opinion that when a non-quantitative separation is required, the elution method (displacement method) may give good results. When incomplete separation into two fractions is sufficient, or when not too complex mixtures have to be analyzed, the continuous introduction method (frontal analysis) may be suitable. However, when complex mixtures have to be investigated or complete separation is aimed at the development method has to be chosen if its use is possible.

Concerning the adsorbents and solvents to be used, no general rules have so far been found. Experiences of other investigators or the trial and error method have to be referred to. Therefore, a review of the literature on

adsorptive percolation of hydrocarbons will be given first. Following this, some experiences with petroleum fractions will be mentioned and, as a further contribution to the question of the efficiency for a special case, a comparison will be made between adsorptive percolation and thermal diffusion. Finally, the applicability of adsorptive percolation to petroleum fractions and the possibilities connected therewith will be summarized. The following survey of the literature has been made according to the methods of percolation used.

The continuous introduction method is applied on a technical scale to remove coloured substances from certain petroleum distillates. The adsorbent used is bauxite or active clay.

Hirschler and Amon[1] showed that continuous introduction of the sample followed by elution with methyl alcohol constitutes a useful purification method for saturated hydrocarbons which are contaminated with homologues. Silica gel and charcoal are suitable adsorbents, whereas alumina is far less effective. A large number of data concerning the adsorption of binary systems of saturated hydrocarbons up to C_{12} is given. The authors show that the method has its limitations when so-called S-type adsorption isotherms occur.

For analytical purposes Mair and White[2] applied the elution method to a mixture of hydrocarbons boiling between 80° and 175° C. Using silica gel as adsorbent and water as eluent (displacer) the mixture could be separated into an aromatic and a non-aromatic fraction. Willingham[3] obtained the same result with a mixture of higher boiling-point hydrocarbons. Mair[4] using methanol as eluant analyzed a synthetic mixture of toluene, cyclohexene, 2 : 2 : 4-trimethylpentene and 2 : 2 : 4-trimethylpentane by percolation through silica gel. Toluene and 2 : 2 : 4-trimethylpentane were recovered in an approximately pure state. Cyclohexene and 2 : 2 : 4-trimethylpentene could not be separated from each other. The elution method is also applied to gaseous hydrocarbons up to heptane. An ingenious apparatus has been constructed by Turner.[5] The adsorption column is filled with pellets of activated carbon. Elution is accomplished by a combined action of heat and mercury. The effluent gases are analyzed by means of heat conductivity cells. Hydrocarbons of different molecular weight may be separated but difficulties are encountered with the separation of isomers. A similar apparatus has been constructed by Tiselius and Claesson.[6]

With the development method the effluents are always diluted by some solvent. Therefore, the method is usually restricted to hydrocarbons of low volatility. Using alumina as adsorbent and benzene as developing solvent, Winterstein and Schön[7] separated polycyclic aromatics. Mair and Forziati[8] carried out a quantitatively controlled adsorptive percolation of a mixture of seventeen hydrocarbons. The mixture was separated into an aromatic and a non-aromatic fraction and the composition found tallied within 1 % with the true composition. Silica gel was used as adsorbent and propane, butane or pentane were the developing solvents. Nederbragt and de Jong[9] obtained complete separation of a mixture of tetracosane and di-*sec.*-butyldecaline using an active earth (Floridine 267) as adsorbent and pentane as developer. With another kind of active earth (Floridine XXF) even the

[1] Hirschler and Amon, *Ind. Eng. Chem.*, 1947, **39**, 1585.
[2] Mair and White, *J. Res. Nat. Bur. Stand.*, 1935, **15**, 51.
[3] Willingham, *J. Res. Nat. Bur. Stand.*, 1939, **22**, 321.
[4] Mair, *J. Res. Nat. Bur. Stand.*, 1945, **34**, 435.
[5] Turner, *Petr. Ref.*, 1943, **22**, 140.
[6] Claesson, *Ann. N.Y. Acad. Sci.*, 1948, **49**, 183.
[7] Winterstein and Schön, *Z. physiol. Chem.*, 1934, **230**, 139, 146.
[8] Mair and Forziati, *J. Res. Nat. Bur. Stand.*, 1944, **32**, 151.
[9] Nederbragt and de Jong, *Rec. trav. chim.*, 1946, **65**, 831.

separation of two naphthenes, viz., octadecylcyclohexane and di-*sec.*-butyldecaline, proved to be possible.

Recently the author[10] showed that introduction of a suitable series of developers may be very useful when a complex mixture of hydrocarbons has to be separated. Out of a mixture of five hydrocarbons, viz., hexadecane, hexadecene, dodecylbenzene, α-propylnaphthalene and 1 : 2-diphenylpropane, all components could be re-collected in their original pure state. Only between the two components last mentioned an intermediate fraction was found. The other components were re-collected quantitatively in a pure state. The adsorbent used was silica gel and the developers used subsequently were pentane, carbon tetrachloride and chloroform. It proved to be possible to calculate the result to be expected from figures obtained on percolation of the pure substances. The result calculated and the result actually found agreed within the experimental error. For further details the original paper should be referred to.

Experimental

Petroleum Fractions.—It would have been of interest to extend the work with pure hydrocarbons previously mentioned to still more complex mixtures of known composition. For temporary lack of suitable pure

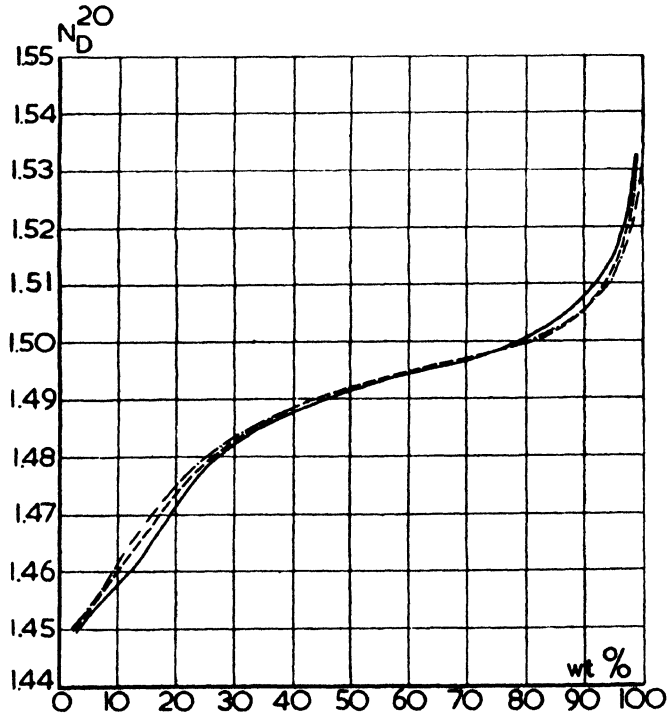

FIG. 1.—Percolated sample: completely hydrogenated lubricating oil fraction. Absorbent: silica gel.

Column int. diam. (cm.)	1·45	2·9	5·8
Length of packing (cm.)	310	302	301
Weight of packing (g.)	336	1345	5242
Density of packing (g./ml.)	0·66	0·67	0·66
Sample weight (g.)	3·754	15·525	60·145
Line	Drawn	Broken	Dash-dot

[10] Smit, *Anal. chim. Acta*, 1948, **2**, 671

hydrocarbons some lubricating oil distillation fractions have now been investigated using the same method. This method may be restated briefly. A glass * adsorption tube is mechanically filled with silica gel (Davison, through 200 mesh). The length of the columns used is about 300 cm. and the cross-sectional area is taken proportional to the weight of sample to be handled. As Fig. 1 shows, the separating efficiency is not affected by the diameter if the length of the column is kept constant and the sample size is taken proportional to the square of the diameter of the column. The weight ratio of sample to silica gel is about 1/100. Before introduction of the sample the column is wetted with the first developer to be used so as to diminish loss of material caused by irreversible adsorption. By doing so losses would be reduced from 4 % without previous wetting to 1 % with pre-wetting. The sample is previously diluted by about ten times its volume of the first developer. Then the sample

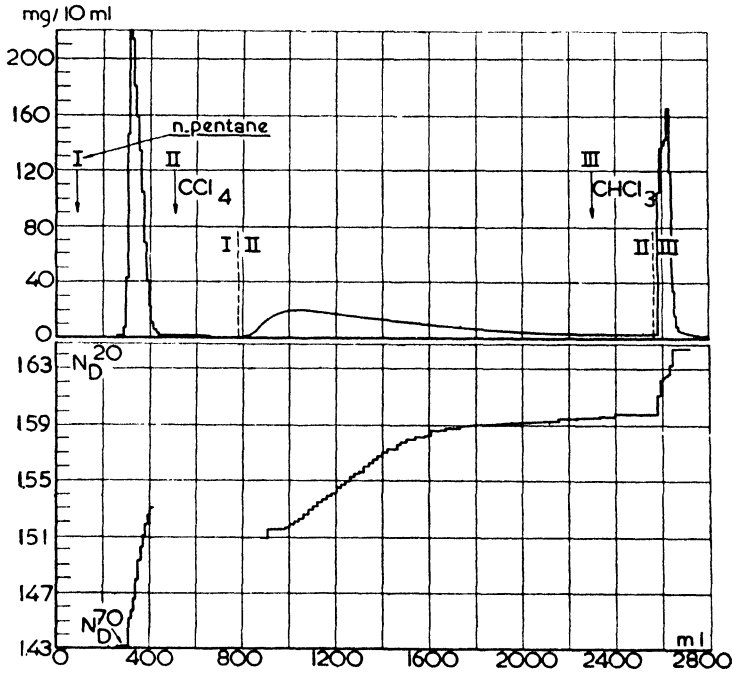

FIG 2.—Result of adsorptive percolation of sample A. (Lubricating oil fraction containing hardly any paraffins.)

is introduced into the column. Subsequently the first developer is forced through the column so that the linear speed of the liquid front does not exceed 2 cm /min. Samples of appropriate volume are collected at the bottom of the column. The solvent is evaporated, the residue is weighed and its refractive index is measured. Introduction of the same developer is continued until evidence has been obtained that the effluent leaves no perceptible residue after evaporation of the solvent. Then the next developer to be used is introduced into the column. The same process is repeated, after which a third developer is introduced, etc. Thus far three developers have been used, viz., pentane, carbon tetrachloride and chloroform. After chloroform has become ineffective the components still retained in the column are eluted by means of ether or acetone. For the determination of the right moment of changing the developer and the sequence of the developers the experiments with pure hydrocarbons [10] have been of great help.

* It may be mentioned here that some oils adsorbed on silica gel may give rise to the formation of coloured products when exposed to daylight.

The results of the percolation of two oil fractions of different origin have been represented (qualitatively) by Fig. 2 and 3. Sample A (Fig. 2) is a lubricating oil fraction which according to ring analysis [11] (the original method has been modified and will be published shortly) contained naphthenes and aromatics and no paraffins. However, the first percolation fractions of this sample solidified at room temperature. Moreover, the refractive index (measured, by way of exception, at 70° C) was 1·431. It was concluded that the very first fractions consisted of paraffins. Thus the presence of a few tenths per cent. of paraffins can be detected in a mixture mainly consisting of naphthenes and aromatics. The following pentane fractions had refractive indices increasing from about

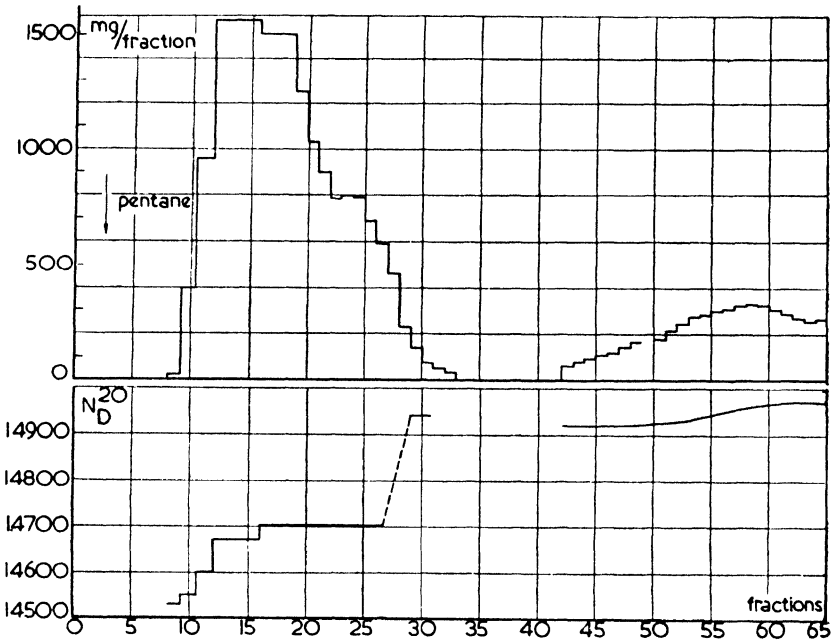

FIG 3.—Result of adsorptive percolation of sample B. (Low boiling-point lubricating oil fraction.)

1·453 to 1·530, suggesting that the main part of the naphthenes was of polycyclic nature.[12] The pentane fractions when examined by an ultra-violet spectrometer showed no trace of aromatics. The fractions obtained with carbon tetrachloride had refractive indices increasing from 1·510 to 1·597. The chromatogram revealed that only a small amount of monocyclic aromatics was present. Indications have been obtained that the carbon tetrachloride fraction contained mainly dicyclic and perhaps some polycyclic aromatics. The refractive indices of the chloroform fractions increased from 1·611 to 1·644. These fractions contained the polycyclic aromatics. After chloroform had ceased to be effective, about 10 % of the oil was still left in the column. This last part was recovered by introducing ether into the column. After evaporation of the solvent a sticky brown mass was obtained which showed a remarkably high sulphur content. Thus it was clear that sulphur compounds and presumably other non-hydrocarbons were accumulated in the last percolation fractions.

As to sample B (Fig. 3), which had a lower mean molecular weight than sample A, only the pentane part of the chromatogram has been represented.

[11] Vlugter, Waterman and van Westen, *J. Inst. Petr. Techn.*, 1935, **21**, 661, 701.
[12] Smittenberg and Mulder, *Rec. trav. chim.*, 1948, **67**, 813, 826.

The remaining part showed no peculiar differences when compared with sample A. According to ring analysis, sample B consisted of paraffins, naphthenes and aromatics. As is seen in Fig. 3, there is a distinct gap in the pentane fractions. First a series of fractions was obtained, the refractive indices of which increase continuously up to 1·470. Then several fractions of pentane were collected leaving no residue after evaporation of the solvent. However, the next pentane fractions after evaporation of the solvent left residues the refractive indices of which start at 1·491 and rise slowly up to 1·500. So a separation between mono and dicyclic naphthenes was obtained here. To confirm this the pentane fractions were examined with an ultra-violet spectrometer. No aromatics could be detected.

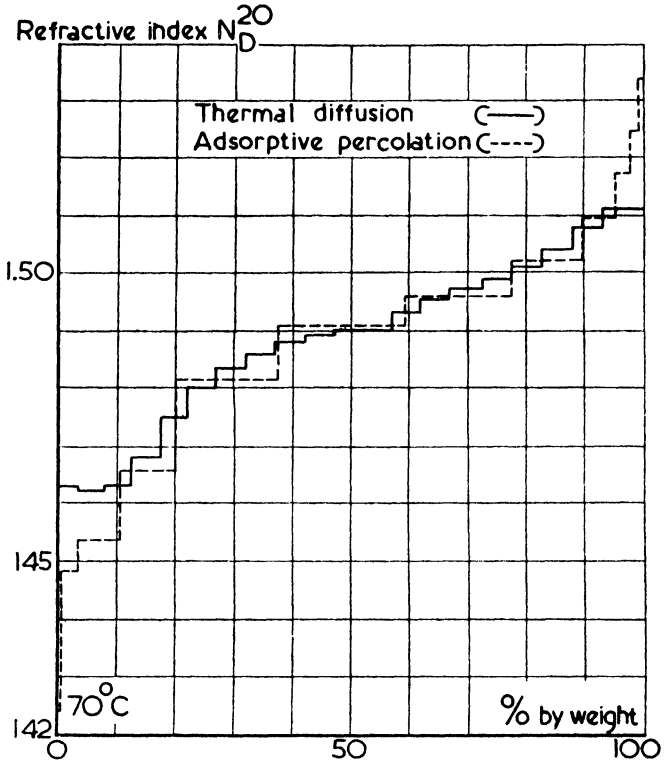

FIG 4.—Comparison of adsorptive percolation and thermal diffusion for the special case of a completely hydrogenated lubricating oil fraction of the same type as sample A.

These two examples of adsorptive percolation show that this method not only permits determination of the ratio between aromatic and non-aromatic compounds, but also gives valuable information about the constituents within one of these groups. Thus adsorptive percolation may be a useful means of characterization. Yet it seemed useful to ascertain whether after a first separation in two groups other physical separation methods might give even more detailed separation between the constituents of one group. Thus, thermal diffusion was compared with adsorptive percolation. Preliminary experiments had shown that thermal diffusion gives no specific separation between aromatics and non-aromatics. Therefore a distillation fraction of oil A (which contains hardly any paraffins) was hydrogenated completely, thus giving a mixture consisting of naphthenes only. One part of the hydrogenated oil was submitted to adsorptive percolation in a silica gel column of length 300 cm. using pentane as developing solvent. The other part was submitted to thermal diffusion.

The thermal diffusion column has a length of 180 cm. It consists of two concentric brass cylinders leaving an annular slit of 0·25 mm. thickness. The surface of the outer cylinder is cooled with tap water. The inner tube is heated by steam, thus causing an effective temperature difference of about 50° C across the slit.

One thermal diffusion run lasts about 240 hr. and requires 5 tons (weight) of steam. The size of the sample is about 40 ml. The adsorption column has an inner diameter of 1·5 cm. The percolation lasts about 8 hr. About 700 ml. pentane is used which may be recovered. The size of the sample is 4 g. If the sample had been of the same amount as used in the thermal diffusion experiments, the diameter of the adsorption column should have been about 4·5 cm. and about 7 l. pentane would have been necessary, whereas the percolation time would have remained the same, viz., 8 hr. The results of both methods are represented graphically in Fig. 4, whence it may be concluded that the separation obtained by adsorptive percolation is in this case somewhat superior to that by thermal diffusion.

Discussion

According to the literature and our own experience it may be stated that quantitative separation of petroleum fractions into an aromatic and a non-aromatic part is hardly a problem nowadays. The elution (displacement) method may give good results when silica gel is used as adsorbent and alcohol, acetone or ether are used as eluants. Water is less suitable. The results may be greatly improved, especially when viscous oils are dealt with by previous dilution of the sample with a mixture of pentane and some other solvent like carbon tetrachloride, chloroform or benzene, which is more strongly adsorbed. A weight ratio of gel/sample above 10/1 is found useful. This method may be also used to accumulate non-hydrocarbons present in oil fractions.

For further separation within the groups of saturated and non-saturated hydrocarbons the developer method is more suitable. This becomes the clearer if the experiments of Hirschler and Amon[1] are taken into account. As yet, percolation through silica gel has shown the possibility of separation between mono and polycyclic compounds, both saturated and non-saturated. However, complete separation has not been established with certainty. As a general feature of adsorptive percolation through silica gel using the developer method, it may be mentioned that the hydrocarbons are arranged according to their refractive indices.

Though silica gel as adsorbent and pentane as developer constitute a good combination to resolve a mixture of saturated compounds, some active earths may presumably give better separation between paraffins and naphthenes.

From separate experiments[10] and the results mentioned in this paper, it may be concluded that carbon tetrachloride has a rather weak developing power, whereas chloroform is adsorbed somewhat too strongly to effect fair separations in the aromatic part, at least when silica gel is the adsorbent. Experience with mixtures of these two solvents has as yet not been very successful. Other solvents have been tried and C_5 olefins might be very suitable but these are polymerized on silica gel. Better results may perhaps be obtained if the aromatic part is re-percolated through alumina as seem to be borne out by the experiments of Winterstein.[7]

When comparing the result of adsorptive percolation with other separation methods applicable in petroleum chemistry, the conclusion arrived at depends on the result desired and the range of molecular weights covered by the constituents of the sample. Up to C_5, low-temperature distillation has several advantages if nearly complete separation is required. From C_6 up to about C_{16}, a combination of distillation and adsorptive percolation may

give good results. But for heavier compounds, distillation permits only a rough separation according to molecular weight, whereas adsorptive percolation is far more selective as to the nature of the compounds. Moreover, with distillation partial decomposition of higher boiling-point compounds can hardly be avoided. Fractional crystallization at low temperatures may be of help in this region but is also less selective. Thermal diffusion is to some extent comparable with adsorptive percolation, except in the separation between saturated and non-saturated compounds.

In conclusion, for petroleum hydrocarbons it may be stated that adsorptive percolation constitutes a very useful, additional separation method, especially in the range above C_{16}. However, the investigation of its possibilities and limitations is far from complete.

Acknowledgment is due to the management of the Bataafsche Petroleum Maatschappij for their permission to publish this work and to the author's colleague Mr. Kramers for permission to make use of results of his experiments on thermal diffusion.

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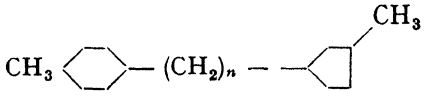
CHROMATOGRAPHIC FRACTIONATION OF BLACK OILS

By A. S. C. Lawrence and D. Barby

Received 1st July, 1949

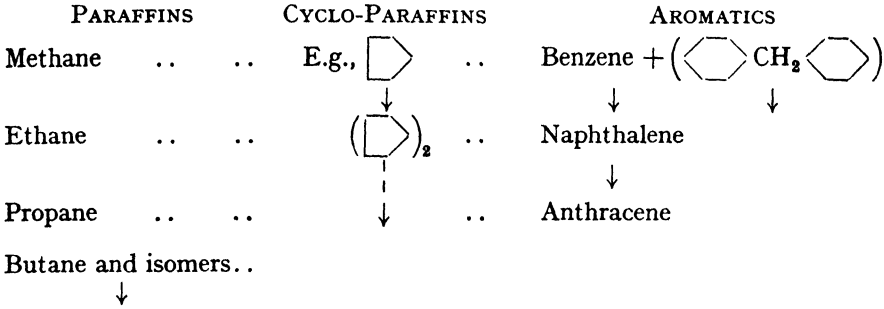
Preliminary attempts at chromatographic fractionation of black oils have given indications that separation into hydrocarbon types is achieved to a great extent and that the method should be of great value in the study of petroleum. The resinous components most strongly adsorbed on alumina and coke contain significant amounts of oxygen.

Very little is known of black oils; they are treated as by-products They were regarded first as mixtures of all possible isomers of paraffins and when it was recognized that the three main classes of hydrocarbons viz., paraffins, naphthenes (i.e., cyclo-paraffins) and aromatic hydrocarbons were all present, the idea arose that the higher oils could not be assessed on any basis of these types because of the existence of mixed molecules. In particular, on this chemical basis it is suggested that all asphalts are different and that there is such an astronomical number of possible components that any examination would be a waste of time. This is true only if the object is to separate such mixtures into their individual chemical components. In view of the evidence and as a result of previous work by one of the writers (A. S. C. L.), it was considered that there is not unlimited mixing of the three types of hydrocarbon in oils of high molecular weight of the type:



although alkyl naphthenes with short alkyl groups are certainly common in petrol fractions. It was further considered that the more serious factor

to be considered was that we have in the simplest case, where there is no mixing of type, three homologous families, viz. :



In very large molecules a small amount of alkyl substitution or linking of rings will not detract much from the ideal type.

With high molecular weight compounds, specificity of solvent action will increase, but even if the higher members of one class are insoluble in a chosen solvent in which another class is soluble, the lower members of the insoluble class will become soluble. It is necessary therefore to carry out 'horizontal' as well as 'vertical' separation. This is important for another reason. The highest molecular weight material in asphalts and black oils, the so-called 'hard asphalt,' is a very powerful absorbent of the lower molecular weight materials which in turn absorb still lower components.

Experimental and Results

Following a number of preliminary experiments, an analysis was made of a Venezuelan fuel oil. The 'horizontal' separation was achieved by distillation in a good vacuum by which four volatile fractions and a residuum were obtained. Each was then fractionated chromatographically by the method of successive elutriation. Finally the whole undistilled oil was fractionated chromatographically for comparison. Powdered coke was used as absorbent at first, but alumina was found to give equally good results and this was used on account of the advantage of its colour.

TABLE I

Fraction No.	Boiling Range °C	% of Oil
54	140–200	18·45
55	200–230	11·06
56	230–290	11·55
57	290–340	11·2
58	Residuum	46·4
117	Whole Oil	—

A battery of tubes was erected each 18 in. long and 1½ in. diam. These discharged into Buchner flasks connected to a filter pump and the solution of the oils was sucked through the columns. This method has the great advantages that the elutriate can be passed from the first column to the second (and the process continued as required), and that where elutriation is very rapid with subsequent solvents, the elutriate can be diluted to 2 % before running through the second and subsequent tubes.

The oils were made up to 2 % solution in hexane and sucked through alumina columns : a colourless solution containing white oil was obtained (α fraction). The alumina was then elutriated with cyclo-hexane which carries through a pale yellow oil with blue fluorescence (β fraction). This is followed by toluene

TABLE II

Fraction	α	β	γ	δ_1	δ_2	ε	ζ	ω
54								
%	86·0	2·22	4·68	Trace	—	—	—	—
n_D	1·4830	1·5664	1·6246	—	—	—	—	—
Dispersion ..	0·0108	0·0202	0·0312	—	—	—	—	—
Colour	None	None	Brown	Brown Green	—	—	—	—
Fluorescence ..	None	None	Blue	None	—	—	—	—
55								
%	72·7	5·06	17·2	2·46	2·52	—	—	—
n_D	1·4952	1·5550	1·6166	1·5953	—	—	—	—
Dispersion ..	0·0099	0·0174	0·0275	0·0237	—	—	—	—
Colour	None	Pale Yellow	Orange Yellow	Yellow	Brown	—	—	—
Fluorescence ..	—	—	—	—	—	—	—	—
56								
%	61·6	2·94	22·2	3·91	2·28	—	—	—
n_D	1·4995	1·5331	1·6095	—	—	—	—	—
Dispersion ..	0·0101	0·0135	0·0259	—	—	—	—	—
Colour	Pale Yellow	Pale Yellow	Red	Brown	Brown	—	—	—
Fluorescence ..	Blue	Blue	Green	Green	None	—	—	—
57								
%	35·3	4·5	43·4	7·66	7·00	—	—	0·834
n_D	1·4910	1·4850	1·5590	1·5730	—	—	—	—
Dispersion ..	0·0081	0·0079	—	0·0174	—	—	—	—
Colour	None	Pale Yellow	Red	Red	Red	—	—	Black
Fluorescence ..	None	Blue	Green	Green	None	—	—	None
58								
%	21·8	2·6	35·4	0·8	10·6	1·8	Trace	23·6
Colour	*	*	Dark Red	Dark Red	Dark Red	Black	Black	Black
117 (Whole Oil)								
%	40·0	11·5	23·0	5·0	7·0	1·5	0·5	7·5
n_D	1·5002	1·5112	—	—	—	—	—	—
Dispersion ..	0·0108	0·0097	—	—	—	—	—	—
Colour	None	Pale Yellow	Red	Brown	Brown	Black	Black	Black
Fluorescence ..	None	None	Green	None	None	None	None	None

* See text.

which removes an orange-brown oil with bright-green fluorescence (γ fraction). When the toluene elutriate runs colourless, chloroform is used: this always gives two fractions, one easily washed out, δ_1, while a sharp brown band moves down the column slowly, δ_2. The first extraction was always complete before the second elutriate reached the bottom. With higher fractions and residua, there still remained material adsorbed on the alumina. This was elutriated successively with carbon disulphide and pyridine both of which gave rapid and sharp separations (ε and ζ fractions). The chloroform, CS_2 and pyridine fractions were dark resins without fluorescence. Any material insoluble in the hexane was named the ω fraction.

Table I shows the fractions obtained by the vacuum distillation of the oil while Table II gives the results of the chromatographic fractionation.

Fig. 1 shows the composition of the oil together with the chromatographic fractionation of the whole undistilled oil. The total α fraction for the fractionated oil is too large because it was not realized in dealing with the two most volatile fractions how difficult is the separation of the α and β fractions, so that these

two α's are slightly yellow due to some β in them. The colour of the higher fractions of the whole oil shows that separation is considerably less sharp, but the greatest difference is in the ω fractions. It might be suggested that distillation has caused polymerization and increased the ω fraction in the distillation residuum, but we do not accept this view. No cracking took place and the ε and ζ fractions of the whole oil contained much black suspended matter which was absent from the corresponding distillate residuum fractions.

Attempts at chromatographic separation at 10 % concentration in hexane failed to give the sharp separation obtained at 2 %, judged by colour.

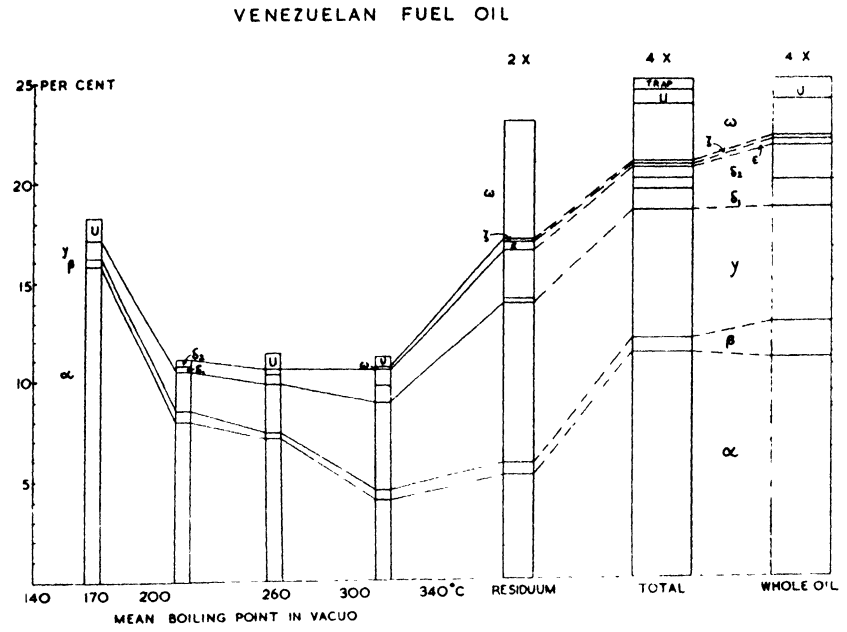


FIG. 1.

The residuum shows well the dual separation, the horizontal and the vertical. The hexane elutriate was evaporated in five successive portions and the residues showed progressive increase of viscosity and refractive index. The β fraction was recovered in three fractions whose viscosity also increased greatly. They were too viscous for determination of refractive index, but their flow was Newtonian as was that of the α fractions.

The fuller characterization of the fractions is being undertaken, but it is considered that the sharpness of the separation as judged by colour and supported by the evidence of refractive index justifies communication of these early results. A residual asphalt from Persian fuel oil gave results very similar to that from the Venezuelan oil. Perhaps the most remarkable feature of this work is the consistency of fractionation found. It is felt that the method should be of the greatest value in studying changes occurring in refinery treatment of oils. It also provides a method for determination of wax in residua which is superior to any method so far proposed. The wax is all found in the α fraction from which it is removed, after evaporation of the hexane, by methyl ethyl ketone. The wax so recovered from the vacuum distillation residuum of a Persian fuel oil had a melting point considerably lower than those of the distillate waxes.

This work forms part of certain researches being carried out for Engineer-in-Chief, Admiralty.

The University,
Sheffield.

CHROMATOGRAPHY OF THE CARBOXYLIC ACIDS

By Harold G. Cassidy and F. H. Max Nestler[1]

Received 13th July, 1949.

The principles and chief methods of application of chromatography as used in the separation of fatty acids are discussed. The applications of elution analysis, frontal analysis, displacement analysis and partition chromatography are reviewed, with attention to their advantages and disadvantages.

The separation of mixtures of homologous carboxylic acids into their components is difficult. The difficulty is greater as the amount of mixture available is smaller, as the mixture is more complex, as the components are more closely related and as the analytical requirements are more stringent. There is at present no way to separate small amounts of fatty acids in mixtures with five or more closely related components with a yield of 99 % of each acid. There is much evidence that this is not an impossible task, however, and excellent separations approaching this acuity have been reported. In this respect chromatography may be used in two ways: as an analytical tool which gives information about *how many*, and which, acids are present in the mixture, and *how much* of each is there; and as a preparative tool, which by the nature of its action goes a step farther and yields all the components of the mixture in pure form.

The second use of chromatography, that for the isolation of the pure fatty acids, was the earliest and is still the most important application. There are situations, as will be pointed out below, in which the first-mentioned use of chromatography is to be chosen. Earlier investigations of the chromatographic separation of carboxylic acids (fatty acids in these cases) were made by Kondo,[2] Manunta,[3] Kaufmann,[4] Cassidy [5] and Caliri.[6] These investigations showed that fatty acids could be separated on a variety of adsorbents such as alumina, frankonite, silica gel and charcoal. On the whole the separations were only fair. Kaufmann established the versatility of the method in extensive applications to saturated and unsaturated fatty acids and glycerides; Cassidy examined the relation between the static isotherms given by individual and mixed fatty acids and their chromatographic behaviour. The methods, though promising, were often tedious.

General Aspects of the Chromatographic Method as Applied to the Separation of Fatty Acids.—Chromatography is one of the separation methods based on a distribution between phases applied in a counter-current manner.[7] The distribution involved in chromatography is that between a bulk phase and an interfacial phase. In partition chromatography the distribution is in some instances between two more or less bulk phases and in others a rather complicated distribution involving bulk and interfacial phases. In any case the method may be compared with others which involve distribution

[1] Present address, Naval Research Laboratory, Washington 20, D.C.
[2] Kondo, *Pharm. Soc. Japan, Trans.*, 1937, **57**, 218
[3] Manunta, *Helv. chim. Acta*, 1939, **22**, 1156.
[4] Kaufmann, *Fette Seifen*, 1939, **46**, 268, and later papers; *Angew. Chem.*, 1940, **53**, 98.
[5] Cassidy, *Dissertation* (Yale, 1939); *J. Amer. Chem. Soc.*, 1940, **62**, 3073, 3076, *ibid.*, 1941, **63**, 2735.
[6] Caliri, *Dissertation* (Fribourg, 1939).
[7] Cassidy (submitted for publication).

between phases.[8] The fundamental fact upon which all of these distribution separations is based is that described by the principle of homology. In the case of adsorption this principle is given restricted expression in Traube's rule: that the extent of adsorption under certain conditions increases regularly along an homologous series. This rule holds well (qualitatively) for adsorption from bulk solution to an air-solution interface, and in many cases also for adsorption to a solid-solution interface. In other cases a reversal of this behaviour is observed, and where the adsorption is more or less dependent on the functional group of the molecule, it does not hold at all.

The rule of Traube may be generalized somewhat: the extent of adsorption at a given interface usually changes in a regular manner from one homologue to another. The chromatographic separation of fatty acids, or of any other substance, rests in part upon this observed behaviour, and where a series of well-separated isotherms are observed for a group of homologues then chromatographic separation may be feasible. Linner and Gortner[9] have published data on the adsorption of carboxylic acids from water onto Norit charcoal. The isotherms of the acids from formic to caproic are well separated, and it should be feasible to separate these acids using this adsorbent, unless other factors prevent (see below). The same may be said for the acids *n*- and *iso*butyric and *n*- and *iso*valeric; and among substituted acids, acetic and glycolic; acetic, glyoxylic and oxalic; caproic, adipic and citric; succinic, malic and tartaric; fumaric, succinic and maleic. In other cases the possibility of a separation is not so promising. Thus with the dicarboxylic acids from malonic to adipic the curves cross, though oxalic is well separated from the others; the propionic and pyruvic curves are fairly close together but well separated from glyceric and lactic which are close together; citraconic and itaconic are close together but mesaconic is better adsorbed, and the isotherm well separated from those of the other two, methyl succinic being less well adsorbed than the other three.

While well-separated isotherms imply chromatographic separatibility this cannot be the whole story, for otherwise separations on alumina, which in equilibrium experiments adsorbs many fatty acids to the same extent, would not be possible. But separations on alumina have been carried out, as has been mentioned above. Another factor which must be present lies in the non-equilibrium nature of the chromatographic adsorption as it is usually carried out. Even though two substances may at equilibrium be adsorbed to the same extent, yet if they are adsorbed at different rates it is to be expected that some separation could be achieved if the chromatography were carried out not too slowly. Thus mixtures which under equilibrium conditions show no preferential adsorption of one component over the other (corresponding, thus, to azeotropes and eutectics in the vapour-liquid and solution-solid distributions) should be separable by non-equilibrium chromatography just as azeotropes and eutectics may be separated, or broken, by non-equilibrium evaporation and non-equilibrium crystallization respectively. Such mixtures have been found in adsorption from some systems in which the adsorptions of two substances have been studied over the entire concentration range.[10] It is to be expected that rates of adsorption would change in a regular manner over an homologous series.

[8] Randall and Longtin, *Ind. Eng. Chem.*, 1938, **30**, 1063. Cassidy, *J. Chem. Educ*, 1946, **23**, 427; *Ann. N.Y. Acad. Sci.*, 1948, **49**, 143. Thiele, *Ind. Eng. Chem.*, 1946, **38**, 646. Benedict, *Chem. Eng. Proc.*, 1947, **43**, 41.

[9] Linner and Gortner, *J. Physic. Chem.*, 1935, **39**, 35.

[10] Very many of these cases are known. For some recent examples see Bartell and Lloyd, *J. Amer. Chem. Soc.*, 1938, **60**, 2120, and Rao and Jatkar, *Quart. J. Indian Inst. Sci.*, 1942, **5**, 73, 80, 87, 91, 98. The correspondence to azeotropes and eutectics has been pointed out.[11]

[11] Cassidy, *Federation Proc.*, 1948, **7**, 464.

Chromatography is essentially a method of using the adsorption distribution in a differential counter-current manner.[7] The procedures which are now commonly used have been given descriptive names by Tiselius and his co-workers, who invented some of them.[12] These procedures are elution analysis, frontal analysis, displacement analysis. An additional method of a somewhat different sort has been invented and developed by Martin and Synge[13] and their co-workers: partition chromatography. The application of these methods and procedures, together with increased understanding of the problems, has improved the separations of carboxylic acids over those achieved in the earlier work referred to above.

Elution Analysis.—The earlier work on the separation of fatty acids cited above was carried out largely by elution analysis, the liquid chromatogram procedure.[14] Ideally, in elution analysis the zones[15] of separated fatty acids are made to march from the column in an ordered array, and are recognized by titration or by some other method. As stated above the method was not very practical as it was developed in the early work; however, the method has the potential advantage that under favourable conditions essentially all of each fatty acid component of a mixture could be obtained free from contamination, in which case three requirements of analysis could be met: the identity of each component and the quantity present in the mixture could be determined, also a considerable fraction of the pure substance itself could be obtained for further characterization. This method is especially applicable to the purification of already separated samples. Thus 0·67 % of ester could be demonstrated in a sample of mixed acids obtained by very careful saponification.[11] The method can probably be applied to mixtures of fatty acids using fractional elution with a graded series of eluents, a method which has been used considerably in the laboratories of Reichstein and of Ruzicka in connection with the chromatography of steroid and other mixtures. A little work of this kind has been done with fatty acids.[5 16] It was observed with fatty acids C_2, C_3 and C_4 that the isotherms on Darco G-60 charcoal from water were well separated; however, elution analysis with water failed to yield a good separation of the three binary and one ternary mixtures investigated.[16] The acids were difficult to elute with water. This procedure verges on displacement analysis in certain cases.

Frontal Analysis.—The examination of mixtures of fatty acids by frontal analysis was reported in detail by Claesson.[17] He reported that by the use of a suitable adsorbent, such as Carboraffin CIV, and absolute ethanol, it was possible to obtain quantitative analyses of fatty acid mixtures containing as many as 6 fatty acids. In one experiment which was quoted a mixture of the *n*- C_8, C_9, C_{10}, C_{12}, C_{14} and C_{16} acids was analyzed. The

[12] Tiselius, in *Advances in Colloid Science*, ed. E. O. Kraemer (Interscience, New York, 1942), p. 81; *Arkiv Kemi, Min. Geol. A*, 1943, **16**, No. 18.

[13] Martin and Synge, *Biochem. J.*, 1941, **35**, 1358.

[14] Zechmeister and Cholnoky, *Principles and Practice of Chromatography* (Chapman and Hall, London; John Wiley and Sons, New York, 1943).

[15] There is some tendency to use the word "band" for the zones on columns and paper strips and sheets. We believe that this should be discouraged if only for the practical reason that the word band is in well-established use in connection with absorption of radiation. It would seem clearer and less confusing to speak of the characteristic *ab*sorption bands observed for a component isolated from a characteristic zone on an *ad*sorption column or paper sheet or strip than to use the word "band" in two different connections. The word seems particularly inapt in connection with the zones on paper.

[16] Nestler and Cassidy, *J. Amer. Chem. Soc.* (in press). This work is part of a programme supported by The Nutrition Foundation, Inc.

[17] Claesson, *Arkiv Kemi, Min. Geol. A*, 1946, **23**, No. 1; *Ann. N.Y. Acad. Sci.*, 1948, **49**, 183.

relative percentages of the components put into the mixture were, respectively, 10, 10, 10, 20, 20, 30; the relative per cent. found were 14, 10, 8, 22, 20, 27. Only a moderate amount of the mixture was needed, and the author states that the method is well suited for the qualitative and quantitative analysis of such fatty acid mixtures where the demands for quantitative accuracy are not too great.

Nestler and Cassidy[16] studied the adsorption of acetic, propionic and butyric acids upon Darco G-60 charcoal from water with a view to devising a simple analytical method for these acids, as well as to testing adsorption and chromatography theories. With frontal analysis, sharp fronts were obtained so that the number of components in the mixture could be clearly detected. However, this system did not give clear evidence that really quantitative analyses could be made with it. It was observed that the threshold, or breakthrough, volume for a given fatty acid changed with concentration of the acid, and that the two values could be related by an equation of exponential form formally resembling the Freundlich equation.

Certain practical limitations seem to be set to the acuity of frontal analyses. With any mixture the most strongly adsorbed component becomes more difficult to detect as its quantity is less or as the total concentration in the solvent is less. Increasing the amount of adsorbent is of no practical value here. The least strongly adsorbed component can be detected in relatively smaller amounts than the most strongly adsorbed. This may be because of the displacing effects of other components. Frontal analysis does have certain advantages. Under the conditions outlined by Claesson[17] the method requires quite small amounts of substance. For example, in one experiment with a mixture of four acids about 0·2 g. was used. Also the method is fairly rapid. With adsorbents which show some irreversible adsorption the method is still applicable where elution or displacement analysis may not be. The method is also applicable with adsorbents on which the fatty acids are adsorbed to some extent on different parts of the surface, so that they do not displace each other.

Displacement Analysis.—Displacement analysis of saturated fatty acids on charcoal was not found possible by Claesson.[17] However, Holman and Hagdahl,[18] using a somewhat different technique,[19] were able to analyze mixtures of fatty acids by displacement analysis. For example, they were able to separate lauric, myristic, palmitic and stearic acids using picric acid as the displacement agent. This work was extended[20] to the separation of saturated fatty acids from C_1 to C_{20} and C_{22}. The separations were improved by using a different charcoal and aqueous alcohol, in which the acids are not too soluble. A higher fatty acid was utilized to displace the mixture of lower acids. Fifteen charcoals were examined as possible adsorbents, and of several of these which had good characteristics Darco G-60 was chosen. Acids C_1 to C_5 were separated from water, with C_6 as the developer; C_6 to C_{10} from 50 % ethanol, with C_{12} as developer; C_{10} to C_{13} from 65 % ethanol, with C_{14} as developer; C_{14} and C_{15} from 80 % ethanol, with C_{16} as developer; C_{16} to C_{20} from 78 % ethanol, 22 % chloroform, with C_{22} as developer. The displacement analysis of saturated, unsaturated and branched-chain fatty acids on silica gel has been reported by Claesson.[21]

Displacement analysis is not applicable when the adsorption isotherms of

[18] Holman and Hagdahl, *Arch. Biochem.*, 1948, **17**, 301.
[19] Hagdahl, *Acta Chem. Scand.*, 1948, **2**, 574.
[20] Hagdahl and Holman, *J. Amer. Chem. Soc.* (in press). Holman and Hagdahl, *J. Biol. Chem.* (submitted for publication). The authors kindly permitted us to read their manuscripts.
[21] Claesson, *Rec. trav. chim.*, 1946, **65**, 571.

the substances are linear.[22] This has been experimentally demonstrated in a clear way by Hagdahl and Holman.[20] They showed that the adsorption isotherms of butyric, caproic and caprylic acids are nearly linear from absolute alcohol onto Darco G-60 charcoal. With this system scarcely any separation of the acids is obtained in an attempt to displace the first two with the third. In 50% aqueous ethanol the isotherms are well curved, and the displacement diagram shows for the different acids well-defined steps which could be recognized interferometrically as well as titrimetrically. Displacement analysis is also inapplicable where the substances are adsorbed on different parts of the surface of the adsorbent, because then one will not displace the other.

Where it is applicable, displacement analysis has a number of advantages. It allows a characterization of the components of the mixture as well as a quantitative determination of their amounts (as does frontal analysis) but in addition, like elution analysis, it yields substantial fractions of the components in pure form. This can be an important advantage since it allows confirmation of the identity of the fatty acid or other component. There is, of course, tailing under the steps of the displacement pattern,[18] but its effect is often of negligible importance.

Partition Chromatography.—This method has been applied to carboxylic acids by Smith,[23] Ramsey and Patterson,[24] Elsden,[25] Isherwood,[26] Gray,[27] Peterson and Johnson[28] and others. It would appear that this is a most promising analytical technique for fatty acids as well as dicarboxylic and substituted acids. The principle of partition chromatography may be applied using columns of adsorbent or paper strips or sheets. Ramsey and Patterson found that formic, acetic and propionic acids could be separated from each other, with butyric and *iso*butyric acids appearing together, when their mixtures were applied to columns of silica with water as the non-mobile phase, and chloroform-butanol as the mobile. Elsden used a silica-water-chloroform system to separate mixtures of acetic, propionic and butyric acids. Stadtman and Barker[29] found that quantitative separation of pentanoic, hexanoic and heptanoic acids and partial separation of heptanoic and octanoic acids could be obtained by the Elsden procedure using 0·6% butanol in cyclohexane as the mobile solvent and Mallinkrodt's A.R. precipitated silicic acid as the support. Peterson and Johnson used Celite as the support, and water or aqueous sulphuric acid as the non-mobile phase. With benzene or chloroform-butanol mixtures as the mobile phase they were able to make separations among the fatty acids from C_1 to C_{10}. In every case, of course, the mobile and non-mobile phases were mutually saturated.

Detailed descriptions have been given by Ramsey and Patterson[24] of techniques whereby the separation of fatty acid mixtures can be extended to acids containing as many as 19 carbon atoms. For formic, acetic, propionic and butyric acids the second solvent is butanol-chloroform, with water as the non-mobile phase. For pentanoic to decanoic acids methanol serves as the supported phase, and 2 : 2 : 4-trimethyl pentane as the second phase. For the acids from hendecanoic to nonadecanoic the supported phase is a mixture of furfuryl alcohol and 2-amino pyridine, and the mobile phase is

[22] Tiselius, *Arkiv Kemi, Min. Geol. A*, 1943, **16**, No. 18.
[23] Smith, *Biochem. J.*, 1942, **22**, P.
[24] Ramsey and Patterson, *J. Ass. Off. Agric. Chem.*, 1945, **28**, 644; 1948, **31**, 139, 164, 441.
[25] Elsden, *Biochem. J.*, 1946, **40**, 252.
[26] Isherwood, *ibid.*, 1946, **40**, 688.
[27] Gray, *J. Expt. Biol.* 1947, **24**, 10.
[28] Peterson and Johnson, *J. Biol. Chem.*, 1948, **174**, 775.
[29] Stadtman (personal communication).

hexane. Butyric and isobutyric acids which with the first set of solvents were not separated were found to be partially resolved by the methanol-*iso*-octane system.

Concluding Remarks.—What has been attempted in the limited space taken is to show examples of what has been done, to show what needs to be done, and to discuss some of the fundamental principles involved, in applying chromatography to the separation of mixtures of carboxylic acids.

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CHROMATOGRAPHIC ANALYSIS OF FATTY OILS

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The application of chromatography to the analysis of fatty oils is briefly reviewed, emphasis being laid on the principles involved. The main advances of the last few years are described or referred to, particular reference being made to the separation of fatty acids from triglycerides and of hydrocarbons from the unsaponifiable matter of natural oils. The use of chromatographic analysis in the separation of the constituent molecules of natural oils is described.

The application of chromatography to the analysis of oils and fats was reviewed by the author [1] three years ago in a paper which traced the history of the subject and described a number of the practical applications of the method. It was emphasized that advances had been based largely on a system of trial and error, and that the principles underlying the subject were only beginning to be capable of formulation. Such formulation seemed to require a knowledge of the relative affinities of adsorbent and solvent for any given molecule, for it was rapidly becoming clear that the molecule is under the influence of competitive forces, and that their resultant determines whether or not adsorption will occur.

The resultant is moreover influenced by the presence of any other compounds which may themselves contribute to the forces acting on the adsorbable molecule, enhancing or diminishing their effect as the case may be.

The fatty oils, fatty acids, fatty alcohols and aliphatic hydrocarbons afford a remarkable set of compounds for the study of chromatography. They offer series of compounds of similar chemical structure in which the variant may be the molecular weight or the degree of unsaturation, or in which the effect of the polar carboxy group may be contrasted with that of a terminal alcoholic group, or again series in which the effect of esterification may be studied. They provide further interest by reason of the extreme difficulties they offer to chemical separation.

With hydrocarbons, the greater the degree of unsaturation, the more strongly is the compound adsorbed; conjugated double bonds lead to

[1] Williams, *Analyst*, 1946, 71, 259.

greater ease of adsorption than do the same number of double bonds separated by a larger number of carbon atoms in the molecule. Thus, of the carotene isomers [2] α-carotene, with 10 double bonds out of 11 conjugated, has least affinity for magnesium oxide ; β-carotene, with 11 conjugated double bonds, is more strongly adsorbed ; and γ-carotene, with 12 double bonds 11 of which are conjugated, is most strongly adsorbed. The introduction of hydroxy groups into the carotenes does not alter the order of adsorption-but it is known that it raises the general level of adsorption affinity ; indeed, increase in oxygen content, in the absence of ester groupings, progressively augments it. Esterification of the xanthophylls or hydroxy-carotenes reduces their adsorption affinity almost to that of the carotenes themselves.

Of the fatty esters, whether of monohydric alcohols, such as methyl alcohol, or of polyhydric alcohols, such as glycerol, the more strongly unsaturated are the more strongly adsorbed on alumina and other adsorbents. Walker and Mills [3] succeeded in separating linseed oil into fractions containing 7, 6, 5 and 4 double bonds per molecule by repeated fractionation on aluminium oxide from *n*-hexane.

If hydroxy groups raise the degree of adsorption, the introduction of free carboxy groups has a pronounced effect in a different direction. The relative ease of adsorption of fatty acids of differing unsaturation is the reverse of that for the corresponding esters, both on alumina and magnesium oxide, the more saturated being more strongly adsorbed. This has been explained by the author by the postulation of polar charges of differing sign and of an adsorbent capable of attracting polar material of either positive or negative charge. Double bonds, of negative charge, and occurring in molecules otherwise non-polar, are attracted in order of increasing unsaturation ; but if the molecules include a terminal group bearing a strong positive charge, such as carboxy group, and the operative affinity is the algebraic sum of the positive and negative charges, increasing unsaturation will lead to decreasing affinity if the positive element is large enough. If, on the other hand, the positive charge is not so large as the least negative charge due to unsaturation, as for instance if the terminal group is alcoholic, the algebraic sum will remain negative, and the order of adsorption will be the same as for the hydrocarbon series.

It has been noted that the reversal of the order of adsorption in a series of compounds may be brought about by changing the solvent ; here again we have an example of the controlling factor being a resultant of more than one force or affinity, and there is clear evidence of an attraction between solvent and solute. Indeed, the interplay of forces of polar character becomes more generally apparent as one widens the field of observation. Chromatographic separations clearly depend on the relative strengths of attractive forces of given substances for different molecules in solution. Equally does the partition coefficient, defining the proportion in which a substance distributes itself between two solvents, depend on similar considerations. And it appears more than likely that solubility in a solvent is itself a function of the same forces.

The separation of fatty acids from triglycerides was readily and quantitatively accomplished by Sylvester, Ainsworth and Hughes [4] and the method has now passed into everyday use by the analyst. It is carried out on a macro scale with quantities of at least a gram of ester or acid, and depends on the fact that fatty acids are so strongly adsorbed on alumina that they cannot be eluted with ether, while fatty esters and the unsaponifiable

[2] Heilbron, *J. Soc. Chem. Ind.*, 1937, **56**, 160T.
[3] Walker and Mills, *J. Soc. Chem. Ind.*, 1942, **61**, 125 ; 1943, **62**, 106.
[4] Sylvester, Ainsworth and Hughes, *Analyst*, 1945, **70**, 295.

matter of fats remains in solution. A single passage of a solution containing triglycerides and free fatty acids in ether through an alumina column gives quantitative separation.

Monoglycerides are more strongly adsorbed by alumina than are diglycerides, and the latter in turn are more strongly adsorbed than triglycerides. Of the fatty acids themselves, the saturated acids may be separated from their mixtures, the higher members of the series being the more strongly adsorbed. This separation has been further studied by partition chromatography by Ramsey and Patterson.[5] Dealing with the acids with from 5 to 10 carbon atoms in the chain, they first prepared chromatographic columns of silicic acid nearly saturated with methanol containing bromocresol green and slurried with *iso*-octane. The acids were dissolved in *iso*-octane and passed through the column, when well-spaced bands were obtained, each being defined by a characteristic threshold value. It was found that in one fractionation each band was contaminated with about 5 % of the next higher homologue. The authors found that when the method was applied to a mixture of butyric and *iso*butyric acids separation was incomplete, the bands overlapping visibly. A single passage would, however, detect about 10 % of the *iso*-acid added to the normal acid or 30 % of the normal acid added to the isomer.

Partition chromatography has also been used by Ramsey and Patterson [6] for the separation of saturated fatty acids with from 11 to 19 carbon atoms in the chain. In this work silicic acid was used as the supporting column and was prepared by the method of Gordon, Martin and Synge.[7] The stationary phase consisted of a solution of 2-aminopyridine in an equal weight of purified furfuryl alcohol, 10 ml. solution being used for 20 g. silicic acid. The treated silicic acid was made to a slurry with *n*-hexane and packed into a column. The mixture of fatty acids was pipetted onto the column and allowed to sink into it under pressure. 1 ml. hexane was similarly pipetted onto the column and allowed to sink in. After a further 1 ml. had been thus added, the tube over the column was filled with hexane. After 30 ml. had percolated the liquid passing through was collected in 2-ml. portions, each of which was titrated with 0·02 N sodium ethylate. A drop of the titration to about 0·1 ml. per 2 ml. fraction was taken as evidence that a particular fatty acid had finished percolating, and the fractions representing that acid were bulked for further examination or fractionation.

Synthetic mixtures of ethyl stearate, oleate, linoleate and linolenate have been studied by Dutton and Reinbold.[8] Dutton had earlier separated equal parts of oleic and stearic acids on carbon, recovering 63 % oleic acid in a pure state and 53 % stearic acid. In this study the authors, however, used alumina, and the columns were developed with a solvent containing 1·75 % diethyl ether in light petroleum. Percolation was speeded by means of nitrogen pressure. Best fractionation was obtained in the stearate-linolenate system, where there is the greatest difference in unsaturation. Poorest fractionation was found in the oleate-stearate, oleate-linoleate and linoleate-linolenate systems, where there is least difference in unsaturation. In accordance with theory, the authors found that the first component eluted from a system was obtained in higher average purity than the second. The percentage recovery of the less strongly adsorbed component was usually of the order of 90, that of the more strongly adsorbed component being generally notably lower.

[5] Ramsey and Patterson, *J. Ass. Off. Agric. Chem.*, 1948, **31**, 164.
[6] Ramsey and Patterson, *J. Ass. Off. Agric. Chem.*, 1948, **31**, 44.
[7] Gordon, Martin and Synge, *Biochem. J.*, 1943, **37**, 79.
[8] Dutton and Reinbold, *J. Amer. Oil Chem. Soc.*, 1948, **25**, 120.

Reinbold and Dutton [9] applied a similar technique to the fractionation of soya bean oil and of ethyl esters prepared from the oil. They found a variable amount of fractionation, dependent on such factors as the quantity of oil adsorbed, the composition of the developing solvent and the activity of the adsorbent. Generally speaking, it may be said that the separations followed the course that would be predicted from knowledge of other similar work. The iodine value of successive fractions obtained from the column rose from 60·8 to a peak value of 184·6. Linolenic acid could not be detected in early fractions, but appeared in a later fraction and then continued to rise in quantity as the separation proceeded.

The use of chromatography has been extended in commercial analysis, chiefly in the field of separating hydrocarbons from triglycerides or fatty acids. This separation was first noted by Drummond [10] and co-worker who separated squalene from the unsaponifiable matter of olive oil by chromatographing a solution of the unsaponifiable matter in light petroleum on aluminium oxide. The method has been used by the author [11] for the removal of hydrocarbons from oxidation and polymerization products in the analysis of used turbine oils, and for the determination of adventitious mineral oil in wool grease.[1] Newberger [12] has worked out a method for the direct separation of mineral oil from wool grease without the previous separation of the unsaponifiable matter. It is quite clear from very many tests in my laboratory that if the unsaponifiable matter extracted from 2 to 5 g. of a fatty oil is dissolved in about 50 ml. light petroleum (b.p. 40°–60° C) and passed through an aluminium oxide column, about 1·5 cm. diam. and 20 cm. long, and the column is washed with from 150 to 200 ml. light petroleum, the percolate contains only hydrocarbons. By this means any quantity of hydrocarbon in excess of about 0·1 % may be separated from a fatty oil.[13]

Adventitious contamination of fatty oils has not been uncommon during the last few years. It is probably extremely rare to find any deliberate admixture, but accidental admixture is sometimes difficult to prevent. Thus, it has happened that on analysis by the method described many vegetable oils have been proved to contain mineral oil. In particular, the admixture has shown itself in linseed oil, teaseed oil, rapeseed oil and whale oil. The test may be applied to sperm oil, where traces of mineral oil are detrimental to the interests of users, and it has been shown that the method is as sensitive as for the other oils mentioned. It may be added that the residue obtained on evaporating the percolate from the chromatographic column has been found, for all the above oils when uncontaminated, to be less than 0·1 % of the weight of oil taken for the test.

161–165, *Rosebery Avenue*,
*London, E.C.*1.

[9] Reinbold and Dutton, *J. Amer. Oil Chem. Soc.*, 1948, **25**, 117.
[10] Thorbjarnarson and Drummond, *Analyst*, 1935, **60**, 23.
[11] Williams, *Analyst*, 1945, **70**, 409.
[12] Newberger, *J. Ass. Off. Agric. Chem.*, 1948, **31**, 670.
[13] Williams, *J. Ass. Off. Agric. Chem.*, 1949, **32**, 668

THE APPLICATION OF PARTITION CHROMATOGRAPHY TO THE SEPARATION OF THE SUGARS AND THEIR DERIVATIVES

By E. L. Hirst and J. K. N. Jones

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An account is given of the various applications of chromatography to the separation of mixtures of sugars and sugar derivatives. Methods of partition chromatography on strips of filter paper and on cellulose columns with automatic devices for collecting fractions are described. The uses of special solvents and developing agents (giving specific colour reactions) are discussed and methods for quantitative separation and determination of sugars on the micro scale are given A list of R_G values for various sugars is included and examples are quoted to show the special values of these methods in the investigation of naturally occurring polysaccharides.

The similarity of the sugars in physical and chemical properties makes it difficult to separate them when mixed, and the lack of suitable methods for the separation and determination of sugars on a micro scale has delayed the progress of many investigations in plant and animal physiology. Earlier workers concentrated their efforts on the use of specific precipitants. For example, phenylhydrazine has been used to determine mannose in the form of its characteristic phenylhydrazone [1] and a solution of benzaldehyde in methanolic hydrogen chloride has been employed for the analysis of xylose as its dibenzylidene dimethylacetal.[2] Colorimetric methods have been developed for the estimation of certain sugars and their derivatives. For example, Percival and Ross [3] have studied the development of colour produced during the reaction of alginic acid with carbazole and sulphuric acid (the Dische reaction) and have shown that the concentration of alginic acid can be determined photometrically. Yet another line of approach has been investigated by Wise [4] and his colleagues, who have used certain micro organisms which ferment specifically sugars such as *D*-glucose, *D*-galactose, *L*-arabinose or *D*-xylose. The residual unfermented sugar can then be estimated by appropriate methods.

In 1939 a new approach to the problem of the separation of the sugars was made by Reich [5] who converted a mixture of glucose and fructose into the corresponding coloured *p*-phenylazobenzoyl derivatives and showed that their separation could be observed visually by chromatography on a column of silica or alumina. Since the description of these experiments many workers have utilized the principle of adsorption chromatography for the separation of the sugars and related substances. This method of separation was utilized by Myrbäck and Tamm,[6] by Freudenberg and Boppel,[7] by Mertzweiler, Carney and Farley [8] and by Coleman [9] to separate the *p*-phenylazo-

[1] Bertrand, *Compt. rend.*, 1899, **129**, 1025. Nowotowna, *Biochem. J.*, 1936, **30**, 2177.
[2] Breddy and Jones, *J. Chem. Soc.*, 1945, 738.
[3] Cameron, Ross and Percival, *J. Soc. Chem. Ind.*, 1948, **67**, 161.
[4] Wise and Appling, *Ind. Eng. Chem.* (*Anal.*), 1944, **16**, 28. Appling, Ratcliff and Wise, *Ind. Eng Chem.* (*Anal.*), 1947, **19**, 496.
[5] Reich, *Biochem. J.*, 1939, **33**, 1000.
[6] Myrbäck and Tamm, *Svensk Kem Tidskr.*, 1941, **53**, 441.
[7] Freudenberg and Boppel, *Ber.*, 1940, **73**, 609.
[8] Mertzweiler, Carney and Farley, *J. Amer. Chem. Soc.*, 1943, **65**, 2367.
[9] Coleman, *J. Amer. Chem. Soc.*, 1942, **64**, 1501.

benzoyl derivatives of the fully and partially methylated sugars. Alumina was used as the adsorbent in these experiments, and the work was extended by Coleman, Farnham and Miller,[10] Coleman *et al.*[11] and Hurd and Zelinsky,[12] who employed similar methods for the separation of simple mixtures of the mono- and di-saccharides. Boissonas [13] has described yet another method for the separation of small quantities of the methylated sugars. They were first hydrogenated to the corresponding hexitol derivatives, and then separated on a column of alumina after conversion to the *p*-phenylazobenzoates, difficulties due to the presence of the different ring forms of the sugars being thus avoided.

McNeely, Binkley and Wolfrom [14] have used Magnesol, a hydrated magnesium silicate, as an adsorbent for the separation of mixtures of the sugar acetates. Since these substances are colourless it is necessary to extrude the column of Magnesol and locate the position of the sugar derivatives by streaking the side of the column with permanganate solution (the Zechmeister brush technique). Lew, Wolfrom and Goepp [15] applied this procedure to the chromatography of unsubstituted sugars and related compounds on a type of fuller's earth, and the method has been utilized to separate the components of cane juice, black strap molasses, the degradation products of streptomycin and many other mixtures of sugars. Using hydrated calcium acid silicate and a similar technique, Georges, Bower and Wolfrom [16] were able to separate mixtures of sugar acetates and of methylated sugars.

The chromatographic separation of starch into its components, amylose and amylopectin, has been achieved by Pacsu and Müller [17] and by Ashford, Evans and Hibbert [18] who have confirmed the earlier results of Tanret.[19] Levi [20] has shown that polysaccharides can be separated on adsorbents such as magnesium oxide and calcium carbonate. Glycogen is easily separated, since it is not eluted by hot water and is located on the column by its white fluorescence. Inulin, on the other hand, shows a blue fluorescence. Mark and Saito [21] have used chromatography to fractionate the acetates of cellulose making use of the observation that materials of lower molecular weight were adsorbed more tenaciously than those of high molecular weight. Dickey and Wolfrom [22] separated the products of acetolysis of cellulose by chromatography on Silene E.F. and Magnesol, derivatives of glucose, cellobiose and cellotriose being isolated.[23]

Tiselius,[24] and Tiselius and Hahn [25] have utilized the principle of the flowing chromatogram to separate complex mixtures of sugars. A 0·5 % aqueous solution of ephedrine was used to develop the sugars adsorbed on a column of activated carbon. The elution and separation of the sugars was followed by observing the change in refractive index of the eluate. The temperature of the entire apparatus was thermostatically controlled to

[10] Coleman, Farnham and Miller, *J. Amer. Chem. Soc.*, 1943, **65**, 1588
[11] Coleman, Rees, Sundberg and McCloskey, *J. Amer. Chem. Soc.*, 1945, **67**, 381.
[12] Hurd and Zelinsky, *J. Amer. Chem. Soc.*, 1947, **69**, 243.
[13] Boissonas, *Experimentia*, 1947, **3**, 1; *Helv. chim. Acta*, 1947, **30**, 1689, 1703
[14] McNeely, Binkley and Wolfrom, *J. Amer. Chem. Soc.*, 1945, **67**, 527.
[15] Lew, Wolfrom and Goepp, Jnr., *J. Amer. Chem. Soc.*, 1945, **67**, 1865.
[16] Georges, Bower and Wolfrom, *J. Amer. Chem. Soc.*, 1946, **68**, 2169.
[17] Pacsu and Müller, *J. Amer. Chem. Soc.*, 1941, **63**, 1168.
[18] Ashford, Evans and Hibbert, *Can J. Res. B.* 1946, **24**, 246.
[19] Tanret, *Compt rend.*, 1914, **158**, 1353.
[20] Levi, *Rev brasil. Chem.*, 1940, **10**, 113.
[21] Mark and Saito, *Monatsh.*, 1936, **68**, 237.
[22] Dickey and Wolfrom, *J. Amer. Chem Soc.*, 1949, **71**, 825.
[23] Montgomery, Weakley and Hilbert, *J. Amer. Chem. Soc.*, 1949, **71**, 1682.
[24] Tiselius, *Advances in Colloid Science*, 1942, **1**, 81; *Kolloid-Z.*, 1943, **105**, 101.
[25] Tiselius and Hahn, *Kolloid-Z.*, 1943, **105**, 177.

avoid intermixing of the layers of separated sugar solutions. A similar procedure was used by Claesson,[26] who showed that synthetic polymers, and derivatives of cellulose such as the nitrate, can be fractionated by chromatography. In the course of this work it was found that with carbon as adsorbent substances of high and low molecular weight were adsorbed less tenaciously than those fractions of intermediate molecular weight.

Trimethyl and tetramethyl methylglucosides have been separated on a column of alumina using a flowing chromatogram.[27] The glycosides were developed with a mixture of ether and light petroleum and the eluate tested for the presence of carbohydrate by the Molisch reagent. A partial separation of the trimethyl derivatives of methyl xyloside and methyl arabinoside was also obtained. Norberg, Auerbach and Hixon [28] observed that 2 : 3-dimethyl-, 2 : 3 : 6-trimethyl- and 2 : 3 : 4 : 6-tetramethyl-glucose could be separated one from another by adsorption chromatography on activated fibrous alumina, using acetone-benzene mixture as developer. The position of the sugars on the column was detected by their fluorescence in ultra-violet light.

With the introduction of partition chromatography (1941) a further method for the separation of the sugars became available. Bell,[29] who was the first (1944) to apply this new procedure, showed that 2 : 3 : 4 : 6-tetramethyl glucose and 2 : 3 : 6-trimethyl glucose could be separated from one another and from 2 : 3-dimethyl glucose by partition chromatography on a column of silica gel with water as the stationary phase, using first chloroform and then chloroform–*n*-butanol as the mobile phase. The tetramethyl glucose travelled rapidly through the column when chloroform was used as eluant, the trimethyl and dimethyl glucose remaining at the top ; on changing the solvent to chloroform–*n*-butanol, the trimethyl glucose moved through the column and appeared in the eluate. The position of the dimethyl glucose which remained on the column was then determined by extrusion and streaking with Molisch reagent.

A further advance came with the application of paper partition chromatography to the analysis of complex mixtures of simple sugars, since this method enables detailed studies of the composition of complex polysaccharides to be performed using only minute amounts of material. Originally, the method of paper partition chromatography was developed by Consden, Gordon and Martin [30] for the analysis of protein and peptide hydrolyzates, but in 1946 it was applied by Partridge [31] to the separation of the sugars. It was shown that very small quantities (*ca* 10 μg.) of sugar mixtures could be separated, and their components identified, by virtue of the difference in partition of the sugars between water held by the cellulose fibres of the paper (the stationary phase) and a solvent such as butanol saturated with water (the mobile phase). The procedure consists in placing a spot of a sugar solution near the top of a sheet or strip of filter paper, the top of which is immersed in a suitable solvent mixture contained in a trough. The trough and paper are then placed in a container so that the paper hangs vertically from the trough, and the atmosphere which surrounds it is saturated with the vapour of the solvents and with water vapour. The solvent is then allowed to advance down the paper until the horizontal front reaches the bottom, when the paper is removed and dried. The sugars are identified by the rate

[26] Claesson, *Chem. Soc. Discussion on Chromatography*, 1948 ; *Ann. N.Y. Acad. Sci.*, 1948, **49**, 183
[27] Jones, *J. Chem. Soc.*, 1944, 333
[28] Norberg, Auerbach and Hixon, *J Amer. Chem. Soc.*, 1945, **67**, 342.
[29] Bell, *J. Chem. Soc.*, 1944, 473.
[30] Consden, Gordon and Martin, *Biochem. J.*, 1944, **38**, 224.
[31] Partridge, *Nature*, 1949, **158**, 270 ; *Biochem. J.*, 1948, **42**, 238.

TABLE I

SOLVENT—*n*-BUTANOL 50 PARTS, ETHANOL 10 PARTS, WATER 40 PARTS (TOP LAYER)

Substance	R_G value	Substance	R_G value
Raffinose	0·001	2 : 4-dimethyl galactose	0·41
Lactose	0·016	4 : 6-dimethyl galactose	0·42
Maltose	0·021	2-deoxy ribose, 2 : 6-dimethyl-galactose	0·44
Sucrose	0·03	4 : 6-dimethyl glucose	0·46
D-gluco-*L-gala*-octose	0·038	2-methyl fucose	0·51
D-gulo-*L-gala*-heptose	0·050	3 . 6-dimethyl glucose	
D-gluco-*D-gulo*-heptose	0·053	3 : 4-dimethyl glucose	
D-manno-*D-gala*-heptose	0·058	4 : 6-dimethyl altrose	0·52
D-gala-*L-gluco*-heptose	0·064	2 : 3-dimethyl mannose	0·54
Turanose	0·060	2 : 3-dimethyl glucose	
Galactose	0·070	4-methyl rhamnose	0·57
D-gluco-*L-talo*-octose	0·082	4 : 6-dimethyl mannose	
Glucose	0·090	3 : 4-dimethyl mannose	0·58
Sorbose	0·10	3-methyl quinovose	0·60
Mannose, manno-heptulose		3 : 4-dimethyl fructose	
D-gala-*L-manno*-heptose	0·11	2-deoxy rhamnose	0·61
D-gulo-*L-talo*-heptose		2 . 3-dimethyl arabinose	
Fructose, Gluco-heptulose	0·12	2 : 3 · 4-trimethyl galactose	0·64
Gulose, Arabinose, Tagatose		2 : 4-dimethyl xylose	0·66
Xylose	0·15	2 4 : 6-trimethyl galactose	0·67
4-methyl galactose	0·16	3-methyl altromethylose	0·68
Altrose	0·17	2 . 3 6-trimethyl galactose	0·71
Idose, 6-methyl galactose	0·18	2 : 3-dimethyl xylose	0·74
Talose, Lyxose	0·19	2 · 4 · 6-trimethyl glucose	0·76
Ribose, Fucose	0·21	3 4 : 6-trimethyl mannose	0·79
2-methyl glucose	0·22	2 : 3 . 6-trimethyl mannose	0·81
2-methyl galactose	0·23	2 · 3 : 6-trimethyl glucose	0·83
Riboketose, Apiose, 2-deoxy-galactose	0·25	1 : 3 4-trimethyl fructose	
3-methyl glucose	0·26	3 · 4-dimethyl rhamnose	0·84
Xyloketose		2 3 4-trimethyl glucose	0·85
6-methyl glucose	0·27	3 4 : 6-trimethyl fructose	0·86
Quinovose	0·28	Cymarose	0·87
Rhamnose	0·30	Oleandrose, 2 : 3 4 6-tetra-methyl galactose	0·88
α-methyl mannoside		Tetramethyl fructopyranose	0·90
3 : 4-dimethyl galactose		2 · 3 4-trimethyl xylose	0·94
4-methyl mannose	0·32	2 3 . 5-trimethyl arabinose	0·95
β-methyl arabinoside		2 · 3 · 4 · 6-tetramethyl mannose	0·96
2-deoxy allose	0·33	2 : 3 · 4 . 6-tetramethyl glucose	1·00
2-methyl β-methyl altroside	0·34	2 . 3 5 6-tetramethyl glucose	
Rhamnoketose, 3 · 6-anhydro-glucose, talo methylose	0·37	2 . 3 4-trimethyl rhamnose	1·01
2-methyl xylose, 2-methyl arabinose	0·38	1 : 3 : 4 : 6-tetramethyl fructose	

The rates of movement (R_G values) of the methylated sugars are determined by dividing the distance the sugars have moved from the starting line by the distance moved by tetramethyl glucopyranose (R_G = 1) under the same conditions For the unsubstituted sugars it is convenient to use rhamnose as standard. A sugar cannot always be identified with certainty by this procedure alone, since a number of the methylated sugars travel to the same or similar position on the paper chromatogram and the R_G values are not exactly reproducible, but alter slightly with variations in apparatus and conditions. We have found, for example, that these values vary slightly from one apparatus (enclosed in a bell jar) to another (enclosed in a tank). Identification can be facilitated, however, by using several standards and calculating the R_G value of the unknown sugar from that of the nearest known sugar on the chromatogram. Wherever possible, the rate of movement of the unknown sugar should be compared on the same chromatogram, with that of a known specimen.

at which they move under standard conditions down the face of the paper (see Table I) (Partridge, *loc. cit.*, Brown, Hirst, Hough, Jones and Wadman,[32] Hirst, Hough and Jones,[33] Jermyn and Isherwood [34]). The positions of the reducing sugars are readily detected by the reduction of ammoniacal silver nitrate (Partridge) or by the colours developed with aniline hydrogen phthalate [34a] or *o*-dinitrobenzene and alkali. They may also be detected and in some cases identified by the colours produced on warming with reagents [35] such as trichloracetic acid and aniline, α-naphthylamine, diphenylamine, dimethylaniline, *o*-anisidine, *o*-toluidine, α-naphthylamine 6 : 8-disulphonic acid or urea. For example, diphenylamine serves to differentiate between trimethyl xylopyranose, which gives an intense mauve colour, and trimethyl arabofuranose, which gives a pale green. Urea gives a black colour only with ketoses. Observation of the colours and examination of their fluorescence under ultra-violet light helps to differentiate between various sugars and their derivatives and this procedure is of particular value in the examination of mixtures of methylated sugars (Hough, Jones and Wadman [36]). Forsyth [37] has used a naphtharesorcinol and hydrochloric acid spray; *m*-phenylene diamine [37a] and benzidine [37b] have also been used. The identification of the sugars is, in some cases, facilitated by an examination of the pattern of sugars produced after epimerization of the unknown sugar with lime water and examination of the products on the paper chromatogram (Hough, Jones, and Wadman [38]).

The separation of sugars by partition chromatography was later used as the basis of a method for the quantitative separation and estimation of the sugars.[34 39] Providing the separation of the sugars is complete, an oxidizing agent that will deal with micro quantities serves for the estimation of each of the components. In order to obtain measurable quantities of sugar, a number of spots of the sugar solution are placed on a line drawn parallel to and about 4 in. from the edge of a sheet of Whatman No. 1 filter paper. The sugars are then separated in the manner described above. The positions of the separated sugars are then determined by cutting narrow strips from the sides and centre of the dried paper, spraying them with a suitable reagent and heating. By reference to the sprayed strips, it is possible to cut the paper into areas, each of which contains one of the separated sugar components. The sugars (*ca.* 0·1–1·0 mg.) are extracted with water in a micro Soxhlet apparatus and are then estimated with either Somogyi's micro copper reagent,[40] with sodium hypoiodite, or with sodium periodate solution.[41] This method has recently been extended to the separation and determination of mixtures of the methylated sugars (Hirst, Hough and Jones [33]) (see Table II). Hawthorne [39] has used a similar method to separate and determine even smaller quantities (40–100 μg.) of sugars. In this instance the sugar solutions (4·0 μl.) were placed on the paper by means of an accurately calibrated micropipette. After separation the sugars were collected in caps

[32] Brown Hirst, Hough, Jones and Wadman, *Nature*, 1948, **161**, 720.
[33] Hirst, Hough and Jones, *J. Chem. Soc.*, 1949, 928.
[34] Jermyn and Isherwood, *Biochem. J.*, 1949, **44**, 402.
[34a] Partridge, *Nature*, 1949, **164**, 443.
[35] Hough, Jones and Wadman (in press).
[36] Hough, Jones and Wadman (in press).
[37] Forsyth, *Nature*, 1948, **161**, 239.
[37a] Chargaff, Levine and Green, *J. Biol. Chem.*, 1948, **175**, 67.
[37b] Horrocks, *Nature*, 1949, **164**, 444.
[38] Hough, Jones and Wadman (unpublished results).
[39] Flood, Hirst and Jones, *Nature*, 1947, **160**, 865; *J. Chem. Soc.*, 1948, 1679. Hawthorne, *Nature*, 1947, **160**, 714.
[40] Somogyi, *J. Biol. Chem.*, 1945, **160**, 61.
[41] Hirst and Jones, *J. Chem. Soc.*, 1949, 1659.

cut in paraffin wax and determined by the method of Linderstrøm-Lang and Holter.[42] In another procedure,[43] the area of the spots produced on spraying the sugars on the paper with Tollens reagent was determined and, by comparison with standards, the amount of sugar estimated.

Separation of the sugars on a micro scale by partition chromatography often facilitates their identification, but the method will not differentiate between the *D* and *L* stereo-isomers of the same sugar. To overcome this difficulty larger quantities of material are necessary and Hough, Jones and Wadman [44] devised an automatic apparatus with the aid of which mixtures of simple sugars can be separated on a semi-micro scale on a column of cellulose using solvents such as *n*-butanol–water mixture, *iso*propyl alcohol–water, acetone–water or ethanol. In this procedure, the mixture of sugars to be analyzed is placed on top of a column of cellulose, which has been treated

TABLE II

% OF SUGARS (CALCULATED AS HEXOSAN, METHYL PENTOSAN AND PENTOSAN RESPECTIVELY) PRODUCED AFTER HYDROLYSIS WITH BOILING N SULPHURIC ACID FOR 5 HR.

Polysaccharide	Galactose	Arabinose	Xylose	Rhamnose
Larch ε-Galactan [45]	82·1	10·6	—	—
Damson Gum [46]	30·8	37·9	4·9	3·3(?)
Cherry Gum (Prunus avium,[47] from Italy)	21·8	48·0	5·5	+
Cherry Gum (from a double white ornamental variety)	24·0	48·8	3·6	+
Peach Gum (from Italy) (Prunus persica)	37·8	38·6	13·8	*ca* 5

Glucose derivatives

Polysaccharide	2·3·4·6-tetramethyl	2 3 : 6-trimethyl	2 : 3-di-methyl	? : 6-di-methyl	mono-methyl
Methylated Waxy Maize Starch	4·2	80·0	4·0	10·0	1·3
Methylated rabbit liver Glycogen	8·7	69·0	8·9	10·8	2·4

For details of the procedure used in the analysis of polysaccharides and their methylated derivatives, see ref. [33].

previously with the developing solvent. The solvent is allowed to percolate down the column and the eluate is collected in an apparatus which changes the receiver automatically at predetermined intervals. The apparatus is designed so that the time intervals may be varied at will and so that four columns can be operated per turntable. In the present design of apparatus, the control unit will operate three tables simultaneously at different speeds, so that no less than twelve analyses can be carried out at one and the same time. Some flexibility is necessary as the rate of flow of solvent through the columns of cellulose varies with the size and density of packing of the column and with the solvent used,

[42] Linderstrøm-Lang and Holter, *C.R. Lab. Carlsberg*, 1933, **19**, No 14
[43] Fisher, Parsons and Morrison, *Nature*, 1948, **161**, 764.
[44] Hough, Jones and Wadman, *Nature*, 1948, **162**, 448.
[45] Campbell, Hirst and Jones, *J. Chem. Soc.*, 1948, 774.
[46] Hirst and Jones, *J. Chem. Soc.*, 1938, 1174.
[47] Jones, *J. Chem. Soc.*, 1939, 558.

In this manner, a large number of fractions of eluate are collected. Spots of the eluate from each fraction are then placed in chronological order on the starting line of a sheet filter paper chromatogram, and the sugars are separated on the paper in the usual way. After drying, their positions are shown by spraying the paper with a suitable reagent, and then heating it. The degree of separation is thereby ascertained and the tubes which contain pure sugars can be selected. Since the composition of mixed intermediate fractions can be determined, a complete analysis of the mixture can be effected. In a similar way, mixtures of the methylated derivatives of sugars may be separated and identified.[48] For such mixtures the solvent *n*-butanol–water is useful for the separation of sugars containing less than 40 % of methoxyl groups and *n*-butanol–light petroleum (b.p. 100°–120°) is preferable for the separation of sugars having a value of OMe greater than 40 %. In some instances, separation of the hydrolysis products of a methylated gum has led to the isolation of small fractions of unknown constitution. The identification of such fractions has been facilitated by demethylation [49] with hydrobromic acid and subsequent analysis of the sugars produced on a sheet paper chromatogram. Tetramethyl glucose is readily converted into glucose, trimethyl xylose into xylose and 2 : 6-dimethyl galactose is converted into galactose by this procedure. Fructose derivatives, however, are destroyed.

Separation on a cellulose column has led to the isolation of *D*-tagatose, *D*-galactose and *L*-rhamnose [50] from the mixture of sugars produced on hydrolysis of *Sterculia setigera* gum, and to the isolation of pure specimens of *D*-quinovose, *D*-fucose, *D*-glucose and an unknown sugar from the hydrolysis products of convulvulinic acid.[51] Formose,[52] the mixture of sugars produced on heating formalin with calcium carbonate, has been shown [53] to contain at least six sugars, some of which are ketopentoses in addition to material of higher and lower molecular weight.

The uronic acids and their derivatives may also be separated on a column of cellulose, a mixture of *n*-butanol, glacial acetic acid, *n*-butyl acetate and water being employed as the mobile phase. The aniline–trichloroacetic acid reagent gives a deep red colour with the methylated derivatives of uronic acids.

The examples given are sufficient to indicate that problems involving the separation and determination of sugars have been very greatly facilitated by the use of partition chromatography and these new techniques which are applicable on the micro scale provide a powerful and much-needed weapon for an attack on many biochemical problems.

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[48] Hough, Jones and Wadman, *J. Chem. Soc.*, 1949, 2511.
[49] Hess and Neumann, *Ber.*, 1935, **68**, 1371. Araki and Hasi, *J. Chem. Soc., Japan* 1939, **60**, 774 (cf. *A.C.S. Abstr.*, 1942, **36**, 4483, 5146).
[50] Hirst, Hough and Jones, *Nature*, 1949, **163**, 177.
[51] Jones and Stacey (unpublished).
[52] von Euler, *Ber.*, 1906, **39**, 39, 48.
[53] Byers and Jones (unpublished).

THE CHROMATOGRAPHY OF PROTEINS. THE EFFECT OF SALT CONCENTRATION AND pH ON THE ADSORPTION OF PROTEINS TO SILICA GEL

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The adsorption of an ovalbumin and a serum albumin preparation on silica gel was dependent upon the salt concentration, and increasing ionic strength in the range of 0·025 to 3 resulted in increased adsorption. The adsorption of two globulin preparations was not so dependent on salt concentration, and the immune globulin showed maximal adsorption at all salt concentrations studied. The adsorption of all the preparations appeared to reach approximately the same maximal values.

The adsorption of the ovalbumin and the immune globulin was also dependent upon the pH, the ovalbumin demonstrating maximal adsorption at pH 4·0 and 5·0, decreasing adsorption at pH 6·0, and little if any at pH 7·0, 7·5, and 8·3, while the immune globulin exhibited maximal adsorption from pH 4·0 to 7·5 and decreased adsorption only at pH 8·3. These results were obtained at an ionic strength of 0·1.

Chromatography of proteins in columns of silica gel and Supercel showed that the serum albumin and immune globulin could be distinctly separated in a solvent of sodium phosphate at pH 7·0 and an ionic strength of 0·1. In chromatograms of human serum under these conditions albumin and globulin were distinctly separated.

Several other substances were studied for their ability to adsorb protein. Although "salting-out" adsorption in paper strips chromatograms was demonstrable, the adsorption by paper in batch experiments was not detectable. Although potato starch manifested adsorption of acid dyes, no adsorption of proteins could be shown. No adsorption of proteins was seen on aluminium hydroxide, but weak adsorption was observed on Supercel and with carbon adsorption was stronger than with silica gel.

The investigation of proteins has depended much upon the analytical procedure available. Chromatographic analysis has provided much help in the study of other substances, for example, the amino acids, yet the application of chromatography to protein has not been extensive. That proteins are adsorbed from aqueous solutions onto various solids is well known, and the phenomenon is frequently made use of in batch operations in purification procedures. The solution is shaken with a certain amount of finely divided adsorbent and the adsorbent removed by filtration or sedimentation. Usually conditions are arranged so that it is contaminating protein which is adsorbed and the substance to be purified remains in solution, since elution of adsorbed protein is frequently difficult and may require the use of solvents which are injurious to the protein. However, such batch adsorptions do not lend themselves easily to analytical work and the advantages of chromatographic analysis are not made use of.

Another approach to the problem was indicated by Tiselius who described "salting-out" adsorption and showed that the presence of salts, such as ammonium sulphate and potassium phosphate, promoted the adsorption of certain proteins and acid dyes onto silica gel and paper.[1] The importance

[1] Tiselius, *Arkiv Kemi, Min. Geol. B*, 1948, **26**, No. 1.

* Address after 1st November, 1949: National Institutes of Health, Bethesda, Maryland.

of salt concentration was shown by examples where adsorption was marked in the presence of salts even though little or no adsorption could be demonstrated in their absence. In the present paper this work has been extended and some of the factors affecting adsorption have been investigated. Some chromatographic analyses are reported.

Experimental

In general, two experimental approaches were employed. In the first, batch experiments were done in which solutions were mixed with an adsorbent until adsorption appeared complete, the adsorbent removed by centrifugation or filtration and the protein concentration determined in the remaining solution. The concentration of protein was measured by determining its optical density in a Beckman spectrophotometer at 278 mμ. This method of measurement imposed certain precautions because the optical density values obtained at this wavelength are increased by the presence of finely divided particulate matter Since it was desirable to have the adsorbent in a finely divided state in order to promote adsorption, it was necessary to employ procedures which removed the adsorbent efficiently. Another source of suspended material was found in experiments where shaking was done in too vigorously a fashion and caused some denaturation to take place, so that a finely divided suspension of insoluble protein resulted. This last source of difficulty could be avoided by carrying out adsorption in flasks which were rotated so that the fluid travelled around the bottom of the flask and kept the adsorbent well suspended but yet no foaming or bubble formation took place. Spectrophotometric measurements were done at 320, 278 and 260 mμ with water in the control cell. Non-specific optical adsorption revealed itself by increased values at 320 and 260 mμ. A ratio between the optical densities at 260 and 278 mμ of less than 0·7 was observed with all clear protein preparations before adsorption. With adsorbed preparations such a ratio indicated satisfactory conditions and was always obtained except at times when very little protein remained in solution.

Shaking was carried out for 30 min. since it was observed that absorption was nearly complete at this time In one experiment with the serum albumin at 0·1 %, pH 7·7 and μ (ionic strength) = 0·125, the amount of protein absorbed after 5, 15, 30, 60 and 120 min. was 27, 30, 32, 33 to 35 %, respectively.

The second general approach employed, and the one toward which the batch experiments were directed, was chromatographic analysis in columns. Appropriate solutions were passed through a column of adsorbent contained in filters which were essentially the same as those described by Claesson [2] but were made of Perspex instead of metal The filters have dimensions which give them satisfactory height/diameter ratios and their volumes are arranged to be multiples of π. Thus a filter whose volume is said to be 250 π has a volume of 250 π mm.[3] The effluent was collected in a fraction collector designed by Claesson (unpublished). With this fraction collector the effluent drains into a container which, when filled, siphons into a test-tube, where the weight of the fluid actuates a wheel which carries a new tube into position below the siphon. With the siphon used the volume drained was about 3½ ml., but the variation between successive drainings was several tenths of a millilitre. This variation affects the results presented in the charted chromatographic analyses by giving some uncertainty to the abscissa value, which is not important in the work here presented. For details on the theory and practice of chromatography as it is carried out here the reader is referred to two reviews.[2,3]

Most of the protein preparations used were obtained through the courtesy of Dr. K. O. Pedersen The immune serum globulin was from humans and was an American commercial product which had been prepared by alcohol fractionation. The serum globulin was a bovine product obtained by precipitation at neutral reaction at ammonium sulphate concentration between 35 % and 45 % saturation. The serum albumin was also a bovine product and was obtained by precipitation with neutral ammonium sulphate at greater than 60 % saturation.

[2] Claesson, *Arkiv Kemi, Min. Geol. A*, 1946, **23**, No. 1.
[3] Tiselius, *Advances in Protein Chemistry*, 1947, **3**, 67.

The ovalbumin was a thrice-crystallized product. All experiments were carried out at room temperature, which varied from 19°–22° C.

Results

Silica Gel as Adsorbent.—Before efforts were concentrated on silica gel, some attempts were made to chromatograph serum proteins in paper strips. Adsorption could be demonstrated when salt concentrations close to salting-out concentration were employed with protein concentration of about 0·1 %. However, it was difficult to work with lower concentration of protein because, after the spreading which always occurred, the boundaries were not clearly visible with the tests used to detect protein, which were the nitrosonaphthol test for tyrosine and bromphenol blue test,[4] both modified for use on paper. Several adsorbents were then compared and it was observed that although salting-out adsorption of acid dyes occurred on starch, salting-out adsorption of protein was weak on this adsorbent. However, adsorption of proteins proved to be strong on silica gel

Preparation of Silica Gel.—A number of samples of silica gel from different manufacturers were investigated, using the coloured protein phycoerythrin. It was found that there was considerable variation between samples but that the chief reason for the variation lay in their alkalinity or acidity. The samples which were strongly alkaline adsorbed phycoerythrin poorly even in the presence of 0·5 M ammonium sulphate, while those which were strongly acid adsorbed strongly even in the absence of ammonium sulphate. Other samples of intermediate reaction adsorbed phycoerythrin only in the presence of ammonium sulphate. Accordingly an available silica gel (acid silicic, powd., Bakers' Analyzed) which appeared satisfactory was selected and used for the rest of the experiments. It was also found that additional grinding increased the speed of absorption. The following method of preparation was adopted and followed for the work hereafter described. The silica gel was ground wet in a ball mill for 8 to 16 hr., suspended in five to ten times its volume of water and allowed to settle overnight. It was then re-suspended in water and the reaction of the suspension adjusted to approximate neutrality with sodium hydroxide. The silica gel was washed until the supernatant was nearly clear by allowing it to settle 12 to 24 hr. and decanting the supernatant. The largest particles were eliminated by discarding the sediment which settled in about ½ hr. From such stock suspensions lots for particular experiments were prepared by suspending an amount of silica gel in the buffer which was to be used, adjusting to the pH desired by the addition of sodium hydroxide or hydrochloric acid, and washing the silica gel three or four times by centrifugal sedimentation and re-suspension in fresh buffer, the final sediment being dried by pressing between filter papers and drying at 105° overnight. The resulting dry powder appeared to be stable in that no change in adsorptive properties was observed during the course of several months.

Effect of Salt Concentration on Adsorption of Proteins.—The effect of salt concentration was investigated with the results which are summarized in Fig. 1. These results were obtained in batch experiments in which 5 ml. solution were mixed for 30 min. with 100 mg. silica gel washed in the buffer and dried as described above. The same solutions to which no silica gel was added served as control, and the amount of adsorption was determined by subtracting the optical density at 278 mμ of the adsorbed solution from the control solution. In order to regulate the pH, all solutions contained sodium phosphate buffer with a ratio of 27/19 of disodium to monosodium phosphate. At $\mu = 0{\cdot}1$ this buffer gives a pH of 6·99. The sodium chloride and sodium sulphate solutions were neutral before the addition of sodium phosphate.

All solutions contained enough phosphate buffer to give an ionic strength of 0·1 except the three shown at the far left in Fig. 1, whose ionic strengths were 0·025. The solutions with ionic strengths greater than 0·1 were obtained by adding sodium chloride or sodium sulphate to the sodium phosphate so that the total ionic strengths were those indicated.

[4] Feigl, *Qualitative Analysis by Spot Tests* (New York, 1947).

The eight salt solutions used had the following salt compositions: (1) 0·001 M monosodium phosphate + 0·008 M disodium phosphate, (2) 0·004 M monosodium phosphate + 0·032 M disodium phosphate, (3) 0·25 M sodium chloride + 0·004 M monosodium phosphate + 0·032 M disodium phosphate, (4) 0·10 M sodium sulphate + 0·004 M monosodium phosphate + 0·032 M disodium phosphate, (5) 0·25 M sodium sulphate + 0·004 M monosodium phosphate + 0·032 M disodium phosphate, (6) 0·50 M sodium sulphate + 0·004 M monosodium phosphate + 0·032 M disodium phosphate, (7) 0·75 M sodium sulphate + 0·004 M monosodium phosphate + 0·032 M disodium phosphate and (8) 1·0 M sodium sulphate + 0·004 M monosodium phosphate + 0·032 M disodium phosphate.

The silica gel was removed from the adsorption mixtures by two centrifugations. The controls were also centrifuged. It will be seen by examination of Fig. 1 that the effect of the salt concentration depends on the protein employed. In the case of ovalbumin there is increasing adsorption in the whole range. On the other hand with the immune globulin little change is seen with variation in ionic strength. The serum globulin falls between these two as does the serum albumin.

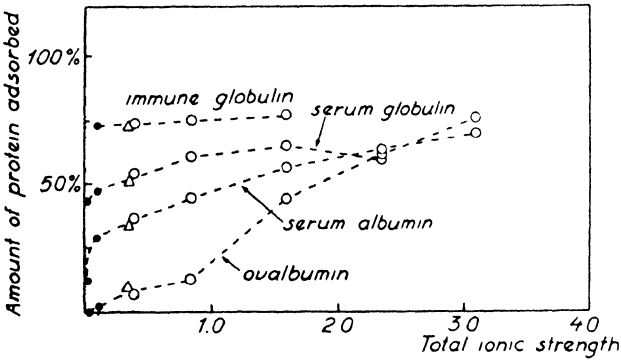

FIG. 1.—The effect of salt concentration on the amount of protein adsorbed onto silica gel from a 0·1 % solution of the protein indicated. The salts used were sodium phosphate (●), sodium chloride + sodium phosphate (Δ) and sodium sulphate + sodium phosphate (○). All solutions contained sodium phosphate at an ionic strength of 0·1, except the three at the far left, where the ionic strength was 0·025. pH 7·0 with $\mu = 0{\cdot}1$.

The serum albumin used behaved in this and other experiments as if it contained two components widely separated in adsorbability. It will be noted that some of the values are not reported for the two globulins. This arises from the fact that at certain salt concentration these proteins were not completely soluble and the control tubes, those containing no silica gel, showed a precipitate before centrifugation.

The most instructive results were those obtained with the immune globulin and ovalbumin, since these illustrated two distinct types of behaviour, one where adsorption was high and unaffected by salt concentration, the other where adsorption was low when little salt was present but which reached a similarly high value with increasing salt concentration.

The Effect of pH on Adsorption of Protein.—The effect of pH on the amount of protein adsorbed onto silica gel is seen in Fig. 2. Again in this experiment 5 ml. protein solution were added to 100 mg. silica gel previously washed in the same buffer. The buffers all had ionic strengths of 0·1 and the pH after addition of the globulin as measured by the glass electrode were 4·00, 5·04, 5·98, 6·99, 7·54 and 8·34 (the ovalbumin solutions did not differ from these by more than 0·03 pH units). They had the following composition: (1) 0·45 M acetic acid + 0·10 M sodium acetate, (2) 0·04 M acetic acid + 0·10 M sodium acetate, (3) 0·070 M monosodium phosphate + 0·010 M disodium phosphate, (4) 0·019 M monosodium phosphate + 0·027 M disodium phosphate,

(5) 0·004 M monosodium phosphate + 0·032 M disodium phosphate and (6) 0·1 M glycine (+ 0·01 M sodium hydroxide) + 0·1 M sodium chloride.

After 30 min. mixing the silica gel was removed by two centrifugations. Protein solutions to which no silica gel was added served as controls, and the amount of adsorption was determined by the difference in optical density at 278 mμ between the adsorbed solutions and their controls.

It may be seen in Fig. 2 that different results were obtained with immune globulin and ovalbumin. Immune globulin showed high adsorption from pH 4 to pH 7·5, while ovalbumin showed decreasing adsorption between pH 5 to 7 and very little adsorption at more alkaline reactions. It may also be noted that the greatest differences between immune globulin and ovalbumin lay in the pH range 7 to 7·5. The data suggest that a high electrostatic charge on a protein molecule prevents its adsorption to silica gel. The same relationship is also suggested by the data showing the effect of ionic strength on adsorption (Fig. 1).

Adsorption Isotherms of Proteins.—Data showing the relationship between the amount of protein adsorbed and the concentration of protein remaining after adsorption is complete may be obtained by two methods, one by varying the protein and the other by varying the silica gel, that is, with

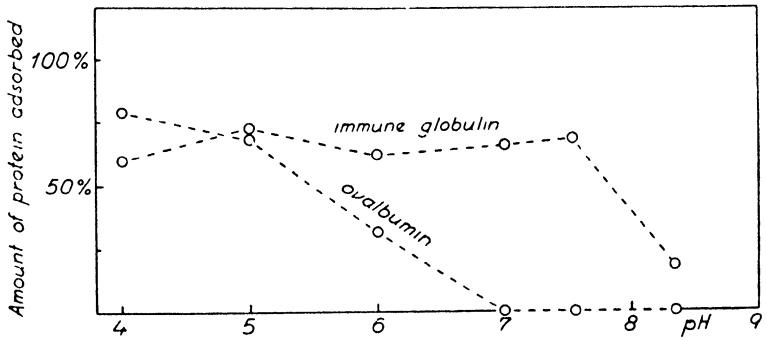

FIG. 2.—The effect of pH on the amount of protein adsorbed onto silica gel from a 0·1 % solution of the proteins indicated. The ionic strength was 0·1 throughout. Acetate buffer was used at pH 4·0 and 5·0, phosphate at pH 6·0, 7·0 and 7·7, and glycine at 8·5.

constant silica gel or with constant protein. Identical results by the two methods would be obtained only when the protein preparation was completely homogeneous. The constant protein method seemed to yield information more useful to our purposes, since with inhomogeneous protein preparation the constant silica gel method gave falsely idealized results. For example, the serum albumin preparation appeared to contain two components, one which was adsorbed very strongly and one which was adsorbed not at all under the conditions employed, and when this preparation was studied by the constant silica gel method the results showed an increasing amount of protein adsorbed in nearly exact proportion to the amount of protein remaining in solution. When the constant protein method was used the results seen in Fig. 3 c were obtained.

The experiments were carried out much as those described before. 5 ml. 0·1 % protein solution were added to a weighed amount of silica gel, the suspension was then rotated 30 min., the silica gel removed by two centrifugations, and the concentration of protein determined at 278 mμ, the amount of protein adsorbed being estimated by subtraction from the value of the control to which no silica gel had been added. The amounts of silica gel used were 5, 10, 20 (or 25), 50, 100 and 200 mg. The concentration of protein has been expressed in terms of optical density units as obtained with the Beckman spectrophotometer, since in this form it is more easily correlated with the chromatographic curves shown below. The value given for adsorption per gram was calculated by multiplying the difference between optical density of adsorbed solution and control by

5 (ml.) and dividing the product by the weight of silica gel which had been used.

The results of Fig. 3 again show the immune globulin and the ovalbumin at two extremes with the serum albumin and the serum globulin lying in between. The immune globulin showed high adsorption in the absence of sodium sulphate and the amount of adsorption was not increased by the addition of sodium sulphate. The ovalbumin, on the other hand, showed no significant adsorption except in the presence of sodium sulphate. The serum globulin showed some increase in adsorption on addition of sodium sulphate. The serum albumin showed considerably increased adsorption with sodium sulphate.

The exact course of the curves close to the origin has not been determined but it is obvious that the amount of adsorption per gram rose very steeply with low protein concentrations. The curves showing adsorption of the serum albumin in the absence of sodium sulphate show an unusual position. This

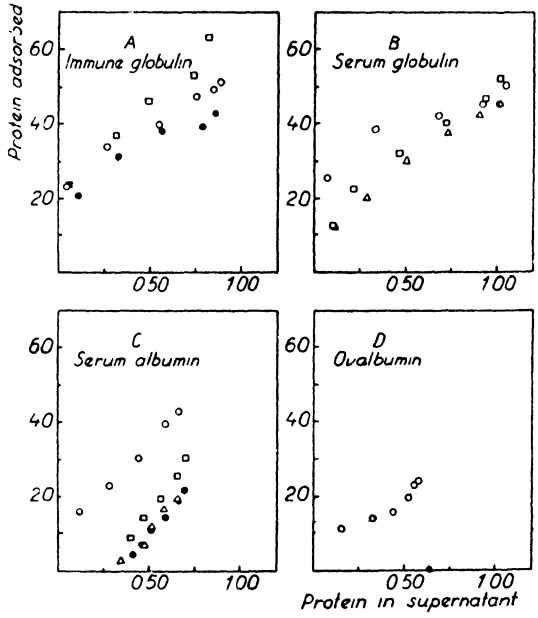

FIG. 3.—Adsorption isotherms obtained by the constant protein method. The protein concentrations after adsorption are plotted against the amount of protein adsorbed per gram of adsorbent. The salts used were sodium phosphate ($\mu = 0{\cdot}1$) at pH 7·0 (□), pH 7·3 (Δ) and pH 7·7 (●), and the same pH 7·7 buffer plus sodium sulphate (○).

presumably arises from the experimental approach (constant protein and different amounts of silica gel) and the inhomogeneity of the preparation, a minor component being strongly adsorbed whereas the major component shows very little adsorption. This supposition is borne out by the chromatogram of this preparation. It should be noted that the irregularities in the values corresponding to high concentration of protein in the supernatant arise from the method used and reflect the uncertainty in estimation of small differences between adsorbed solution and control.

Column Experiments.—The column experiments reported were done with frontal analysis, whereby a solution of protein is passed through the column until adsorption is complete, and the effluent contains as much of the material being adsorbed as does the solution entering the column. Although each component is not isolated separately, and after the first component each succeeding component must be contaminated with the preceding, such a procedure proved very helpful in preliminary orientation.

The column was filled with a mixture containing equal parts by weight of Supercel and silica gel prepared as described above. The filling was accomplished by preparing a thin paste of the Supercel and silica gel by adding to the dry powder enough of the solvent (solution without the protein) to yield a paste from which the small bubbles produced by mixing could rise to the surface within a few seconds. A column with high resistance to flow appeared usually to have been caused by the accidental inclusion of air bubbles in the adsorbent. It was observed that rapid flow-rates obscured the development of steps and a rate of one drop in 50 to 60 sec. was eventually adopted. Such a slow rate made it necessary for 24 to 48 hr. to elapse before the experiment was concluded. Under such conditions bacterial contamination appeared in the solution in the reservoir and in the collected effluents. To prevent the disturbances arising

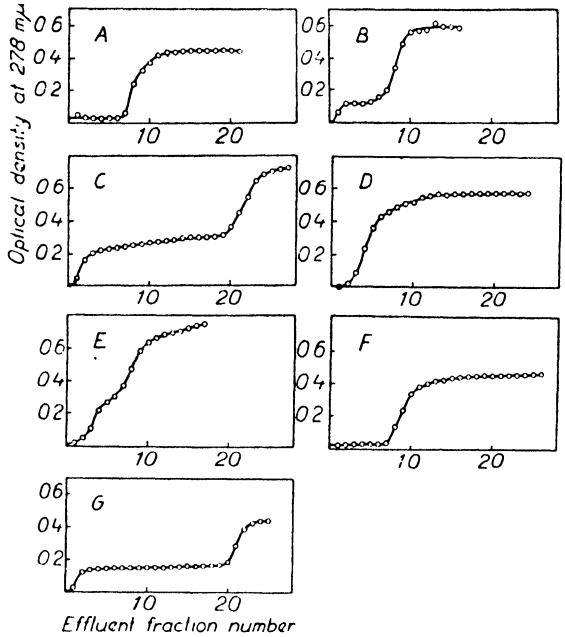

FIG. 4.—Frontal analysis chromatograms of several protein preparations and mixtures of them. The solvent was pH 7·0 sodium phosphate, μ = 0·1 + 0·1 % formalin in all cases except D and E, where it was pH 7·7 sodium phosphate, μ = 0·1 + 0·5 M sodium sulphate + 0·1 % formalin. A. 0·05 % immune globulin. Filter volume 250 π. B. 0·05 % immune globulin + 0·05 % serum albumin. Filter volume 250 π. C. 0·05 % immune globulin + 0·05 % serum albumin. Filter volume 500 π. D. 0·1 % serum albumin. Filter volume 250 π. E. 0·05 % immune globulin + 0·05 % serum albumin. Filter volume 250 π. F. 0·1 % fetuin. Filter volume 500 π. G. 0·05 pea-seed globulin. Filter volume 500 π.

from bacterial growth 0·1 % formalin was incorporated in the solution. Little effect on adsorption appeared to result, and adsorption isotherms of the immune globulin on silica gel in pH 7 sodium phosphate, μ = 0·1, with and without 0·1 % formalin were nearly identical.

Not much pressure was needed to achieve the flow-rates desired, and accurate control of the pressure was necessary. The effluent was collected in the fraction collector in successive 3½ ml. amounts and its protein content estimated by measurement of optical density at 278 mμ. A measurement was also made at 320 mμ in order to gain an impression of the amount of adsorbent or other interfering material which might be present. The spectrophotometric measurements were done with the solvent in the control cell. Spectroscopic analysis from 240 to 370 mμ was then carried out on samples from representative portions of the chromatogram.

Some of the chromatograms are given in Fig. 4. Most of the experiments were carried out in sodium phosphate, with a proportion of disodium to monosodium phosphate of 27/19, $\mu = 0{\cdot}1$ and pH 6·99. 0·1 % formalin was also added.

Fig. 4 A shows that when 0·05 % of immune globulin was introduced at the top of the column, the effluent first contained no protein but that after 7 volumes or 24 ml. ($7 \times 3\frac{1}{3}$ ml.) protein began to appear and that after a total of 12 volumes the adsorbent had become saturated and the effluent contained globulin in the same concentration as the solution being introduced (the optical density of the control at 278 mμ was 0·467). On the other hand, when the serum albumin in 0·05 % concentration was studied under the same conditions the effluent showed nearly maximal protein even in the first volume. With the serum albumin the optical density of the effluent at 278 mμ never reached that of the original solution, but instead rose to a value about 80 % even when the experiment was continued for 48 hr. This indicated the presence of a component responsible for about 20 % of the optical density which was very strongly adsorbed, in confirmation of the impression gained in work reported earlier in the paper.

When a mixture of 0·05 % serum albumin and 0·05 % immune globulin was studied the pattern seen showed two distinct components as in Fig. 4 B and 4 C. In 4 B a 250 π filter was used and the immune globulin appeared in the 5th or 6th tube although the serum albumin had been present from the 1st tube. In 4 C a 500 π filter was used and the appearance of the globulin was accordingly delayed although more so than was expected from 4 B, since it did not appear until the 20th tube. The identity of the components in the chromatogram in 4 C was confirmed by precipitation with ammonium sulphate solutions. Aliquots from various tubes were mixed with 4 M ammonium sulphate to give 2 M and 3 M solutions Thus in 3 M ammonium sulphate precipitate was obtained with the 5th, 10th, 15th, 20th and 25th tube, but not the 1st, whereas in 2 M ammonium sulphate precipitate was obtained with only the 25th.

The effect of increasing the salt concentration is shown in Fig. 4 D and 4 E where it is seen that in 0·5 M sodium sulphate the serum albumin adsorbs sufficiently to give a retention volume of about 10 ml. ($3 \times 3\frac{1}{3}$ ml.). The mixture of serum albumin and serum globulin is not well resolved in 4 E where the serum albumin appeared in about the 3rd tube and the immune globulin in the 6th. The identity of the components was again checked with ammonium sulphate. 3 M ammonium sulphate gave precipitate with material from the 5th, 10th, and 15th tube but not the 1st, whereas the 2 M salt produced precipitate with the 10th and 15th, but not the 1st and 5th.

Ovalbumin also showed adsorption in column experiments in the presence of salt, and in one experiment, the tracing of which is not given here, showed a retention volume of 20 ml. ($6 \times 3\frac{1}{3}$ ml.) with 0·1 % ovalbumin in pH 7·7 sodium phosphate, $\mu = 0{\cdot}1 + 0{\cdot}75$ M sodium sulphate + 0·1 % formalin (pH 7·7 before addition of sodium sulphate), and a 250 π filter.

Chromatograms of the serum globulin preparation indicated the preparation to be heterogeneous since the curve rose more or less linearly from the origin to a maximal value in about the 10th tube.

In Fig. 4 F may be seen the chromatogram obtained with 0·1 % fetuin, which shows only one component with a retention volume of about 26 ml. ($8 \times 3\frac{1}{3}$ ml.). In Fig. 4 G is given the pattern of a preparation of pea-seed globulin obtained from C.-E. Danielsson. Two components are seen, but only the second appeared to be protein since the first component showed a spectrophotometric curve with a maximum at 260 mμ. Other evidence suggests that the poorly adsorbing component is a nucleic acid, or related product,* but not enough of the material resulted from this experiment to identify it. The two seed globulins, legumin and vicilin, were apparently both contained in the second chromatographic component.

Several samples of human serum were studied by this method. The pattern seen in Fig. 5 is representative of the results obtained and is instructive because

* C.-E. Danielsson, personal communication.

the serum contained agglutinins for *Brucella abortus*. The results of precipitation with 2 M and 3 M ammonium sulphate solutions are also presented. The serum was diluted 1/77 times in pH 7 sodium phosphate, $\mu = 0{\cdot}1 + 0{\cdot}1$ % formalin and the solution showed an optical density at 278 mμ of 1·27. The results considered together show the albumin appeared soon and by the 3rd tube was near its maximum. Thereafter a small inhomogeneous component appeared from about the 5th to the 10th tubes; it did not appear to be precipitable with 3 M ammonium sulphate. After the 10th tube the globulin began to appear and increased in amount up to the 20th tube. The brucella agglutinins were first detected in the 15th tube and increased thereafter. A strongly adsorbed component, which did not appear in the eluate, was responsible for the difference between the highest reading on the eluate (1·15) and that of the unadsorbed solution (1·27). Although the experiment does not appear to yield novel information regarding the composition of human serum, it does illustrate some of the possibilities of the chromatographic analysis of complex biological mixtures and the advantages that the operator has in having the fractions separated in test-tubes

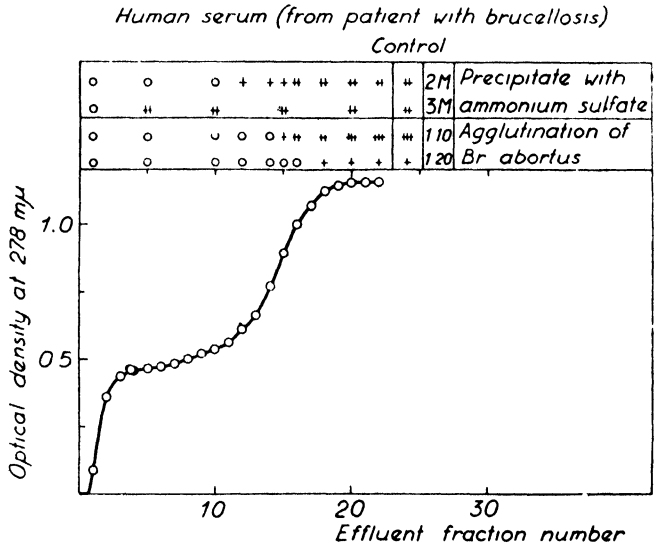

FIG. 5.—Frontal analysis chromatogram of human serum containing antibodies for *Brucella abortus*. Solvent: pH 7·0 sodium phosphate = 0·1 + 0·1 % formalin. Filter volume 500 π.

Comparison with other Adsorbents.—A few experiments using other adsorbents were carried out. It has already been stated that paper and starch showed no detectable adsorption in batch experiments. For example, in one experiment 5 ml. 0·1 % serum albumin in 2 M ammonium sulphate were treated with 0·5 g. filter paper and another 5 ml. with 0·5 g. potato starch and no detectable adsorption occurred. Under the same conditions over 93 % of the serum albumin was adsorbed on silica gel.

Aluminium hydroxide was also inactive. In experiments, in which 5 ml. quantities 0·1 % ovalbumin and 0·1 % immune globulin in pH 7 sodium phosphate, $\mu = 0{\cdot}1$, or in pH 7·2 sodium phosphate, $\mu = 0{\cdot}1 + 0{\cdot}5$ M sodium sulphate, were treated with 100 mg. aluminium hydroxide (powd., Bakers' Analyzed), for 30 min., no detectable adsorption occurred.

Supercel and carbon (Norit FHX Special) were tested and found to adsorb proteins under similar conditions. Quantitative results were not achieved because of difficulties encountered in removing the adsorbent, but comparison with

results with silica gel indicated that in terms of adsorption per gram of adsorbent the carbon adsorbed two to three times as much protein as did silica gel, whereas the Supercel adsorbed only 1/10 to 1/20 times as much protein as did silica gel. With both carbon and Supercel the presence of 0·5 M sodium sulphate increased adsorption of ovalbumin and serum albumin but not of immune globulin. A few column experiments with these adsorbents have also been done with results that also show the high adsorptive capacity of carbon and low adsorption of Supercel.

Discussion

In the work reported here some of the factors affecting adsorption of proteins to silica gel have been investigated and the role the factors play in chromatograms has been studied. It was seen in the range of ionic strength from 0·025 to 3·1 that the adsorption of the serum albumin and ovalbumin preparation increased with increasing ionic strength, while the adsorption of the serum globulin and immune globulin preparation was not much affected by ionic strength. The maximal amount of adsorption of the albumins and globulins studied was about the same, but with the albumins maximal adsorption was seen only with high salt concentrations. The difference in adsorption of albumin on the one hand and globulin on the other was greatest in the presence of salt of low ionic strength.

The effect of pH on adsorption was also different for ovalbumin and immune globulin. With ovalbumin, high adsorption was observed at pH 4 and 5 and adsorption decreased rapidly above pH 5 until it was scarcely measurable at pH 7 and above. The immune globulin behaved differently in that high adsorption was manifested from pH 4 to pH 7·5. The greatest difference between ovalbumin and immune globulin was thus seen in the range of pH 7·0 to some point below pH 8·3.

The relationships between salt concentration and pH and adsorption show that the optimal conditions for separation of albumin from globulin are a low ionic strength, of 0·1 or less, and a pH of about 7 to 8. It would also be suggested that for the separation of globulins from one another a higher pH might be needed, and that for the chromatographic separation of albumins from one another a lower pH or higher ionic strength would be of advantage.

That the electrokinetic potential of the protein molecule is of great importance in determining its adsorption to silica gel is indicated by the ionic strength—pH relationships. That this could be a concept of some functional value is illustrated by the results reported by Delbrück with a bacteriophage and sintered-glass filters,[5] by Reiley with Rous chicken sarcoma virus and celite,[6] by Davenport and Horsfall with PVM virus and lung particles,[7] by Reiley *et al.* with melanized particles from mouse melanomas and celite,[8] and by Leyon with murine encephalomyelitis and paper.[9] In all these cases elution was successful with distilled water or very weak salt solution, while adsorption had taken place from salt solutions of not more than physiological concentration.

The adsorption isotherms showed in general a steep rise with low protein concentrations. A shape such as this is helpful for obtaining sharp fronts but does not indicate satisfactory conditions for elution analysis when the same solvent is used. The isotherm curves were of aid in calculating the retention volume of proteins in column experiments. For example, in Fig. 4 A it could be calculated that the protein step would appear in the

[5] Delbrück, *J. Gen. Physiol.*, 1940, **23**, 643.
[6] Reiley, *Science*, 1948, **107**, 573.
[7] Davenport and Horsfall, *J. Expt. Med.*, 1948, **88**, 621.
[8] Reiley, Hesselbach, Fiala, Woode, and Burk, *Science*, 1949, **109**, 361.
[9] Leyon, *Arkiv Kemi* (in press).

8th or 9th tubes, and in Fig. 4 D in the 4th or 5th tubes. (The amount of silica gel in a 250 π filter averaged about 0·32 g.)

Chromatography was carried out in columns containing silica gel, to which Supercel was added to decrease resistance to flow. With a solvent containing sodium phosphate at an ionic strength of 0·1 and pH 7·0, frontal analysis distinctly separated serum albumin and globulin. The patterns obtained with human serum illustrate some of the possibilities of the method, and its applicability to the study of biologically active materials.

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THE APPLICATION OF CHROMATOGRAPHY TO AMINO ACIDS AND PEPTIDES

BY TUDOR S. G. JONES

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An account is given of the application of the chromatographic methods, adsorption, ion exchange and partition chromatography in its forms using paper and columns of silica gel, starch and kieselguhr to amino acids and polypeptides. Paper chromatography is considered in greater detail because of its wide application in this field. Attention is given to the conditions employed, including the influence of the paper support, the solvent systems used and their direction of flow, and to methods of detection. The use of kidney *D*-amino acid oxidase for the determination of configuration is described in greater detail than hitherto. A discussion of the mechanism of the method is followed by many examples of specific applications.

The following account of the application of the chromatographic method to the separation, identification and determination of the amino acids and peptides includes work published up to July, 1949. Not every article which has appeared is cited, and where no reference is given, the statement is derived from the author's own unpublished work. The subject will be treated under three divisions: (i) adsorption chromatography, (ii) ion exchange, (iii) partition chromatography, although no implication is made that these divisions are mutually exclusive or that the mechanisms involved follow strictly the title of the division. The following authors have published reviews mentioning amino acids and peptides in the following divisions: all methods, Martin and Synge,[1] Cannan,[2] Strain [3]; ion exchange, Tompkins,[4] Applezweig,[5] Kunin [6]; paper partition, Consden [7]; adsorption, Tiselius.[8]

[1] Martin and Synge, *Advances in Protein Chemistry*, II, 1.
[2] Cannan, *Ann. N.Y Acad. Sci.*, 1946, **47**, 135.
[3] Strain, *Anal. Chem.*, 1949, **21**, 75.
[4] Tompkins, *J. Chem. Educ.*, 1949, **26**, 32, 92.
[5] Applezweig, *Ann. N.Y. Acad. Sci.*, 1948, **49**, 295.
[6] Kunin, *Anal. Chem.*, 1949, **21**, 87.
[7] Consden, *Nature*, 1948, **162**, 359.
[8] Tiselius, *Kolloid-Z.*, 1943, **105**, 101.

1. Adsorption Chromatography.—The conditions under which amino acids are adsorbed from solution, using the term in its classical sense, were defined as early as 1917,[9] but little advantage was taken of this discovery for the analytical separation of amino acids until the work of Tiselius [10 11] laid the foundations for the use of charcoal, particularly as a component for his group separation [12] (see §2). At about the same time a number of workers used activated alumina as the adsorptive material, but the applications belong more properly to the next section. This material has been used, however, for separation of tryptophane, tyrosine and phenylalanine.[13] The adsorptive chromatography of coloured derivatives of amino acids, using basic zinc carbonate,[14] has been taken up by Reich, who used alumina to separate small quantities of a few amino acids in the form of their *N*-azobenzene *p*-sulphonyl [15] or *N*-2 : 4-dinitrophenyl [16] (DNP) derivatives of their methyl esters. Sanger [17] has used the partition column, described in §3 (*a*), for end-group analysis, which employs the DNP amino acids, and it is generally considered that the silica gel used functions largely as an adsorptive medium. This method has been used to determine the free amino groups in gramicidin *S*,[18] in insulin,[17] in salmine,[19] and in polymyxin *D*.[51] The use of a mixture of Lloyd's reagent and kieselguhr permitted the separation of basic and neutral amino acids.[20]

2. Ion-exchange Methods.—Ion-exchange materials have, with few exceptions, only recently been applied to amino-acid separations. Folin used natural, and later, synthetic zeolites to remove bases from biological media and Whitehorn [21] showed that passage of the solution through a column of the zeolite was more effective than simple agitation with it, since reversal by the liberated sodium ions was prevented. Thus originated ion-exchange chromatography, but little application was made to amino-acid separations. Titania gel [22] found a limited use but acid-washed alumina has been used in a number of different applications. The ionizable chloride groups, introduced by washing alumina with HCl, may be exchanged by the anions of dicarboxylic amino acids in solution in water or 80 % alcohol.[23] Glutamic acid may be eluted by acetate buffer and aspartic acid subsequently by caustic soda, a process difficult to understand. "Basic alumina," an analogous cation-exchange material, has been applied to the separation of basic amino acids. In the presence of formaldehyde, the neutral amino acids are rendered more acidic and the carboxyl group is strong enough to exchange with the Cl^- ions of acid-washed alumina. Lederer and Tchen [24] found that di- and tri-peptides, glycine, serine and β-alanine but not α-alanine were retained from a mixture with other amino acids in the presence of formalin. Acid peptides are adsorbed from water, glycyl peptides from 1 % formalin and other neutral peptides from 10 % formalin, while the adsorption of aromatic neutral peptides on carbon completes the group separation.[25]

[9] Abderhalden and Fodor, *Fermentforsch*, 1917, **2**, 74.
[10] Tiselius, *Arkiv. Kemi, Min. Geol. B*, 1941, **15**, No. 6.
[11] Tiselius, *Science*, 1941, **94**, 145.
[12] Tiselius, Drake and Hagdahl, *Experientia*, 1947, **3**, 21.
[13] Schramm and Primosigh, *Ber B*, 1943, **76**, 373.
[14] Karrer, Keller and Szönyi, *Helv. chim. Acta*, 1943, **26**, 38
[15] Reich, *Biochem. J.*, 1948, **43**, xxxviii.
[16] Reith, *ibid.*, 1949 (in press).
[17] Sanger, *ibid.*, 1945, **39**, 507.
[18] Sanger, *ibid*, 1946, **40**, 261.
[19] Tristram, *Nature*, 1947, **160**, 637.
[20] Bergdoll and Doty, *Ind Eng. Chem.* (*Anal.*), 1946, **18**, 600.
[21] Whitehorn, *J. Biol. Chem.*, 1923, **56**, 751.
[22] Johnston, cited by Cannan.[1]
[23] Turba and Richter, *Ber. B*, **75**, 1942, 340. Wieland, *ibid.*, 1001. Wieland, *Naturwiss*, 1942, **30**, 374.
[24] Lederer and Tchen, *Biochim. Biophys. Acta*, 1947, **1**, 35.
[25] Jutisz and Lederer, *Nature*, 1947, **159**, 445. Dubnoff, *J. Biol. Chem.*, 1941, **141**, 711. Archibald, *J. Biol. Chem.*, 1944, **156**, 121. Freudenberg, Walch and Moltor, *Naturwiss.*, 1942, **30**, 87.

The introduction of anion-exchange resins of high capacity has interested a number of workers in this field,[26] who have presented detailed theoretical [2] and practical studies. Consden, Gordon and Martin [27] adopted the principle of establishing a constant pH value in finely ground Amberlite IR-4 and in this way were able to effect a complete separation of glutamic, aspartic and cysteic acids from a wool hydrolyzate. These acidic amino acids were first separated from the neutral and basic acids on a column at pH 3 to 4, eluted in small volume by displacement by N HCl, placed on a column already brought to pH 2·5 by washing with 0·003 N HCl and developed with the same solution. The recoveries were not quantitative. These results are interpreted as involving a depression of the iso-electric point of the acids when adsorbed, since the optimum pH for separate elution should be pH 4·5, at which pH the acids are actually retained. The same resin was used by Drake,[28] who eluted the glutamic acid with 0·05 % acetic acid and aspartic acid with N HCl. An automatic sampling device for applying small portions of the effluent to paper for ninhydrin reaction and matching enabled quantitative determinations to be made.

Cation-exchange resins were originally applied to separate basic from neutral amino acids in analysis, but difficulties were evident as it was not appreciated that the neutral acids have a distinct affinity for the sulphonic groups of the resins used [2] With the intention of developing preparative rather than analytical methods, Partridge and Westall [29] have investigated the behaviour of Zeo-Karb 215, a sulphonic acid resin. In this method all the amino acids are adsorbed on the acid resin and, in order to avoid the difficulties of selective elution, the displacement technique of Tiselius and Claesson [30] was employed. Changes in the composition of the effluent were measured by a combination of methods . measurement of conductivity or pH, paper chromatography or measurement by titration of small successive fractions. A detailed consideration is given of many of the factors involved. The retention isotherms for single amino acids take the form of the Langmuir equation. Effective separation is achieved if the iso-electric points of the components differ by more than 0·5 to 1·0 pH unit. Use is made of the graphical method of Tiselius [31] for calculating the concentrations of a given displacing agent necessary for a given concentration of the issuing amino acid. In a subsequent paper [32] we are promised data on the separation of complicated mixtures including protein hydrolyzates

Perhaps the most valuable feature of the ion-exchange methods is the scale on which they may be applied, and there is no reason why this may not be increased to full plant operation An application of this sort is the isolation of the polypeptide antibiotics, the polymyxins, from the crude culture fluid in which they are elaborated.[33] Another application in this field was the separation of the amino acids shown to be present in polymyxin *A* (Aerosporin) [34] This led to the isolation of an unknown amino acid retained on Zeo-Karb 215 in the presence of ammonia and displaced by sodium hydroxide, and of obviously basic character. The attempted isolation of γ-aminobutyric acid, found by paper chromatograms to be present in molasses, using the carboxylic cation exchanger Zeo-Karb 216, was not successful.[35] This may have been due to the effect of large concentrations of sugar on the pK of the amino acid comparable with the effects of acetone [12]

Esters of polysaccharides with polybasic acids, such as cotton succinate or phthalate, have been suggested for use as carboxylic cation exchangers [36]

[26] Turba, Richter and Kuchar, *ibid*, 1943, **31**, 508. Englis and Fiess, *Ind Eng Chem*, 1944, **36**, 604 Cleaver, Hardy and Cassidy, *J. Amer. Chem. Soc.*, 1945, **67**, 1343. Cannan, *J. Biol. Chem*, 1944, **152**, 401.
[27] Consden, Gordon and Martin, *Biochem. J.*, 1948, **42**, 443.
[28] Drake, *Nature*, 1947, **160**, 602
[29] Partridge and Westall, *Biochem. J.*, 1949, **44**, 418.
[30] Tiselius and Claesson, *Arkiv Kemi, Min. Geol. B*, 1942, **15**, No 18.
[31] Tiselius, *ibid A*, 1943, **16**, No 18.
[32] Partridge, *Biochem J.*, 1949, **44**, 521.
[33] Jones, Johnston and Hood, *Brit. Pat. Appl.* 1994/49.
[34] Jones, *Biochem. J.*, 1948, **42**, xxxv.
[35] Jones (unpublished observations).
[36] McIntire and Schenk, *J. Amer. Chem. Soc.*, 1948, **70**, 1193.

The capacity of these materials can never be very high, but the properties of a material of a form familiar as cotton wool, and its relative insolubility, render it worthy of trial on very small-scale separations.

The separation of amino-acid mixtures into groups for subsequent analysis has been achieved by the methods outlined in these two sections. The use of acid alumina with formaldehyde [35] and the use of a hydrogen-exchange resin by Block [37] to separate basic from neutral amino acids have been described in principle already and systems are available [38 12] by which four groups may be obtained. For analytical purposes, more specific separations may be achieved by partition methods, without group separations, but comparative investigation of the two methods remains to be done.

3. Partition Chromatography.—(*a*) SILICA GEL AND DIATOMACEOUS EARTH.—Taking advantage of the gradation in partition coefficients of the acetylamino acids, Martin and Synge [39] devised a counter-current machine for their separation, and used it in the analysis of hydrolyzates of wool. This idea was soon modified to form the well-known partition chromatogram,[40] and a scheme of analysis for certain acetylamino acids was devised.[41] This was modified later by Tristram.[41a] The method has been used for the analysis of hydrolyzates of wool,[40 41] gelatin,[41 42] gramicidin,[43 44] tyrocidine,[41] myosin and fibrin,[45] gramicidin *S*,[46] methylated proteins,[47 48] and salmine.[19] The preparation of silica gel of the non-adsorptive nature necessary for the method has not proved to be straightforward,[19] and this is one factor which has tended to prevent its rapid development. Difficulties in the preparation of silica gel suitable for separating DNP-amino acids are overcome by the use of buffered columns [49] according to Blackburn,[50] who specifies solvents suitable for separating different groups of DNP-acids.

Diatomaceous earth has been used as a substitute for silica gel [51] and has found application in the analogous problem of the separation of the penicillins.[51] Its application to the determination of *N*-dinitrophenyl derivatives of amino acids in hydrolyzates combines the use of a separation technique with the possibilities of a sensitive absorptiometric method. It has been so applied to the determination of both free amino groups and the individual amino acids in polymyxin *D*.[51] Wieland and Fremery [51a] have described the use of partition columns of silica gel for the separation of the coloured copper complexes of amino acids. The amino groups are in combination with the copper atoms and adsorption is absent.

(*b*) STARCH CHROMATOGRAPHY.—A development of paper partition chromatography, which is treated in the next sections, that is, chromatography on starch, was introduced in order to increase the scale on which free amino acids may be separated.[52 44] In the hands of Moore and Stein [53 54 55] it has been converted into a tool for the complete separation and determination of the amino acids in

[37] Block, *Proc Soc Exp. Biol. N.Y.*, 1942, **51**, 252.
[38] Fromageot, Jutisz and Lederer, *Biochim Biophys Acta*, 1948, **2**, 487.
[39] Martin and Synge, *Biochem. J.*, 1941, **35**, 91.
[40] Martin and Synge, *ibid*, 1358.
[41] Gordon, Martin and Synge, *ibid.*, 1943, **37**, 79, 313; 1944, **38**, 65.
[41a] Tristram, *ibid.*, 1944, **38**, 721.
[42] Gordon, Martin and Synge, *ibid.*, 1943, **37**, 92.
[43] Gordon, Martin and Synge, *ibid.*, 1943, **37**, 86.
[44] Synge, *ibid.*, 1944, **38**, 285.
[45] Bailey, *Advances in Protein Chemistry*, 1945, **1**, 289.
[46] Synge, *Biochem. J.*, 1945, **39**, 363.
[47] Gordon, Martin and Synge, *ibid.*, 1943, **37**, 538.
[48] Blackburn, Consden and Phillips, *Biochem. J.*, 1944, **38**, 25.
[49] Levi, *ibid.*, 1948, **43**, 257.
[50] Blackburn, *Nature*, 1949, **163**, 955.
[51] Bell *et al.*, *Ann. N.Y. Acad. Sci.*, 1949, **51**, 897.
[51a] Wieland and Fremery, *Ber. B*, 1944, **77**, 234.
[52] Elsden and Synge, *Biochem. J.*, 1944, **38**, ix.
[53] Moore and Stein, *Ann. N.Y. Acad. Sci.*, 1948, **49**, 265.
[54] Stein and Moore, *J. Biol. Chem.*, 1948, **176**, 337, 367.
[55] Moore and Stein, *ibid.*, 1949, **178**, 53.

a protein hydrolyzate. Indeed, the much analyzed proteins, β-lactoglobulin and bovine serum albumin, have been examined by the method with results comparable with the best recorded.[56] Another example of its use was the separation, on a considerable scale from a variety of sources, of a peptide,[57] thought to be reasonably homogeneous. The amino-acid composition of this peptide was also determined by this method. Another application was the demonstration of the conversion of lysine to α-amino-adipic acid by guinea-pig liver.[58] The disadvantage of the starch column is its exceedingly slow rate of flow and means must be provided for collecting many small fractions of equal volume for the quantitative application.

(*c*) CONDITIONS OF PAPER CHROMATOGRAPHY.—The theory of paper partition chromatography is covered by other papers in this Discussion, but it is appropriate to treat here those aspects special to amino acids and peptides and to describe findings which may have a bearing on the method as a whole. The problems involved in the paper chromatography of amino acids, for which the method was originally introduced,[60] namely, the choice of paper and solvent, the methods of recognition and determination and the effects of physical conditions, are the same problems as are met with in the examination of other classes of compounds. The choice of paper is limited to what is available commercially, and little modification is possible apart from washing to remove undesirable contaminants, or impregnation with such substances as buffering materials or even hydrophobic substances such as rubber.[61] In general, a given grade of paper behaves uniformly, except for occasional unexplained variations, which cannot be ascribed to any known cause except the lack of uniformity of the paper batch. A conspicuous example is the variation [62] in the intensity of fluorescence [63] of amino acids, even on consecutive sheets of paper drawn from a single batch. The explanation of this lies in the practice of the manufacturer of bulking the output of a number of cutting machines from a number of different rolls of paper. It is thus possible that a single batch may incorporate paper originating from several different batches of raw material, without influence on the mechanical properties of the paper as filter paper but having an important effect on the properties needed for a chromatographic support. The simple solution is to specify paper for chromatography and a leading manufacturer, we are assured, will see that a given batch at least comes from one source of raw material. For uniformity, the direction of flow of the solvent relative to the direction of cut of the paper [64] is important With rectangular sheets, this point scarcely arises, but with square sheets a direction should be chosen following observation of the texture. Another factor in the choice of a paper is the degree of separation achieved in a given time with a given solvent. With the solvents in common use, the tendency is to use Whatman 4 paper. The texture of the paper has an influence on the separations achieved and it has been found that Whatman 7 paper gives with amino acids round spots showing less dispersion in the direction of flow.

The choice of solvents for amino acids follows that of the originators [60] of the method, with few modifications, the commonly used solvents for two-dimensional work being the collidine fraction of coal tar distillate, and phenol. The collidine fraction is a mixture and, if pure collidine and lutidine are available, equal parts of these [65] will reproduce the standard chromatogram maps published [60 65] The addition of ammonia to phenol causes the basic amino acids to take up positions on the two-dimensional chromatogram well removed from the acidic acids.[61] Alkaline phenol tends to oxidize easily,

[56] Stein and Moore, *J. Biol. Chem.*, 1949, **178**, 79.
[57] Borsook, Deasy, Haagensmit, Keighley and Lowy, *ibid.*, 1949, **179**, 705
[58] Borsook, Deasy, Haagensmit, Keighley and Lowy, *ibid.*, 1948, **173**, 424.
[59] Randall and Martin, *Biochem. J.*, 1949, **44**, ii.
[60] Consden, Gordon and Martin, *ibid.*, 1944, **38**, 224.
[61] Boldingh, *Experientia*, 1948, **4**, 270.
[62] Woiwod and Jones (unpublished).
[63] Phillips, *Nature*, 1948, **161**, 53.
[64] Bull, Hahn and Baptist, *J. Amer. Chem. Soc.*, 1949, **71**, 550.
[65] Dent, *Biochem. J.*, 1948, **43**, 169.

giving dark fronts, and various expedients, such as addition of KCN, cupron, oxine and displacing the atmosphere by coal gas, have been devised to minimize the oxidation and metallic interference. Collidine interferes with the quantitative determination of amino acids by Woiwod's [98] method and needs periodical purification and may with advantage be replaced by butanol equilibrated with aqueous acetic acid,[67] different concentrations of acetic acid from 5 % to 25 % being of use for the separation of different mixtures. The overlapping of the basic amino acids with other amino acids is negligible if neutral phenol follows butanol–acetic acid in a two-dimensional chromatogram, so that the addition of ammonia becomes a disadvantage to be avoided The addition of oxine to a good commercial grade of phenol enables clean chromatograms to be produced and renders special purification unnecessary. Workers who have standardized their chromatograms using collidine and alkaline phenol will be loath to change to different solvents, but newcomers are recommended to try both systems. It is of interest that Phillips [68] finds butanol-20 % acetic acid to be superior to other solvents for the peptides of an enzymic digest of insulin. Tertiary amyl alcohol in the presence of diethylamine has been found by Work [122] to effect the separation of leucine, *iso*leucine and phenylalanine.

Williams and Kirby [69] have described a modification of the method of Consden, Gordon and Martin,[60] in which the solvent is caused to move over the paper by capillary ascent. The paper, in the form of an open cylinder secured by staples along overlapping edges, is stood on end in a dish of solvent. Other modifications to this method have been described,[70] designed to render the paper, wet with solvent, sufficiently rigid to stand. It is here considered that nothing is simpler for two-dimensional work than the original apparatus, in one of its slight modifications described by Dent [65] or Woiwod,[71] that of the latter being very similar to that used by the author.

Upward flow may be used with advantage for narrow strips in very simple apparatus and in the test-tube.[72] Its use for thick paper is described in §3 (*g*).

(*d*) Recognition of Amino Acids and Peptides.—The method universally adopted for the recognition of amino acids and peptides on paper is the ninhydrin reaction, the reagent being applied to the paper as a solution in dry or aqueous butanol or chloroform in the form of a spray.[60] The paper is then dried and the colour developed at slightly elevated temperatures for amino acids or higher temperatures up to 100° for peptides. The reaction is said to go to completion more rapidly if the final development is carried out in steam [64] The reagent may be incorporated in the solvent,[73] but a disadvantage of this is the cost With relatively volatile solvents, the preliminary drying of the paper may be avoided by evaporation in a stream of air to a state sufficiently dry to avoid diffusion of the spots, spraying with ninhydrin and subsequent development The addition of small amounts of collidine [71] or pyridine [68] to the ninhydrin solution causes a differentiation of the colour of the spots as an aid to recognition, while the presence of phenol renders the colour more uniform and suitable for densitometry [64] The ninhydrin method is not specific for α-amino acids; on paper, positive reactions are given by many bases, although with diminished intensity, as well as by β- and other amino acids. The sensitivity of the ninhydrin reaction for a number of amino acids has been compared by Pratt and Auclair.[68a] Other methods of recognition applicable to all amino acids are the naphthoquinone-β-sulphonate reagent,[74] which gives spots of less intensity than ninhydrin, and the fluorescence in filtered ultra-violet light shown by amino acids and peptides,[63] but which is also less sensitive than the ninhydrin method, needs careful drying of the paper and is subject to considerable variation with the

[67] Partridge, *Biochem J*, 1948, **42**, 238. Jones, *ibid.*, xxxv.
[68] Phillips, *Biochim. Biophys. Acta*, 1949, **3**, 341.
[68a] Pratt and Auclair, *Science*, 1948, **108**, 213.
[69] Williams and Kirby, *ibid*, 1948, **107**, 481
[70] Wolfson, Cohn and Devaney, *ibid.*, 1949, **109**, 541.
[71] Woiwod, *J. Gen. Microbiol*, 1949, **3**, 312.
[72] Rockland and Dunn, *Science*, 1949, **109**, 539.
[73] Nicholson, *Nature*, 1949, **163**, 954.
[74] Folin, *J. Biol. Chem.*, 1922, **51**, 377, 393.

paper batch. Special methods have been described for the recognition of specific amino acids, e.g., the use of *o*-phthaldehyde for glycine [76]; potassium iodoplatinate [77] and the sodium azide-iodine [78] reaction for sulphur-containing amino acids; treatment with periodic acid followed by Nessler's reagent for hydroxyamino acids [7]; diazotized sulphanilic acid for tyrosine and histidine [80]; *p*-dimethylamino-benzaldehyde for tryptophane [7]; 2 : 4-dinitrophenylpyridinium chloride for reduced tryptophane [7]; *p*-nitrobenzoyl chloride and pyridine for *N*-methylamino acids.[83] Iodine sublimed on to the paper or sprayed on in alcoholic solution gives temporary spots of a brown colour,[84] which soon fade by evaporation and lead to no interference with the subsequent use of another reagent. Another method consists in treatment of the paper with a 5 % solution in chloroform of copper acetylacetonate,[85] which gives a quantitative conversion of the amino acid to its copper complex. Excess of the reagent is removed by chloroform and the copper in the complex is revealed by treatment with 1 % rubeanic acid in acetone. This and similar methods may be used to distinguish between α-amino acids and others which give the ninhydrin reaction.

An elegant method due to Synge [85a] is the use of the *D*-amino acid oxidase of sheep's kidney for the determination of the configuration of amino acids on paper chromatograms. The method may be used for leucine, valine, threonine and serine but has failed with *D*-αγ-diaminobutyric acid and would presumably fail for other diamino acids. The method was applied to the valine residues of gramicidin by Synge and for the demonstration of the presence of *D*-leucine and *L*-threonine in polymyxin *A* (Aerosporin) by Jones [86] The method has lately been published by Synge, but a description of it would find a place here. A chromatogram is prepared from a suitable quantity of the amino-acid mixture to give about 25 μg of the one to be tested. On the same paper are placed 25 μg. of the *D*- and *L*-forms of the acids, and graded mixtures of them. The amino acid spots are marked by their ultra-violet fluorescence and the paper in their vicinity is sprayed with a solution of the *D*-amino acid oxidase. The damp paper is then kept for three hours in an oxygenated water-saturated atmosphere at 40° C. It is then dried in a hot room, sprayed with ninhydrin in water-saturated butanol, and the colour developed at 40° C. Amino-acid mixtures containing the *D*-form will be found to yield spots of diminished intensity. The enzyme solution is prepared according to the directions of Negelein and Brömel,[87] proceeding as far as their step 5. Instead of preparing the solid, the solution is dialyzed against 0·2 M pyrophosphate buffer, and the co-enzyme is added as described in the paper This preparation gives little or no background.

(*e*) Quantitative Paper Chromatography.—The introduction of partition chromatography was a great stimulus to the established interest in the analysis of proteins and other nitrogenous biological products, and attempts were soon made to apply quantitative methods to the materials separated on the paper chromatogram, both *in situ* and after removal from the paper. Included in the former are visual matching of the spot formed with ninhydrin with a spot due to a known quantity of the amino acid,[60 80] photoelectric determination of the optical density of the coloured spot,[64 89] and measurement of the area of the spot.[90] Another *in situ* method of great sensitivity results from the incorporation

[76] Patton and Foreman, *Science*, 1949, **109**, 339
[77] Winegard, Toennies and Block, *ibid.*, 1948, **108**, 506
[78] Chargaff, Levine and Green, *J. Biol. Chem.*, 1948, **175**, 67.
[80] Dent, *Biochem. J.*, 1947, **41**, 240.
[83] Plattner and Nager, *Helv chim. Acta*, 1948, **31**, 2203
[84] Brante, *Nature*, 1949, **163**, 651.
[85] Wieland and Fischer, *Naturwiss*, 1948, **35**, 29.
[85a] Synge, *Biochem. J.*, 1949, **44**, 542.
[86] Jones, *Biochem J.*, 1948, **42**, lix
[87] Negelein and Brömel, *Biochem Z.*, 1938/9, **300**, 225
[88] Polson, Mosley and Wyckoff, *Science*, 1947, **105**, 603
[89] Fosdick and Blackwell, *ibid.*, 1949, **109**, 314. Block, *ibid*, 1948, **108**, 608
[90] Fisher, Parsons and Morrison, *Nature*, 1948, **161**, 764. Brimley, *ibid.*, 1949, **163**, 215.

of radioactive carbon,[91] sulphur [92] or iodine [93] in the amino acid or a derivative. The spot may then be detected by autoradiography [94] and determined quantitatively by the usual methods.[92 93] Isotopic *p*-iodobenzenesulphonyl derivatives of any amino acids may be employed.[95] The ninhydrin spot may be removed from the paper,[96] or the spot may be marked by a minimal quantity of ninhydrin and then removed,[97] and the coloured ninhydrin product determined absorptiometrically. Another reaction applied to amino acids removed from the paper is the formation of the copper complex by reaction with copper phosphate suspension,[98] which may be formed in the eluate by simple mixture of copper sulphate and Na_2HPO_4 titrated to pH 10·2. The copper in the complex may then be determined absorptiometrically by one of the usual colour reactions,[98] or the complex itself may be determined polarographically.[100 101] In the first method the excess copper phosphate must be removed by filtration, but this is unnecessary in the second since insoluble materials are not reduced at the dropping mercury cathode. One advantage of the absorptiometric method is that, since the copper equivalent to the amino acid is determined, the colour produced is substantially the same for most common amino acids and mixtures such as leucine-*iso*leucine-phenylalanine, valine-methionine and glycine-serine which occur on single dimensional chromatograms may be determined. Collidine interferes with this method by preventing the quantitative removal of leucine,[99] at least, and this is another reason for its replacement by butanol–acetic acid mixture. Phenol has no deleterious effects, provided that it is properly removed by evaporation. The polarographic method determines the diffusion current of the copper complex of the amino acid and, in general, this differs for different amino acids in a manner mainly dependent upon the size of the complex, and independent of the nature of the amino acid. For example, glycine gives a higher diffusion current than leucine or lysine, which are approximately equal. All these amino acids yielded complexes, at the pH employed, containing one atom of copper to two molecules of the amino acid. In contradistinction to this, $\alpha\gamma$-diaminobutyric acid forms a complex with copper which contains one molecule per atom of copper ; this smaller complex yields a much higher diffusion current than glycine. It was found possible to soak off the amino acids in 0·18 M Na_2HPO_4 solution, and addition of an equal volume of copper phosphate suspension was all that was necessary before placing the solution in the polarograph cell. This reagent enabled a reproducible blank diffusion current to be measured, contrary to Martin and Mittelmann's [100] experience with the reagent of Pope and Stevens.[102] It has been found advantageous to attempt the elimination of variations caused during the running of the chromatogram. Block,[89] using a densitometric ninhydrin method, measures five consecutive strips containing the amino acids. Woiwod [99] prepares a chromatogram of a large number of identical amounts of the mixture, placed on a starting line parallel with the shorter side of a 22 in. × 18½ in. sheet of paper. A strip is then cut so as to include the line of spots corresponding to one amino acid, determined by fluorescence, and the amino acid removed by soaking in water is reduced to dryness by evaporation under reduced pressure. The amino acid is then treated with the copper reagent.

(*f*) THE MECHANISM OF THE METHOD AS APPLIED TO AMINO ACIDS AND PEPTIDES. —In applying the above methods to the examination of biological preparations of single substances, the following considerations are relevant. In the first

[91] Calvin and Benson, *Science*, 1949, **109**, 140. Fink and Fink, *ibid.*, 1948, **107**, 253.
[92] Tomarelli and Florey, *ibid.*, 1948, **107**, 630.
[93] Taurog, Chaikoff and Tong, *J. Biol. Chem.*, 1949, **178**, 997.
[94] Fink and Fink, *Science*, 1948, **107**, 253. Fink, Dent and Fink, *Nature*, 1947. **160**, 801.
[95] Keston, Udenfriend and Levi, *J. Amer. Chem. Soc.*, 1947, **69**, 3151.
[96] Naftalin, *Nature*, 1948, **161**, 763.
[97] Landua and Awapara, *Science*, 1949, **109**, 385
[98] Woiwod, *Nature*, 1948, **161**, 169 ; *Biochem. J.* (in press)
[99] Woiwod (private communication).
[100] Martin and Mittelmann, *Biochem. J.*, 1948, **43**, 353.
[101] Jones, *ibid* , 1948, **42**, lix
[102] Pope and Stevens, *ibid.*, 1939, **33**, 1070.

place the purity of these materials is often difficult to achieve and equally difficult to establish. When therefore faint spots appear on chromatograms, consideration must be given to the question whether they are due to components of the molecule, e.g., in the case of a protein hydrolyzate, the amino acids. A knowledge of the order of the molecular weight of the substance under investigation will enable a decision to be taken whether the faint spots observed could represent a minimum of one unit amino acid in the molecule. The finding of small amounts of material on paper chromatograms, prepared from biological mixtures in positions usually occupied by standard amino acids, should only be taken as a challenge to attempt the isolation of the material on a scale sufficient to prove its identity by the established procedures. One important result to be obtained from paper chromatograms is the demonstration of the absence, in significant amount, of a particular amino acid.[103] In the field of such complexity as the composition of proteins, the value of limiting the number of the components can scarcely be over-emphasized.

In their original publication, Consden, Gordon and Martin [60] held that the paper played the role of an inert support. Whatever the mechanism with other materials, it is certain that some adsorption of amino acids on the paper takes place. Evidence for this is found if sufficient paper is cut from a chromatogram and eluted, when amino acids which have passed over the strip will be found in the eluate. Moore and Stein [53] consider that with starch chromatograms adsorption plays a prominent part and cite experiments in which no immobile phase was employed and the results of which were comparable with those of partition chromatograms. The separation of the anions of amino acid and peptide salts from the cations would argue a selective 'adsorption' for the cation, and a mechanism for this is available in the carboxyl groups present in the partly degraded cellulose of the paper. The displacement of certain amino acids by slightly slower ones, present in large amount, has been ascribed to a kind of salting-out process; it could equally well be a displacement development on an ion-exchange medium. Whatever the mechanism, the separation of the salts as ions has a distinctly practical significance. Some salts such as picrates, picrolonates or double salts are very readily dissociated on the paper and bases applied as these salts are found to have separated. On the other hand, sulphates of basic amino acids as well as of other bases, such as ergometrine, are found to be moved by such solvents as butanol–acetic acid at rates much slower than the corresponding hydrochlorides, tartrates, maleates or the free bases. Free sulphuric acid in excess is found to have a high R_F value in these solvents, and it is of advantage to remove sulphuric acid or sulphates before attempting to separate basic amino acids.

Another consequence of the mechanism of the chromatographic process is the loss which occurs in the progress of the amino acid along the paper.[99] In many publications, this loss is not mentioned and in others it is buried in the losses consequent upon the series of operations made to form a quantitative estimate Many methods proposed attempt to overcome this defect by a comparison of the unknown with known amounts applied to the paper at the same time. It appears that losses occur to an extent roughly proportional to the distance travelled on the paper [98] It has been reported that the loss which occurs on separating metallic cations is uniform along the paper strip. This may be due to the acidic solvents employed or to a different mechanism for the separation. It is to be hoped that some method which is exact and does not involve removal from the paper will be applied to this problem to divide the possible losses into their components.

(*g*) THICK PAPER.—The use of paper chromatograms for preparative separations, although discussed by Consden, Gordon and Martin,[60] has received little development, probably because of the introduction of the starch column. Two methods are available: (i) to use many sheets of paper and (ii) to use thicker paper. The above authors mentioned Whatman Accelerator paper as a possible support, but this proved too brittle for use with descending flow. With *upward* flow, however, difficulties due to this cause are overcome, and reasonable one-dimensional separation of small numbers of amino acids which normally separate

[103] Dent and Rose, *ibid*., 1948, **43**, liv

well can be achieved. One method is to clamp the sheet of paper at the upper edge between strips of plate glass and hang it so that the lower edge rests in the solvent in the trough One sheet of paper will easily accommodate 20 mg. lots of each amino acid distributed along a line parallel with the lower edge, and the bands of amino acids will usually be less than 1 cm deep.

(*h*) SPECIFIC APPLICATIONS: AMINO ACIDS.—The most spectacular applications of paper chromatography have been in the field of polypeptide antibiotics and among those examined in this way are the polymyxins,[105] gramicidin *S* [107] and the licheniformins.[108] The enniatins,[109] while not polypeptides but cyclic esters, can be discussed with this class. The amino acids present in these antibiotics have been identified and new natural ones discovered, and the proportions present and their configurations determined. The new amino acids are αγ-diaminobutyric acid, found in polymyxin *A* (Aerosporin) by its characteristic position among the basic amino acids and the characteristic colour with ninhydrin,[110] and the *N*-methyl amino acids, methyl valine, methylleucine and methyl*iso*leucine, found in the enniatins. The occurrence of more than one polypeptide antibiotic synthesized by a single strain of an organism (the licheniformins) and by different strains (the polymyxins) is revealed when their amino-acid composition is determined. The other antibiotics present, so far as is known, no unusual qualitative features, and the reported occurrence of *D*- and *L*-forms [111] of the same amino acid in one molecule is resolved by the discovery that the antibiotic (gramicidin) is a mixture.

The identification of free amino acids in normal [112] and abnormal animal tissues, including tumours,[113] in the secretions of normal [92 114] and diseased mammals [114 103 115] and in the hæmolymph of insects,[116] serves as an introduction to the enormous scope opened out for future investigation. In the plant world, the following of amino-acid metabolism by ordinary paper chromatograms [117] and those obtained after introduction of radioactive tracer elements [94 91] has been begun. The amino-acid compositions of biologically important proteins and polypeptides, including the hormone insulin,[68 118] the enzymes lysozyme,[119] and streptokinase, the tissue peptide of Borsook,[57] the protamine salmine,[120] and the fibrous proteins wool and silk,[88] have all been recently examined by this means.

In the study of bacteria, the smallness of the sample necessary for examination will prove to be the decisive factor in the development of qualitative and quantitative methods Already studies have appeared on the occurrence of free amino acids in bacterial cells,[121 122] on the amino-acid composition of hydrolyzates,[121 122 123] on the amino-acid changes in the medium during growth, including uptake and secretion,[124] and the composition of bacterial products such as diphtheria toxin and toxoid [122] and, of course, the antibiotics mentioned

[105] Jones, *Ann. N.Y. Acad. Sci*, 1949, **51**, 909.
[107] Consden, Gordon, Martin and Synge, *Biochem. J.*, 1947, **41**, 596.
[108] Work (private communication).
[109] Plattner and Nager, *Helv. chim. Acta*, 1948, **31**, 2192.
[110] Catch and Jones, *Biochem. J.*, 1948, **42**, lii.
[111] Christensen, *J. Biol. Chem.*, 1943, **151**, 319; 1944, **154**, 427. Christensen and Hegsted, *ibid*, 1945, **160**, 593.
[112] Awapara, *J Biol Chem.*, 1949, **178**, 113; *Arch Biochem.*, 1948, **19**, 172.
[113] Roberts and Tishkoff, *Science*, 1949, **109**, 14.
[114] Dent, *Biochem J.*, 1947, **41**, 240.
[115] Ames and Risley, *Proc Soc. Exp. Biol., N.Y.*, 1948, **68**, 131. Young and Homburger, *Fed Proc*, 1948, **7**, 201.
[116] Raper and Shaw, *Nature*, 1948, **162**, 999 Finlayson and Hamer, *ibid.*, 1949, **163**, 843.
[117] Dent, Stepka and Steward, *ibid*, 1947, **160**, 682. Stepka, Benson and Calvin, *Science*, 1949, **109**, 385
[118] Woolley, *J. Biol. Chem.*, 1949, **179**, 593.
[119] Fromageot and de Garilhe, *Biochim. Biophys Acta*, 1949, **3**, 82.
[120] Hamer and Woodhouse, *Nature*, 1949, **163**, 689.
[121] Proom and Woiwod, *J. Gen. Microbiol.*, 1949, **3**, 319.
[122] Work, *Biochim Biophys. Acta*, 1949, **3**, 400.
[123] Polson, *ibid.*, 1948, **2**, 575.
[124] Linggood and Woiwood, *Nature*, 1949, **163**, 218

above. In large-scale work the production of toxic filtrates of enhanced titre has followed the determination of the amino-acid requirements of *Corynebacterium diphtheriæ*.[125] The quantitative examination of a bacteriophage showed that its composition was not significantly different from that of its host.[126]

Another field in which the amino-acid composition of a hydrolyzate is proving of value is that of the ergot alkaloids.[127] These are characterized by the presence in all members of *D*-proline, while the individuals have either *L*-leucine, *L*-phenylalanine or *L*-valine in peptide combination with the proline and either pyruvic acid or dimethylpyruvic acid. Even the purest preparations have been found to have a trace or more of amino acids pertaining to some other member of the group, although in less than one molecular proportion (cf. §3 (*f*)). In addition, examination of the crude as well as purer preparations of the alkaloid enables a good indication to be obtained of the members of the series present.

Amino acids, whose presence has not been confirmed by isolation, have been reported to occur on paper chromatograms of natural products. Potato juice,[117] pea juice,[128] molasses[129] and portal blood[130] are thought to contain γ-amino-butyric acid, the α-isomer of which has been considered to be present in the urine in Fanconi syndrome,[114] in portal blood,[130] in hydrolyzates of *Escherichia coli*[131] and free in *C. diphtheriæ*.[125] Ackerman and Kirby[132] consider that *E. coli* contains pantonine rather than α-aminobutyric acid and Proom and Woiwod[121] have failed to detect any new amino acids in the hydrolyzates of twelve species of bacteria. This illustrates the principle, enunciated above, that isolation must be the criterion for the actual presence of amino acids detected in minor amounts on paper chromatograms.

An advantage of the method is the ability to confirm the absence of an amino acid from a natural product. One of the earliest applications was to show that *nor*leucine is not present in spinal cord, and that the material long considered to be this amino acid, isolated by Thudichum in 1880, was actually *DL*-leucine.[133] Bence Jones protein has been shown to be devoid of methionine.[103]

(*i*) PAPER CHROMATOGRAPHY OF PEPTIDES.—Perhaps the most valuable use for paper chromatography in the future will be the detection and identification of peptides. Already important results have been obtained for two classes of peptides, the natural peptides synthesized by bacteria, including antibiotics, and the peptides produced by hydrolysis of natural products. The simple peptides were shown by Consden *et al.* to run on phenol–collidine two-dimensional chromatograms as elongated spots, almost as satisfactorily as do amino acids. Their positions are rather sensitive to slight changes in conditions. The use of butanol–acetic acid mixtures, either in one- or two-dimensions, has proved superior to either collidine or phenol for peptides such as the polymyxins[34 105] or for peptides produced by enzymic degradation of insulin.[68] The identification of peptides depends upon their amino-acid composition as revealed by removal from the paper, followed by hydrolysis and identification of the amino acids produced.[134] Difficulties have frequently been encountered in removing peptides in micro-quantities of water and the method adopted by the author is to soak the paper in larger quantities of water and remove this by evaporation under diminished pressure. It is a safe working rule to assume any unknown spot to be a peptide until shown to be completely resistant to acid hydrolysis. A serious difficulty when working with partial hydrolyzates is the larger number of peptides encountered, but colour is some guide to their separate identity.[68]

An early example of the second class mentioned above was the identification of the order of the amino acids in gramicidin *S*[134]; four dipeptides and two tripeptides were identified, showing that five amino acids were joined in a cyclic

[125] Linggood and Woiwod, *Brit. J. Expt. Path.*, 1948, **29**, 283.
[126] Polson and Wyckoff, *Science*, 1948, **108**, 501.
[127] Foster, MacDonald and Jones, *J. Pharm. Pharmacol.*, 1949 (in press).
[128] Brown, Cook and Stewart, *Biochem. J.*, 1948, **43**, xx.
[129] Jones (unpublished).
[130] Dent and Schilling, *Biochem. J.*, 1949, **44**, 318.
[131] Polson, *Nature*, 1948, **161**, 351.
[132] Ackerman and Kirby, *J. Biol. Chem.*, 1948, **175**, 483.
[133] Consden, Gordon, Martin, Rosenheim and Synge, *Biochem. J.*, 1945, **39**, 251.
[134] Consden, Gordon, Martin and Synge, *ibid.*, 1947, **41**, 596.

peptide structure. More complex structures such as insulin, trypsinogen, the gramicidins and many others are now under investigation in a number of laboratories.

The application to the antibiotic field was the first application to the higher peptides. It was found that admittedly impure preparations of polymyxin *A* (Aerosporin) gave single spots when chromatographed in butanol–acetic acid mixture, although stationary in butanol.[84] Material obtained from different strains of the organism could be distinguished either by the different positions taken up on the paper, or by differences in their amino acid composition, and this formed the basis of their classification.[135 105] The polymyxins were recognized by the ninhydrin reaction or by fluorescence. The purest available specimens of different members of the series appear to be composite, e g., using Whatman 4 paper and butanol–15 % acetic acid, polymyxins A and C, but not B and E, appear to be composite, while in butanol–25 % acetic acid, the latter are composite while the first two give single spots.[136] Whatman 7 paper gives better separations of the latter.

Another important class of natural polypeptides contains those synthesized by bacteria in their normal course of growth. Proom and Woiwod [121] have shown that when grown on a standard casein hydrolyzate medium many species of bacteria produce material appearing on the chromatogram as strong ninhydrin-positive spots in positions distinguishable from the standard pattern of amino acids obtained for the basal medium. A number of them have yet to be proved to be polypeptide, but certain of them yield amino acids on hydrolysis. Their appearance in the medium is always associated with the disappearance of one or more amino acids. The presence of polypeptides in the medium may be group-, genus-, or species-specific and may provide data for a classification associated with their nitrogen metabolism. The interior of the bacterium does not appear to contain polypeptide.

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[135] Jones, *ibid.*, 1948, **43**, xxvi.
[136] Jones and Wilkinson (unpublished)

SEPARATION OF BASES AND AMINO ACIDS BY DISPLACEMENT CHROMATOGRAPHY ON ION-EXCHANGE COLUMNS

BY S. M. PARTRIDGE

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Displacement chromatography possesses special advantages for preparative procedures since the maximum use is made of the adsorptive capacity of the column. In the separation of amino-acid mixtures, columns packed with synthetic resinous ion exchangers may be used and since these are capable of adsorbing large amounts of electrolyte, the throughput of a single column of moderate size may be very large indeed.

Protein hydrolysates may be fractionated by first removing the aromatic amino acids (tyrosine and phenylalanine) by adsorption on charcoal and then displacing the remaining mixture from a column packed with a cation-exchange resin by means of a strong base such as ammonia or sodium hydroxide. A single passage serves to break down the mixture into seven or eight groups of components, each group containing a simple mixture capable of further separation either by conventional methods or by variations of the initial procedure.

The object of the work which forms the subject of this paper was primarily to develop a preparative scale procedure for the isolation of bases and amino acids from biological extracts of various kinds and from the hydrolysis products of proteins. The use of ion-exchange separations for the quantitative analysis of such mixtures has not yet been examined in any detail.

The principle of displacement development was introduced by Tiselius [1] in 1943 and has been applied with success to chromatographic separations on adsorbents such as charcoal and alumina. The work reviewed here, which has been published (in part) in a series of papers [2 3 4] was based very largely on the theoretical background developed by Tiselius and Claesson.[5]

The displacement method applied to ion-exchange columns is of particular value for preparative work since very large amounts of material may be placed on the column. The usual procedure is to pass into the filtration tube sufficient of the mixture of organic electrolytes to saturate about

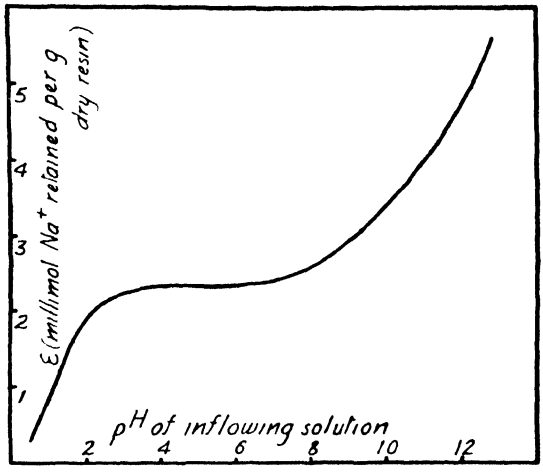

FIG. 1.—Zeo-Karb 215; adsorption of Na^+ from a range of buffers containing NaOH (0·053 N) adjusted to pH 12·1–pH 1·1 by the addition of acids.

one half of the column. Where a cation-exchange resin is used the displacement developer is a solution of a base that has a higher affinity for the column material than any of the components of the mixture. This is introduced onto the top of the filtration tube and, as it displaces the components down the column, they separate into discrete bands, and arrange themselves in order so that each component is displaced by the next strongest base.

1. Properties of the Resin in Relation to the Displacement Chromatogram.—(*a*) The ion-exchange resin that has so far been adopted for most of the experiments with amino acids is the Permutit product Zeo-Karb 215. The properties of this resin may be described by reference to a titration curve (Fig. 1). In carrying out experiments designed to assess the behaviour of resins we have usually preferred to rely on direct observation of columns rather than on equilibrium studies. Thus the points given in Fig. 1 were determined by

[1] Tiselius, *Ark. Kemi, Min. Geol. A*, 1943, **16**, No. 18.
[2] Partridge and Westall, *Biochem. J.*, 1949, **44**, 418.
[3] Partridge and Brimley, *Biochem. J.*, 1949, **44**, 513.
[4] Partridge, *Biochem. J.*, 1949, **44**, 521.
[5] Claesson, *Ark. Kemi, Min Geol. A*, 1946, **23**, No. 1.

measuring the breakthrough volume of various buffers containing a fixed concentration of sodium hydroxide adjusted to various pH values between 1·1 and 12·1 by the addition of various acids. The curve is obviously a titration curve, but differs from the usual form in that the concentration of sodium ion is constant. The portion of the curve between pH 1 and pH 7 is due to the titration of the sulphonic acid radicles of the resin, and that between pH 7 and pH 12 is due to the titration of phenolic hydroxyl groups. Thus between pH 1 and pH 7 the resin reacts as though it were mono-functional, but at higher pH values a range of weakly acidic groups is titrated. The curve suggests that some of the phenolic groups are more strongly acid than would be expected from the published pK value of phenol, and this may be due to modification of the phenolic groups by oxidation.

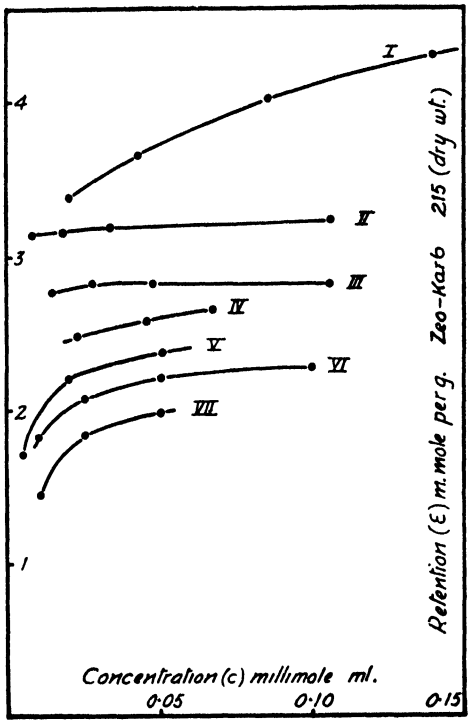

FIG 2.—Retention of a number of bases and amino acids on Zeo-Karb 215 (80–100 mesh). The experiments were carried out with a column 8·5 mm. diam. containing 1·44 g. resin (dry wt.). I, ammonia; II, lysine; III, histidine; IV, creatinine; V, glycine; VI, glutamic acid; VII, aspartic acid.

(*b*) Fig. 2 shows a number of adsorption isotherms obtained with various bases and amino acids, again by measurement of the breakthrough volume in column experiments. The adsorption of the various components is clearly determined to a great extent by the pH of the solution applied to the column, and the amount of each component adsorbed follows, more or less, what would be expected from the titration curve. All the natural amino acids are adsorbed from pure solution by Zeo-Karb 215, including aspartic and glutamic acids, the isotherms of which lie below that of glycine.

By use of the graphical method of Tiselius and Claesson [5] the curves given in Fig. 2 may be used for the determination of the concentration at which the components in a displacement experiment leave the column. This method has been found to give good results with mixtures of amino acids displaced with barium hydroxide or ammonia.

(*c*) Fig. 3 shows the results of one of the earliest experiments in which the displacement technique was applied to the problem of separating organic bases on a column packed with a cation-exchange resin (Bendall, Partridge and Westall).[6] A solution containing glycine and creatinine was applied to the column and was displaced with ammonia. The shape of the fronts and the position of the bands were determined by continuous measurement of electrical conductivity and pH of the solution flowing from the column. The results obtained by this method were afterwards checked by estimation of the content of glycine and creatinine in fractions cut from the effluent. The results obtained in this experiment, which was carried out with a column 10 cm. high, were quite typical, and similar results are obtained in experiments with a larger number of components, although in this case longer columns are necessary.

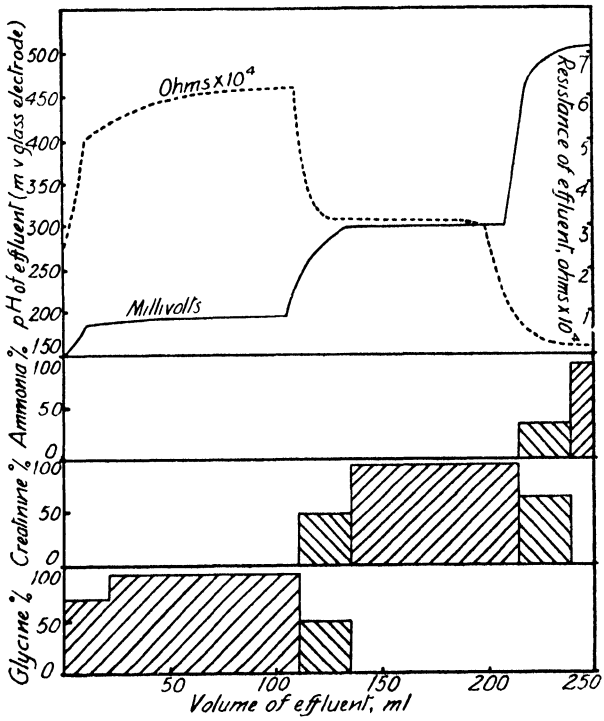

Fig. 3.—Separation of glycine and creatinine on a column of Zeo-Karb 215 (80–100 mesh). The upper part of the figure shows the changes in conductivity and pH of the effluent and the lower part the results of chemical analysis of successive fractions. The analytical results are expressed as percentage of the expected concentration of the component as calculated from adsorption isotherms determined in independent experiments.

- - - - ohms × 10⁴, ——— millivolts.

(*d*) Obviously, in displacement experiments the yield of pure component obtained depends upon the sharpness of the boundaries of the bands. The effect of various factors on the widths of the boundaries has therefore been studied in some detail and Fig. 4 shows the effect on the width of the boundary of ammonia fronts due to changes in the particle size of the resin and the rate of flow. The co-ordinates of this diagram need some explanation: Fig. 5 is a diagrammatic representation of the concentration changes in the effluent from a column as the solution of a base is passed through it at uniform concentration. The effluent at first consists of pure solvent and the volume of

[6] Bendall, Partridge and Westall, *Nature*, 1947, **160**, 374.

effluent measured to the point of first breakthrough of solute is V_0; V_{90} is the volume of liquid measured to the point at which the concentration of the effluent solution reaches 90 % of that flowing into the column. V_e is the retention volume of the column and is estimated by fixing the position of the line DB such that the area ABC equals the area ADE. Thus the weight of solute adsorbed by the resin is given by CV_e, where C is the concentration of the inflowing solution. The values for V_0, V_{90} and V_e must be corrected for a small volume V_t representing the volume of water contained in the apparatus before the experiment begins.

It follows from proportionality that if the length of the column is L cm. and the width of the boundary of the band due to the solute is λ_{90} then

$$\frac{\lambda_{90}}{L} = \frac{V_{90} - V_0}{V_e} \quad . \quad . \quad . \quad . \quad . \quad (1)$$

The volumes in the right-hand side of this equation are readily measured, and thus λ_{90} may be evaluated. It should be pointed out, however, that boundaries

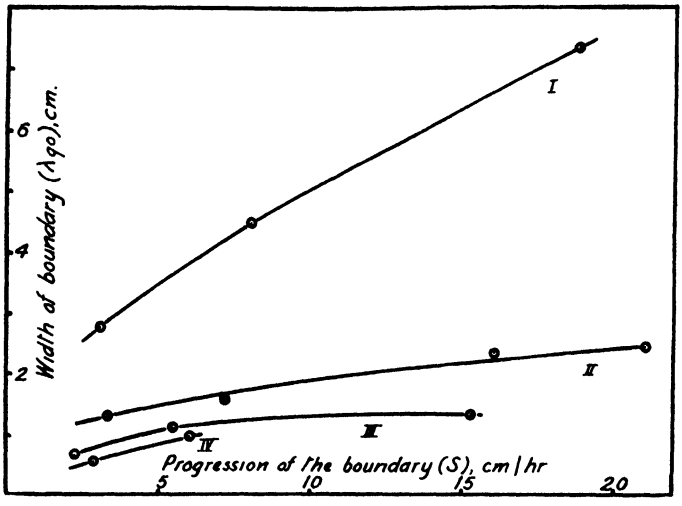

FIG. 4.—Showing the effect on the width of the boundary of an ammonia front due to changes in the particle size of the resin and the rate of progression of the boundary down the column. Zeo-Karb 215 : I, 20–40 mesh/in. ; II, 40–60 mesh/in ; III, 80–100 mesh/in. ; IV, 100–120 mesh/in.

in experimental columns are frequently more or less oblique and this obscures the physical meaning of the boundary width when the measured values of λ_{90} are of the same order as the distortion produced by irregularities in packing. Boundary widths greater than 3–4 cm. may be measured with a fair accuracy, and in this case λ_{90} may be correlated directly with the rate of the exchange reaction taking place on the resin. If the downward rate of progression of the boundary is S cm./hr. and the boundary width is λ cm. then the time (in hours) required for completion of the exchange reaction on any particle of resin is λ/S.

Inspection of Fig. 4 shows that the boundary given by dilute ammonia solution is very roughly proportional to the rate of progression S except at low values of λ_{90}. The boundary width varies with a power of the particle diameter and, from the data, there appears to be no advantage (under the conditions envisaged) in using finer resin than 80–100 mesh/in. In general, resin of this particle size has been used for fractionation experiments with small columns, but in experiments with long columns, where the resistance of the resin bed to the flow of solvent is an important consideration, we have usually used 40–60 mesh/in. resin. The rates of flow in these experiments have been adjusted to correspond with rates of progression of the boundaries of 10–15 cm./hr.

The values of λ_{90} given in Fig. 4 refer to the boundary of the ammonia front when the ammonium ion is displacing the hydrogen ion from the acid form of the resin. However, λ_{90} may also be measured for the case where one base displaces another from the column. Thus λ_{90} may be measured for the glycine-ammonia boundary, by saturating the column with glycine and displacing it with ammonia. Generally, such boundaries are a little wider than those obtained with the hydrogen form of the resin, but all such boundaries are of the same order of width. Thus when a series of components are displaced down a long column, and sufficient time and column length have been allowed to ensure that separation is complete in each case, the widths of the boundaries between the various components are of the same order, and in most experiments with *weak bases* the values vary from 0·5 cm. to 3 cm. It should be made clear, however, that the symbol λ_{90} refers specifically to the condition of flowing equilibrium after separation is complete, and that the conception serves little useful purpose where two components of similar affinity for the resin undergo a progressively increasing separation as they pass down the column.

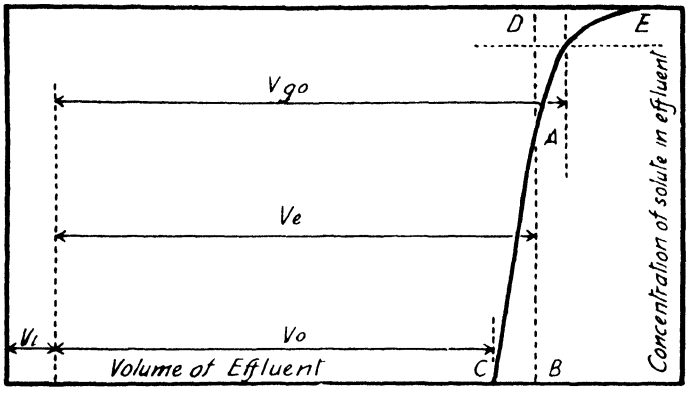

FIG 5.—Concentration changes in the effluent from a column packed with a cation-exchanger during the passage of a solution of a base (diagrammatic) An explanation of the symbols appears in the text.

(*e*) With Zeo-Karb 215 it is found that where strong bases are used the values for λ_{90} may become very large indeed, and for that reason it is impracticable to use this resin for bases stronger than ammonia. The reason for this effect of strong bases is clear from the experiment illustrated in Fig 6. The curves show the shape of the boundaries obtained by passing through a column a series of buffers containing equal concentrations of sodium ion, but adjusted to different pH values by use of a range of weak acids. It will be seen that the boundaries are very sharp from pH 1–7 but get progressively wider as the pH rises from 7–12. At high pH values the boundaries are obviously quite useless for the purpose of separation experiments. If this diagram is compared with the titration curve (Fig. 1), it will be seen that the boundaries become more diffuse as the phenolic hydroxyl groups are titrated. Thus in alkaline solution the resin behaves as if it were a mixture of two ion exchangers, one reacting very rapidly and the other very slowly. This feature of the resin is a serious disadvantage since it limits the range of pH over which practical operations are possible.

2. Separation of the Amino Acids of a Protein Hydrolyzate.—When the number of components in the mixture is small the various boundaries flowing from the column may be located by following changes in electrical conductivity and pH by means of cells placed in the path of the effluent, but with a complex mixture such as a protein hydrolyzate this method is insufficiently sensitive and it is necessary to resort to other means of following the changes in composition of the effluent. In recent work the method adopted has been

to divide the effluent arbitrarily into a large number of equal fractions, and to carry out on each fraction a qualitative analysis by means of the filter-paper technique described by Consden, Gordon and Martin.[7]

In a typical experiment the HCl-hydrolysis product of commercial egg albumen (64 g.) was fractionated on a column of Zeo-Karb 215. Since the amino acids tyrosine and phenylalanine behave irregularly on the column, these were first removed by adsorption on prepared charcoal. A partial recovery of tyrosine and phenylalanine was obtained by eluting the charcoal with aqueous phenol-acetic acid.[8] Treatment with charcoal had a further advantage since soluble humin was removed and the charcoal filtrate was clear and colourless.

The column of Zeo-Karb 215 was in two sections, each 82 cm. high and each containing 200 g. of the resin. The mixture of amino-acid hydrochlorides (containing a small excess of hydrochloric acid) was applied to the column, and the mixed band so obtained was developed by displacement with 0·15 N NH_3; the effluent solution was collected by means of an automatic arrangement in 64 fractions, each of 90 ml. The flowing chromatogram was analyzed by paper

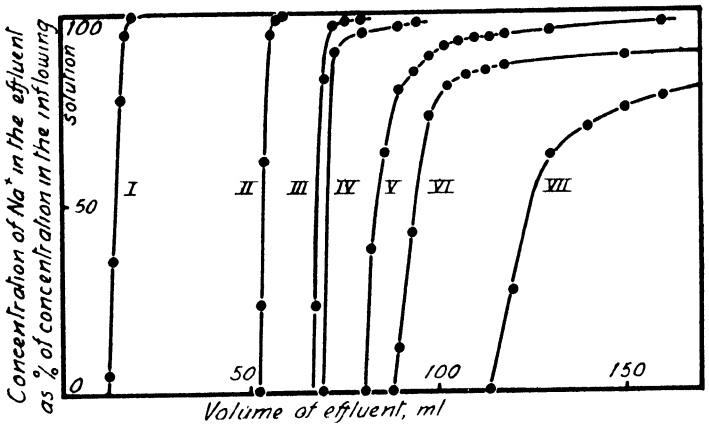

FIG. 6.—Volume-concentration curves showing the shape of the fronts obtained on Zeo-Karb 215 (80–100 mesh/in.) with buffer solutions containing Na^+, 0·53 N and various anions. I, NaCl + HCl (pH 1·1); II, NaCl + HCl (pH 2·1), III, NaAc + HAc (pH 4·7), IV, NaH_2PO_4 + NaOH (pH 6·9), V, boric acid + NaOH (pH 9·35); VI, boric acid + NaOH (pH 11); VII, NaOH (pH 12·5).

chromatography and the result showed the presence of seven discrete bands: I, aspartic acid; II, glutamic acid, serine and threonine; III, glycine and alanine; IV, valine and proline; V, leucine, *iso*leucine, methionine and cystine; VI, histidine and glucosamine, VII, lysine. Arginine has a greater affinity for the resin than ammonia and thus it remained on the column.

The results of the separation are illustrated by Fig. 7, which is a diagrammatic representation of the paper chromatograms. It will be observed that the overlapping portions between the various mixed bands are all of similar width. This indicates that sufficient column length was allowed to complete the separation between the mixed bands; there is indication of partial separation of some of the components within the mixed bands but, obviously, a column of great length would be required to effect the complete separation of all the components of a protein hydrolyzate in a single passage.

However, displacement chromatography may be used to effect a further separation of the components of the individual bands by modifying the experimental conditions. Some of the possible modifications that have been investigated are as follows: (1) the use of a water-soluble organic solvent such as ethanol or acetone in order to modify the ionization of the carboxylic acid groups.

[7] Consden, Gordon and Martin, *Biochem. J.*, 1944, **38**, 224.
[8] Schramm and Primosigh, *Ber.*, 1943, **76**, 373.

in the amino acids.[4] (ii) The choice of a solvent mixture having a water-solvent partition in favour of the amino acid it is desired to separate.[4] (iii) The choice of a second ion-exchanger with different properties from that effecting the first separation (e.g., the use of an anion-exchange column to follow a separation carried out on a cation-exchange column).[4] (iv) The properties of one or more of the components may be modified by chemical means.[4]

The actual isolation of the amino acids in a crystalline form is a very simple matter since they appear in the effluent solution in an isoelectric condition, substantially free from salts With aspartic acid, this appeared (in the experiment described above) at a concentration rather in excess of its solubility, and a heavy crop of crystals formed in the receivers soon after they were filled. The other amino acids and amino-acid mixtures were recovered by evaporating the solution to small bulk and even without recrystallization, they were in a

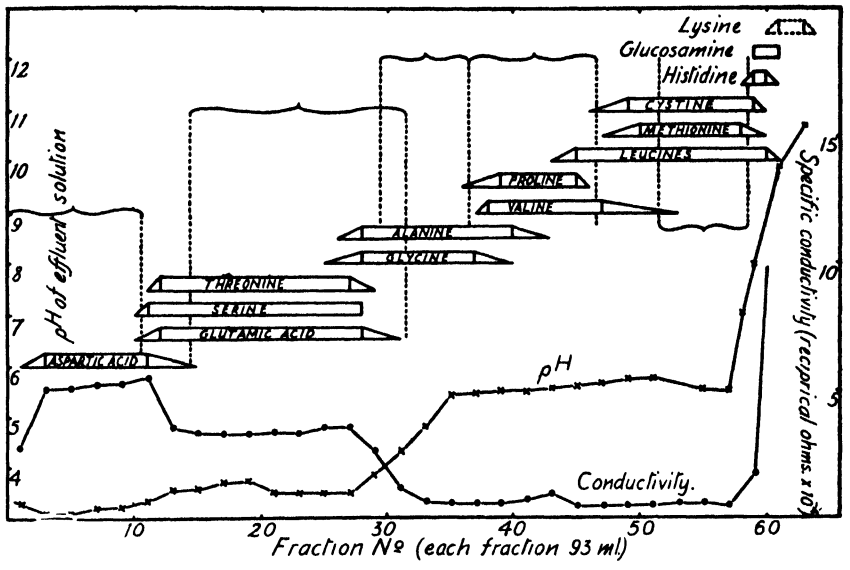

FIG. 7.—Fractionation of a protein hydrolyzate. Analysis of the effluent obtained from a column of Zeo-Karb 215 by displacing the mixture of amino acids with dilute ammonia solution The upper portion of the figure shows an analysis carried out by means of filter-paper chromatography, while the lower portion shows the corresponding variations in pH and conductivity. The brackets include fractions collected together for treatment on secondary columns

—●— conductivity, — × —, pH

substantially pure condition. In all, 46 % of the original protein dry weight was recovered as crystalline amino acids in this experiment, either as the pure substances or as simple mixtures, but it is obvious that this recovery could be very considerably increased by use of longer columns and by returning the mixed fractions forming the boundaries of the bands for refractionation in subsequent experiments.

At the inception of these experiments it was not expected that separations would take place among the homologous mono-carboxylic mono-amino acids since they are alike in acidic and basic dissociation. It has been pointed out[9] that when the displacement developer is a free base, the order of displacement reflects the ability of the stronger base to control the pH of the aqueous phase and thus to depress the cationic form of the weaker bases. However, Davies[9]

[9] Davies, *Biochem. J.*, 1949, **45**, 38.

showed that while the controlling equilibrium in the case of the amino acids may be represented by

$$A^+ \rightleftharpoons A^{\pm} + H^+, \quad . \quad . \quad . \quad . \quad (2)$$

in addition there may be at least two further factors affecting the adsorption of an amino acid by the resin: (i) van der Waals' forces between the resin and the cation A^+ or the neutral molecule $A^{\pm}$ and (ii) the valency of the cation. From the fact that, in protein hydrolyzates, separations occur between alanine and valine and between valine and leucine it appears that the effect of van der Waals' forces is not inconsiderable.

3. Displacement with Solutions of Salts.—Since the mechanism of adsorption by the resin depends upon more than one physical property, it is clear that by altering the environment of the ions in the ambient phase it would be possible to exaggerate the effect of one of these properties at the expense of the others and thus to produce conditions in which the order of displacement is altered. Thus by using a dilute solution of a salt as displacement developer the pH of the ambient fluid would be lowered and eqn. (2) would lie entirely to the left

When a solution of a salt is introduced onto a column containing an adsorbed mixture of amino acids, the cations of the salt are adsorbed and the anions are released together with the cations of the displaced amino acids. The high acidity thus set up results in the mechanism depending upon eqn. (2) becoming inoperative and the mutual displacement of the amino acids is thus controlled by mechanisms (i) and (ii) only. In this case, as would be expected, the divalent ions are strongly adsorbed and the observed order of appearance of the cations in the effluent when 0·1 M NaCl is used as the displacement developer is as follows: glucosamine, Na^+, leucine, histidine, lysine. Thus by use of a salt instead of a free base as displacement developer the order of glucosamine and leucine is reversed and this effect has been used to secure the separation of both glucosamine and histidine from the hydrolysis product of a protein.[10]

4. Use of Resins of the Sulphonated Polystyrene Type.—The effect of phenolic hydroxyl radicles in modifying the rate of exchange under conditions of high alkalinity is a serious disadvantage and is inherent in resins of the sulphonated phenol-formaldehyde type. The presence of such groups prevents the effective use of the phenol-formaldehyde resins for separations involving the important amino acids lysine and arginine, and in order to avoid this difficulty, the use of sulphonated polystyrene resins is being investigated. The experiments are being carried out in collaboration with Dr. Pepper and his colleagues at the Chemical Research Laboratory, Teddington. The results to date show that not all styrene polymers are effective, and that the rate of exchange depends very critically upon the degree of cross-linking of the resin. Lysine and arginine have been separated effectively both from the mixture in pure solution and from the products of protein hydrolysis, but it proved necessary to use a lightly cross-linked form of the resin, and this introduced difficulties due to the high degree of hydration of such samples. Resins containing only a small percentage of the cross-linking monomer, divinyl benzene, suffer large volume changes with changes in the ionic strength of the liquid phase, and special precautions are necessary to prevent disturbance of the column-packing during the course of the fractionation.

General Conclusions.—Although the work is still incomplete it is already clear that the displacement technique shows considerable promise as a systematic method for the separation of organic electrolytes on a preparative scale. The commercial cation-exchange resin Zeo-Karb 215 has been found satisfactory as a column-packing for the primary separation into groups, but where strongly basic substances are involved the procedure could with advantage include passage of the mixture through a short column packed with a calculated weight of sulphonated polystyrene resin,

[10] Partridge, *Biochem. J.*, 1949, **45**, 459.

in order to retain the more basic components. The polystyrene column would then be displaced with sodium hydroxide solution and the Zeo-Karb column by means of ammonia.

With a system such as a protein hydrolyzate, once the primary separation has been carried out, the resulting fractions contain a few components only and are easily dealt with, either by the conventional methods of fractional crystallization or solvent extraction or by further column procedures. Where further chromatography is indicated it is of interest that this may frequently be carried out by use of the same resin adopted for the primary separation, merely by altering the environment of the ions in the solution phase. Thus displacement chromatography offers a considerable advantage over the process of distillation, which it otherwise resembles, since environmental factors may be varied at will, and the best use may therefore be made of differences in the chemical structure of the components in order to separate them. The procedure has been tested to some extent by carrying out the fractionation of the products of hydrolysis of a protein, and in the initial work with a small column, some 45 % of the original weight of protein was recovered either as pure amino acids or as simple mixtures which contained no contaminating impurities and were readily crystallized.

This review was prepared as part of the work of the Food Investigation Organization of the Department of Scientific and Industrial Research.

Low Temperature Station for Biochemistry and Biophysics, Cambridge.

CHROMATOGRAPHY OF STEROIDS AND OTHER COLOURLESS SUBSTANCES BY THE METHOD OF FRACTIONAL ELUTION

By T. Reichstein and C. W. Shoppee

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The method for the separation of coloured substances called chromatography was developed and so named by Tswett (1906), but attracted no attention for a long time until revived by R. Kuhn and E Lederer.

Many methods have been suggested for the separation of substances which are colourless and give no visible reaction upon irradiation with ultra-violet light. This paper deals only with the technique of fractional elution as broadly applied by us respectively since 1933 and 1939, using almost exclusively Al_2O_3 as adsorbent. The technique is quickly acquired and gives reliable results to those not deterred by its laboriousness.

The mixture of substances is adsorbed on Al_2O_3 from a suitable solvent (as high up in the series given below as solubility allows), and then fractionally eluted by consecutive treatments with solvents of increasing eluting power. The following sequence of solvents has been proved efficient: pentane, petroleum ether, benzene, ether, chloroform or acetone (possibly ethyl acetate), methanol (only rarely with addition of water or acetic acid). Each fraction is evaporated separately, weighed and, if possible, characterized by melting point or other suitable data.

Chromatography is an indispensable tool in steroid chemistry. In rare cases compounds are altered or destroyed during the procedure The technique described is especially suitable for crystallizable, colourless compounds Acids and polyphenols react with and remain firmly attached to Al_2O_3 For the separation of such compounds use of SiO_2, active carbon, in some cases acid-washed Al_2O_3, etc., or partition chromatography is indicated.

The present paper contains some observations on the practical procedure for chromatography by fractional elution (*Durchlaufmethode*) as applied by us since 1933 and 1939 respectively.

The Russian botanist Tswett observed in 1906 that mixtures of coloured substances could readily be separated by filtration of a solution through a column of a suitable absorbent followed by subsequent washing of the column with appropriate solvents. The components were thereby concentrated in clearly defined zones, and could be obtained in a pure state by dissection of the extruded column. Tswett named this procedure chromatography, but it remained unnoticed until R. Kuhn and E. Lederer realized its significance and commenced to make full use of it. Since then chromatography has proved itself to be one of the most important techniques for separation and purification.

It is clear that the principles used by Tswett are also applicable to the separation of colourless substances, but that any separation of the components achieved cannot directly be observed. In individual cases this difficulty may be overcome by irradiation with ultra-violet light and observation of the resulting fluorescence. When this is not possible various other methods have been suggested :

(i) Arbitrary dissection of the column into small sections and examination of the material eluted from the individual sections.

(ii) Painting of a narrow strip along the extruded column with reagents, e.g., potassium permanganate (brush method).

(iii) Addition of, and so simultaneous chromatography with, coloured dyestuffs (indicator method).

(iv) Addition of fluorescent substances to the adsorbent ; quenching of the fluorescent often occurs at the enriched zones.

For the separation of colourless compounds we have invariably preferred to use the method of fractional elution, the technique of which will briefly be described. This method is quite well known, but in our opinion too little employed ; it is relatively time-consuming, but gives good results and is extremely reliable. For separations in the laboratory quantities of 30 g. or less are suitable ; larger quantities require semi large-scale equipment for the distillation of solvents. In regard to small quantities, in theory there is no lower limit, but in practice the separation of quantities less than 10 mg. is infrequent because the compounds obtained are then insufficient for micro-analysis. As adsorbent we have used almost exclusively aluminium oxide ; this is suitable for both neutral and basic substances and also for simple phenols. Acids and polyphenols react chemically and are extremely difficult to remove from the column ; for such substances the use of silica, charcoal, acid-washed aluminium oxide or the employment of partition chromatography may be more suitable.

Experimental

Preparation of Al_2O_3.—Activated and partly standardized Al_2O_3 is available commercially. Equivalent and very active preparations are obtained by heating technical pure $Al(OH)_3$ for about three hours with stirring at 380°–400° ; such preparations always contain free alkali (or sodium carbonate) which, however, is usually not deleterious, and in general give the best separations. In certain cases these preparations are too active and lead to condensation of ketones or aldehydes, elimination of alcoholic hydroxyl groups, etc., but by homogeneous addition of moisture their activity can be reduced and standardized (e.g., by the use of selected dyestuffs under defined conditions [1]).

Alkali-free Al_2O_3.—For sensitive substances (ketones, lactones, readily hydrolizable esters, etc) neutralized aluminium oxide is used. Aluminium oxide, prepared in the laboratory by activation at 380°–400° or obtained commercially, is boiled repeatedly with distilled water until the extract is neutral ; the filtered material is then washed with methanol and reactivated at 160°–200°

[1] Brockmann and Schodder, *Ber.*, 1941, **74**, 73.

(internal temperature) at 10 mm. pressure. Preparations reactivated at 200° are too active for many purposes, and a temperature of 180° is generally sufficient. The product still contains traces of alkali, which are not harmful, and is rather less effective than the alkali-containing oxide in regard to separation.

Neutralization is more rapidly achieved by neutralization of the first aqueous suspension with dilute nitric acid (use of hydrochloric, sulphuric or acetic acid is rather more dangerous) followed by boiling with distilled water, treatment with methanol and reactivation as above. The product contains nitrate ions, which are not always harmless.

Regeneration.—Used aluminium oxide is repeatedly extracted, first with boiling methanol and then with boiling water (with addition of some sodium hydroxide if necessary) and subsequently treated as described above Slight discoloration of the regenerated material is of no importance

Solvents.— The following solvents are most frequently used and in the given serial order of increasing elutary power: pentane, petroleum ether, benzene, ether, chloroform (or acetone), methanol or ethanol (possibly mixed with ethyl acetate-water or ethyl acetate-water-acetic acid) All should be dry and freshly distilled; commercial chloroform is stabilized by addition of 1 % ethanol, which is not removed.

Pure methanol, water, etc., are used but little, these solvents dissolve quantities of aluminium oxide, from which the fractions obtained must be separated.

If the mixture to be separated is soluble in petroleum ether, the column is prepared in petroleum ether, in general, the column is prepared in that solvent which, in the above series, precedes the solvent in which the mixture to be separated is soluble.

Preparation of the Column.—A glass tube with a tap (no grease!) or with a fused-in sintered-glass plate (porosity 4) is used (cf. Fig 1), and for 1 g of substance 30 g Al_2O_3 is employed The following sizes of tube are convenient

Amount Al_2O_3 (g.)	Internal diam. (mm)	Usable length (mm)	Bore of tap (mm)
1	8	110	3
2	10	130	3
4	13	160	4
8	16	200	4
15	20	250	4
30	25	300	6
60	32	400	6
125	40	500	6
250	50	600	8
500	65	750	8

The tube is selected so that, when the appropriate quantity of aluminium oxide has been introduced, it is less than half-full

After insertion of cotton-wool, a porcelain filter-plate or disc of wire-gauze, fitting the tube as accurately as possible, is introduced, followed by an accurately cut circle of filter-paper. The tap is then closed, and the tube filled completely with the chosen solvent. When all air-bubbles have been removed, highly purified sand is poured in to give a layer of approx. 1 cm thickness, which is covered with an accurately cut circle of filter-paper, 30 g. Al_2O_3 are now introduced in a fine stream and allowed to settle, a process which may be promoted sometimes by gentle tapping. The tap is opened, the solvent run out and the tube repeatedly refilled with the filtrate until the aluminium oxide ceases to pack together: it should be noted the column must *always* remain covered with solvent. The column is covered with a circle of filter-paper, on which is placed a small wad of cotton-wool to prevent disturbance of the column by swirling. Finally, the solvent is allowed to run out until the aluminium oxide just remains covered, and the column is ready for use. Gentle positive pressure may be used to accelerate the production of the column and the subsequent chromatography, and is readily applied by means of the simple apparatus indicated in Fig. 1.

The procedure using a tube with a sintered-glass plate is similar but simpler. The tube is filled with solvent and the aluminium oxide introduced directly onto the sintered-glass plate ; gentle positive pressure is applied as shown in Fig. 1, and solvent allowed to run out until the column remains just covered. The pressure supply is disconnected, the tube refilled with the solvent and the process repeated until the column ceases to settle ; the column is covered with a circle of filter-paper on which is placed a small wad of cotton-wool, and the solvent expelled until the column remains just covered.

The prepared column should be free from pores and should show no cracks ; suction is dangerous and easily leads to the formation of cracks in the presence of volatile solvents, and positive pressure should always be employed in preference to suction.

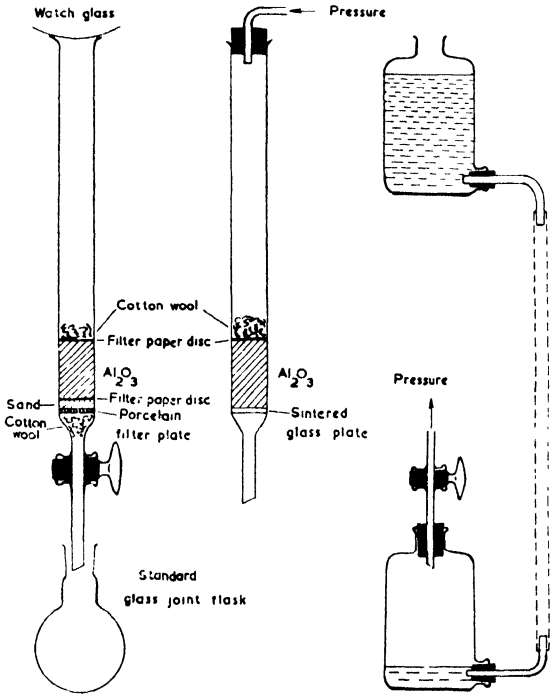


FIG. 1.

The Actual Chromatography.—In the case of mixtures soluble in petroleum ether, 1 g. of the substance is dissolved in 100 ml. petroleum ether, the solution allowed to percolate under gravity or gentle positive pressure through the column and the filtrate collected as Fraction 1. Further 100 ml. portions of petroleum ether are allowed to percolate through the column, each fraction being collected separately, evaporated * and weighed. When the residue obtained by evaporation weighs less than 50 mg , the ascent of the solvent series is commenced by use of benzene-petroleum ether mixtures (starting with 10 %–20 % benzene) for percolation. The proportion of benzene is gradually increased to 50 %, and then directly to 100 %, i e., pure benzene Direct passage from one pure solvent to another should never occur because cracks in the column can arise ; when passing to ether and especially to methanol, very small proportions of these solvents (1 %, 2 %, 5 %, 10 %) must be used at first.

With a little practice, it is almost always possible to divide a mixture into about 10 or more fractions, of which each should weigh not more than 10 % of

* Standard-joint flasks and an all-glass apparatus are convenient.

the weight of the original mixture; if some fractions exceed this limit, they must be rechromatographed separately.

For substances insoluble in petroleum ether, a suitable solvent must be chosen and the procedure is somewhat as follows: suppose, for example, the substance is insoluble in petroleum ether and in benzene, but soluble in chloroform. The column is prepared in benzene; 1 g. of the mixture is dissolved in 10 ml. chloroform and the solution diluted with 90 ml. benzene. If a part of the dissolved material is thereby precipitated, the suspension is allowed to settle and the clear solution resulting is poured onto the column. The precipitated material is then redissolved in 10 ml. chloroform, 90 ml. benzene added, and the treatment repeated until all has been dissolved; if this takes too long, the proportion of chloroform can be increased, e g., 20 ml. chloroform + 80 ml. benzene.

The individual fractions obtained by evaporation of each eluate are examined separately; it is usually possible to decide which are alike on the basis of m p. crystal form, colour reactions, spectra, etc. Fractions containing the same compound are then united.

It is generally desirable to complete a chromatographic separation in a single day; for rapidity in working, a set of three flasks used in rotation—one for collection of a filtrate, one for evaporation of a filtrate and one with the residue being examined and transferred to a small boiling tube—is helpful. For quantities larger than 1 g, an accurate trial separation of a small sample is to be recommended, because short-cuts often become evident and can be applied to the treatment of the main bulk of material with considerable saving of time.

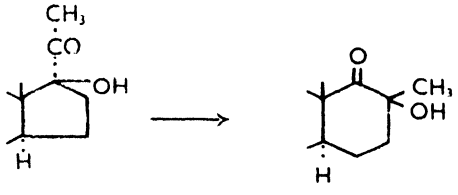
In almost every chromatographic separation, there are observed lightly coloured zones caused by accidental impurities These should be accurately horizontal; inclined zones or areas exhibiting downward displacements indicate that the column was poorly constructed, and the separation is then generally unsatisfactory.

Presentation of Results.—Experimental descriptions of chromatographic separations frequently omit the essential details; the weight and activity of the Al_2O_3 employed and possibly the dimensions of the column should be specified, whilst the medium in which the column was constructed and the volume(s) of eluants should be stated. The details of the various fractions may either be tabulated [2] or may be set out in the form of a graph.[3]

Discussion

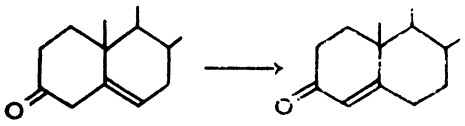
By chromatography of coloured substances, it has been possible to achieve results obtainable by no other method. The modification here described is especially suitable for the separation of colourless substances capable of crystallization because by means of melting points the progress of the separation can readily be followed without appreciable wastage of material; in this connection, the use of a micro-m.p. apparatus is particularly desirable. Provided some suitable control is available, the method can equally well be applied to the separation of liquids. Steroids, though colourless, are generally crystalline substances and in this field chromatography has been used with great success: it is in fact almost essential In a few cases only have difficulties been encountered, e g:

1. 17-Hydroxy-20-ketones, especially those of the 17-*iso*pregnane series, undergo molecular rearrangement to give *D*-homo-ketones. This can largely be avoided by use of neutralized Al_2O_3 of low activity and dry solvents, and is completely avoided by use of the 17-acetates.

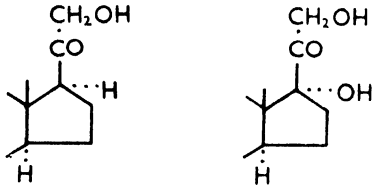


[2] Reichstein and von Euw, *Helv. chim. Acta*, 1941, **24**, 247. Shoppee, *ibid*, 1940, **23**, 925; *J. Chem. Soc.*, 1946, 1138; 1948, 1032; 1949, 1671.
[3] Barton and Miller, *J. Chem. Soc.*, 1949, 337.

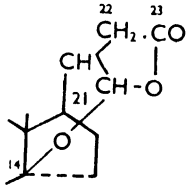
2. Δ^5-3-ketones undergo rearrangement to Δ^4-3-ketones by use of alkali containing Al_2O_3, but not when neutralized Al_2O_3 is employed.



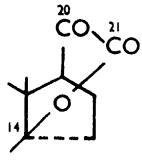
3. Free hydroxy-ketones of the types illustrated often undergo considerable loss. This cannot completely be avoided by use of neutralized Al_2O_3, and it is best to chromatograph the 21-acetates.



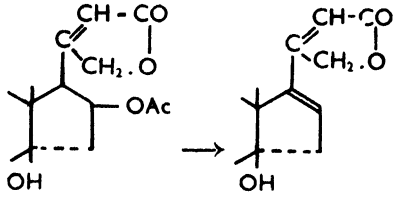
4. 14β: 21-oxido-23→21-lactones ("*iso*-compounds") derived from heart poisons of the digitalis and strophanthus types undergo large losses probably as the result of facile fission of the 23→21-lactone ring; such losses are decreased but not completely eliminated by use of neutralized Al_2O_3.



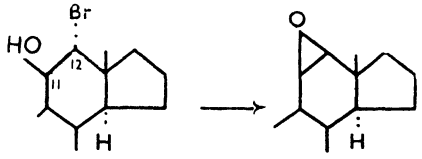
5. 20-Keto-21→14-lactones suffer very large losses which cannot be avoided even by use of neutralized Al_2O_3.



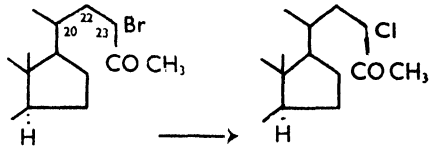
6. 16-Acetoxy derivatives of the type of oleandrigenin or digitoxigenin acetate eliminate one molecule of acetic acid by chromatography on many, but not all, preparations of Al_2O_3.



7. 11 : 12-Bromhydrins of the type illustrated eliminate hydrogen bromide to give 11β : 12β-oxides by filtration over alkaline Al_2O_3, but are unaffected by neutralized Al_2O_3.



8. The 23-bromo-24-ketone shown was found on attempted purification by chromatography on Al_2O_3, which had been neutralized by treatment with dilute hydrochloric acid, despite extensive washing with water after neutralization, to undergo ion exchange to give the corresponding chloro-ketone.



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PARTITION CHROMATOGRAPHY OF THE TERTIARY AMINE SALTS OF THE PENICILLINS

By T. Leigh

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Partition chromatography of the penicillins using a mixture of a tertiary amine and water adsorbed on silica gel as the stationary phase and water-immiscible organic solvents as the mobile phase has been investigated. On applying a solution of the free acids to such a column the penicillins are converted to, and emerge as, the tertiary amine salts. *n*-Heptyl, *n*-amyl, and *p*-hydroxybenzylpenicillins were readily isolated in the pure state from mixtures of these penicillins with Δ^2-pentenyl- and benzylpenicillins. Resolution of the last two penicillins was very poor owing to the similarity of the partition coefficients of their amine salts.

The results are compared with those obtained with the phosphate buffer–silica gel–ether system. The microchromatographic assay of Goodall and Levi was used to follow the progress of the separations. Five penicillins of purity > 99·7 % have been isolated and the characteristics of these virtually pure penicillins are appended.

Early attempts to separate mixtures of penicillins by partition chromatography were based upon the method devised by Martin and Synge [1] for the separation of the amino acids. These failed because of the low solubility and pronounced instability of the free penicillin acids in water. The difficulties were overcome by Levi and Terjesen [2] who applied a solution of the penicillin acids in ether to a column composed of silica gel impregnated with

[1] Martin and Synge, *Biochem. J.*, 1941, **35**, 1358.
[2] Levi and Terjesen, *Brit. Pat* 569,844.

an aqueous phosphate buffer solution and this type of partition chromatography has since been extensively used for the separation of the penicillins produced by the fermentation of *P. notatum* and *P. chrysogenum*.

The major constituents of the mixture of penicillins produced by the submerged fermentation of *P. chrysogenum* Q 176 in a corn steep liquor-lactose-phenylacetic acid medium are benzylpenicillin (penicillin-II or G), Δ^2-pentenylpenicillin (I or F), *n*-amylpenicillin (dihydro F) and *n*-heptylpenicillin (K) and it was required to isolate a few grams of each in the pure state to allow the determination of their more important physical and biological properties. Partition chromatography using potassium phosphate buffer and moist ether as the mobile phase afforded four fractions in each of which one penicillin preponderated. *n*-Heptyl- and benzylpenicillin were readily separated from *n*-amyl- and Δ^2-pentenylpenicillin and rarely contained more than a few per cent. of the latter, but the resolution of *n*-amyl- and Δ^2-pentenylpenicillin was much less efficient and the *n*-amylpenicillin fraction was often heavily contaminated with Δ^2-pentenylpenicillin. The sparing solubility of certain amine salts of benzylpenicillin and the relatively much greater solubility of the corresponding salts of *n*-heptylpenicillin in solvents such as acetone and ethyl acetate suggested that the separation of the penicillins might be more easily accomplished by partition chromatography of these salts using water adsorbed on silica gel as the stationary phase and a water-immiscible solvent as the moving phase.

On applying a solution of a tertiary amine salt of the mixed penicillins to such a column the tertiary amine was adsorbed by the silica and the penicillins travelled down as the free acids, separation was poor and some decomposition of the penicillin acids occurred.

Moist silica gel is capable of adsorbing appreciable amounts of organic bases. Thus one part of silica gel impregnated with 70 % of its weight of water adsorbed 0·05 part of N-ethylhexamethyleneimine when treated with a solution of the base in an organic solvent ; the base was not removed on washing the silica with neutral organic solvents and a slurry of the treated silica with water had a pH of 7·5–8·0. Chromatogram columns of silica impregnated with water and various amines were prepared and solutions of the penicillins, either as their amine salts or as the free acids, in suitable solvents were charged. On eluting these columns the penicillins passed through in the same order as through a phosphate buffer–silica gel–ether column and emerged as the tertiary amine salts. Because of the low solubility of some of the mixed penicillin salts it was found more convenient to use solutions of the free acids. In the latter case the penicillins are converted to their amine salts at the top of the column by combination with the amine contained on the silica gel. The efficiency of the chromatographic separation did not vary with the method of application. Owing to the instability of the penicillins towards primary and secondary amines only tertiary amines were used. Triethylamine, N-ethylpiperidine and N-ethylhexamethyleneimine were employed for the preparation of the chromatographic columns and ethylene dichloride, chloroform, ethyl acetate and *n*-butanol were tried as eluting agents.

n-Heptylpenicillin was very easily separated from the other penicillins by using any combination of the above solvents and bases. For the separation of *n*-amylpenicillin from the remaining penicillins it was necessary to use a solvent in which the penicillin salt had only a sparing solubility compared with its solubility in water and the most efficient system of those examined was that with N-ethylhexamethyleneimine and wet ethyl acetate. For the isolation of those penicillins with the saturated side chains, i.e., *n*-heptyl- and *n*-amylpenicillins, this method proved to be more efficient than partition

chromatography of the mixed acids with phosphate buffer and ether. Comparison of the two types of partition chromatogram showed that for the same ratio of penicillin to weight of silica gel the resolution was sharper and a smaller number of chromatographic separations were required for the isolation of the pure penicillins. Less destruction occurred and the total recovery of penicillin was 10 %–20 % higher than with the phosphate buffer-ether column. The higher recovery is probably associated with the fact that during the separation the penicillin is wholly in the form of the stable tertiary base salt and not as the free acid. The efficiency of the separation was the same whether the starting material was a mixture of the pure penicillins or a crude mixture containing some 40 % of biologically inactive impurities. Improved separation from impurities was also obtained with this type of partition chromatogram. Thus pure crystalline salts of *n*-heptylpenicillin were readily obtained from crude penicillin mixtures but the corresponding fractions from the phosphate buffer–silica–ether columns could not be induced to crystallize owing to heavy contamination with impurities.

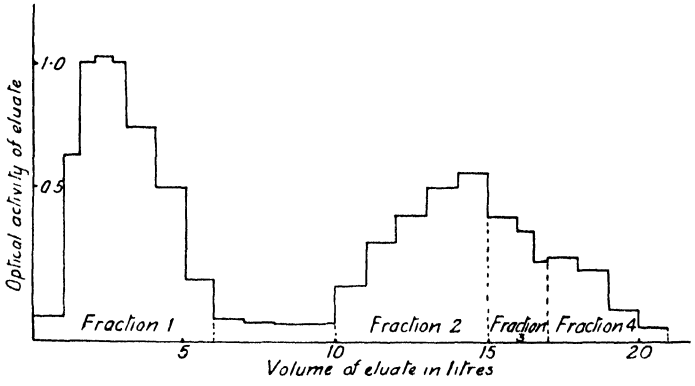

FIG. 1—Fractionation of mixed penicillins.
N-ethylhexamethyleneimine salt partition chromatogram.

The tertiary amine–water–organic solvent systems described above failed to separate Δ^2-pentenylpenicillin from benzylpenicillin despite the very great difference in solubility of their salts in solvents such as acetone and ethyl acetate. It was presumed that a similar relationship existed between the solubilities of these salts in water and their partition coefficients between water and ethyl acetate at pH 7·0 were found to be approximately equal. Since excellent separation of these two penicillins can be achieved by means of the phosphate buffer–silica–ether column [3] no attempt was made to devise another partition system.

The method has only been used for the separation of the penicillins, but it should be capable of adaptation to the separation of other mixtures of organic acids by the choice of suitable amines and eluting agents and, if necessary, replacement of the stationary phase by other polar solvents and might be of particular value in chromatography of mixtures of acids which, like the penicillins, are only stable in the form of their salts in hydroxylic solvents.

Experimental

In a typical experiment a stirred suspension of silica gel (500 g.) in ethyl acetate (1500 ml.) was treated with water (350 ml.) over ½ hr. and N-ethylhexamethyleneimine (25 ml.) was added to the slurry. The suspension was

[3] Boon, Calam, Gudgeon and Levi, *Biochem. J.*, 1948, **43**, 262.

charged to a chromatogram tube, allowed to settle 1 hr., and the effluent ethyl acetate recycled till the column depth (37 cm.) was constant. A mixture of crystalline sodium penicillins (15 g) was dissolved in water, the solution was acidified and the acids were transferred to ethyl acetate (100 ml). This solution was run on to the column which was then developed and eluted with wet ethyl acetate. Twenty 1000 ml. fractions were collected from the moment the ethyl acetate solution was run on to the column. The penicillin content of the fractions was conveniently estimated by measuring the optical activity in a 2 dm. tube using sodium light and the relationship between optical activity and fraction number is shown in Fig. 1.

The first 6 l of eluate contained almost pure *n*-heptylpenicillin, the next 4 l. had almost zero optical activity and were discarded, the eleventh to the fifteenth litre constituted the *n*-amylpenicillin fraction and the last 5 l. contained

TABLE I

FRACTIONATION OF MIXED PENICILLINS—N-ETHYLHEXAMETHYLENEIMINE SALT PARTITION CHROMATOGRAM

	Percentage of starting material	Percentage composition Penicillin				
		p-hydroxy-benzyl	benzyl-	Δ^2-pentenyl-	*n*-amyl-	*n*-heptyl-
Starting material	—	0·3	4·7	20·0	38·0	37·0
Fraction 1	34	—	0·5	0·5	0·7	98·3
Fraction 2	31	—	0·6	11·0	88·0	0·4
Fraction 3	10	—	6·0	34·0	60·0	—
Fraction 4	13	—	13·0	75·0	12·0	—
Total recovery of penicillin	88					

The above percentages are expressed on a weight basis.

largely Δ^2-pentenylpenicillin together with some benzylpenicillin. Four fractions were evaporated to dryness under reduced pressure and the composition of the fractions determined by the microchromatographic method of Goodall and Levi.[4] Details of the composition of the starting material and the four fractions are given in Table I.

The *p*-hydroxybenzylpenicillin was left near the top of the column; it was quite free from contamination by other penicillins. *n*-Heptylpenicillin of purity $> 99{\cdot}9$ % was obtained by rechromatographing fraction 1. The overall yield of pure *n*-heptylpenicillin from the starting material, including a recrystallization of the product from the second chromatogram, was 68 %. A further chromatogram with fraction 2 yielded *n*-amylpenicillin of purity 96 %, the remaining 4 % consisting of Δ^2-pentenylpenicillin and a final chromatogram yielded *n*-amylpenicillin of purity 99·7 %. After conversion to the sodium salt and recrystallization the overall yield of pure *n*-amylpenicillin from that contained in the starting material was 26 %.

The progress of the fractionation of a similar mixture of penicillins by the phosphate buffer–silica gel–ether method described by Boon *et al.*[3] using amounts of penicillin and silica gel comparable with those used in the N-ethylhexamethyleneimine salt partition chromatogram is indicated in Fig. 2.

[4] Goodall and Levi, *Analyst*, 1947, **72**, 277.

In the preparation of this type of column it was found advantageous to ' pour ' the column with ethyl acetate instead of the denser ethylene dichloride used by the above authors. Silica gel columns prepared in this fashion showed no tendency to ' crack ' after replacement of the first solvent with ether. The eluate was divided into three fractions as indicated in Fig. 2, and a fourth fraction was obtained by washing the silica with water. The penicillin contained in the aqueous extract was transferred to ether and the four ethereal solutions were dried, concentrated under reduced pressure and treated with triethylamine to precipitate the penicillins. Details of the composition of the starting material and the four fractions are given in Table II.

Total recovery of penicillin from these columns averaged 70 % when the separation was performed at room temperature (23° C). The yield was increased to over 80 % when the partition was effected at 5° C. Further purification of the *n*-heptylpenicillin fraction was accomplished by raising the pH of the potassium phosphate buffer to 6·9 and after a second column treatment the amylpenicillin content was reduced to 2 %. A second pH 6·5 phosphate buffer column treatment

TABLE II

FRACTIONATION OF MIXED PENICILLINS—pH 6·5 PHOSPHATE BUFFER–SILICA–ETHER CHROMATOGRAM

	Percentage composition Penicillin				
	p-hydroxybenzyl-	benzyl-	Δ^2-pentenyl-	*n*-amyl-	*n*-heptyl-
Starting material	2·4	11·0	28·0	28·0	30·6
Fraction 1				14·0	86·0
Fraction 2			21·0	71·0	8·0
Fraction 3		5·0	78·0	17·0	
Fraction 4	2·0	73·0	25·0		

of the *n*-amylpenicillin fraction yielded a main fraction containing 90 % of this penicillin and 10 % of Δ^2-pentenylpenicillin and a third column gave *n*-amylpenicillin of 97 % purity. Almost pure benzyl- and Δ^2-pentenylpenicillins were obtained on rechromatographing fractions 3 and 4 respectively.

Two criteria were used for assessing the purity of the individual penicillins. Each penicillin was examined for contamination by other penicillins by the microchromatographic assay of Goodall and Levi.[4] This method readily detected the presence of other penicillins when these were present to the extent of more than 0·1 %. The second criterion was the specific rotation and each penicillin was recrystallized till this had a constant value. With the exception of Δ^2-pentenylpenicillin the molecular rotations of the penicillins were the same within the limits of experimental error (± 0·5 %). In the following descriptions of the properties of the penicillins isolated by the above methods the potencies were determined against *S. aureus* N.R.R.L. 313 and an untyped strain of *B. subtilis* by the cylinder plate method and against *S. aureus* N.C.T.C. 6571 by the turbidimetric method, using pure sodium benzylpenicillin as standard. All melting points are corrected.

n-HEPTYLPENICILLIN.—The anhydrous sodium salt melted with decomposition at 185° and had $[\alpha]_D^{23}$, 296° (C = 1·0 % in water) or a molecular rotation of 107,000°. Triethylammonium *n*-heptylpenicillin melted sharply without obvious decomposition at 117°. Microchromatographic assay showed the material to be free from other penicillins and the potencies of the anhydrous sodium salt were : *S. aureus* N.C.T.C. 6571, 2850 u/mg. ; *S. aureus* N.R.R.L. 313, 2255 u/mg. ;

B. subtilis, 845 u/mg. The corresponding *n*-heptylpenillic acid had m.p. 179° (with decomposition; put in bath at 150°) and $[\alpha]_D^{23}$, 489° (C = 1 % in 0·1 N sodium bicarbonate). Confirmation of the identity of the penicillin was obtained by hydrolysis to *n*-octoic acid in 80 % yield.

n-AMYLPENICILLIN.—The triethylamine, N-ethylpiperidine and N-ethylhexamethyleneimine salts were extremely difficult to crystallize and the only pure crystalline salt prepared was the sodium salt: m.p., 188° (decomp.); $[\alpha]_D^{23}$, 319° (C = 0·5 % in water); molecular rotation, 107,200° By microchromatographic assay it contained not less than 99·7 % of *n*-amylpenicillin. Potencies of the anhydrous salt were: *S. aureus* N.C.T.C. 6571, 1680 u/mg, *S. aureus* N.R.R.L. 313, 1670 u/mg.; *B. subtilis*, 660 u/mg. A preparation of the penillic acid from this material had m.p. 182° and $[\alpha]_D^{23}$, 534° (C = 1·0 % in water). *n*-Caproic acid was obtained in 80 % yield by acid hydrolysis of the penicillin.

Δ^2-PENTENYLPENICILLIN.—This penicillin was isolated by means of the phosphate buffer chromatogram. The sodium salt had m.p. 212° (decomp.); $[\alpha]_D^{23}$, 296° (C = 1·0 % in water) or a molecular rotation of 98,900°. Partition chromatography of the N-ethylhexamethyleneimine salt between ethyl acetate and water and subsequent recrystallization of this salt yielded crystals of the same molecular rotation. The N-ethylpiperidine and N-ethylhexamethyleneimine

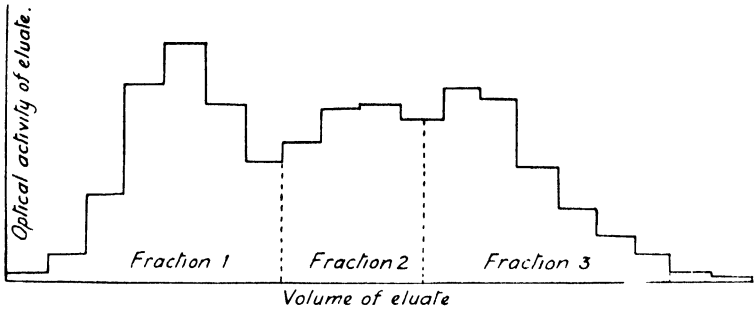

FIG. 2—Fractionation of mixed penicillins.
pH 6·5 phosphate buffer–silica–ether chromatogram.

salts melted without obvious decomposition at 131° and 115°, respectively. Examination by the microchromatographic assay method indicated a Δ^2-pentenylpenicillin content of not less than 99·9 %. The potencies of the anhydrous sodium salt were: *S. aureus* N.C.T.C. 6571, 1930 u/mg, *B. subtilis*, 970 u/mg. Catalytic hydrogenation by the method of Catch, Cook and Heilbron [5] yielded *n*-amylpenicillin and treatment of the penicillin in aqueous solution with mercuric chloride [6] yielded β-hexenoylaminoacetaldehyde which was identified as the dinitrophenylhydrazone.

BENZYLPENICILLIN.—The pure N-ethylpiperidine salt had m.p. 163°. Sodium benzylpenicillin melted with decomposition at 225°; $[\alpha]_D^{23}$, 303° (C = 1·0 % in water) or $[M]_D^{23}$, 107,900°.

p-HYDROXYBENZYLPENICILLIN.—Unlike the foregoing tertiary amine penicillin salts triethylammonium *p*-hydroxypenicillin melted with effervescence (143°). The sodium salt, $[\alpha]_D^{23} = 290°$, $[M]_D^{23} = 107{,}700°$, had the following potencies:—*S. aureus* N.C.T.C. 6571, 1090 u/mg.; *B. subtilis*, 766 u/mg. The *p*-hydroxybenzylpenicillin content was > 99·9 %.

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Research Laboratories,
Hexagon House,
Manchester, 9.

[5] Catch, Cook and Heilbron, *Brit. Pat.* 584,852. Improvements in and relating to manufacture of penicillin derivatives.
[6] *The Chemistry of Penicillin* (Oxford).

BIOLOGICALLY ACTIVE SUBSTANCES IN LIVER EXTRACT

By E. Lester Smith

Received 14th July, 1949

Chromatography has been invaluable in the isolation of the anti-pernicious anæmia factor (vitamin B_{12}) from liver. Adsorption chromatography of aqueous solutions on charcoal, silica, alumina and aluminium silicate has been employed, also partition chromatography on moist silica, starch and filter-paper, using butanol and other solvents. Propanol (*n*- or *iso*-), though completely miscible with water, gives satisfactory partition chromatograms with damp silica as base. Adsorption on the silica base is superimposed on partition and affects the rate of travel of bands on the chromatograms; nevertheless, partition remains the dominant factor.

Partition chromatography of purified liver extracts on silica, or starch, often reveals two pink zones, the faster one is due to vitamin B_{12}, the other to a related substance. On rechromatographing either zone, both reappear; this is presumably due to loose association between the two pigments.

The liver is a remarkable storehouse of vitamins and growth factors, and chromatography has been used in isolating many of them. The fat-soluble vitamins A and D have, for example, been concentrated chromatographically from fish-liver oils. Vitamins of the B group including vitamin B_1 and pteroylglutamic acid have been concentrated from mammalian liver by chromatography of aqueous extracts on ion-exchange resins and other substances. The present paper is concerned with the anti-pernicious anæmia factor, vitamin B_{12}, and certain related factors.

Vitamin B_{12} was first isolated in crystalline form by Rickes *et al.*[1] by methods that have not yet been disclosed. Within a few weeks it was independently isolated at Glaxo Laboratories[2,3] following progressive purification by adsorption and partition chromatography. Subsequently it has been isolated by Ellis, Petrow and Snook[4] after chromatography on a different adsorbent, namely, aluminium silicate. Details of our methods of isolation, and an account of the properties of this remarkable red cobalt complex of exceptional biological potency, will be published elsewhere.

Certain features of the chromatographic procedures, however, have more general interest and application. A chromatogram is usually made in a tube very much greater in height than in diameter. With a finely divided adsorbent charcoal, however, we have often found it expedient to make these dimensions of the same order, or even to have the diameter much greater than the height; for instance, we have used beds only a few cm. in depth and up to a metre or more in diameter. Our experience with vitamin B_{12} has paralleled that with penicillin.[5] In both instances a chromatographic effect can be demonstrated, even with such shallow beds of charcoal; a higher proportion of the active principle is adsorbed from a crude aqueous liquor by passing it through a pre-formed bed than by stirring it with the same amount of charcoal; similarly, less loss of active principle occurs on washing the bed

[1] Rickes, Brink, Koniuszy, Wood and Folkers, *Science*, 1948, **107**, 396.
[2] Lester Smith and Parker, *Biochem. J.*, 1948, **43**, P. 8.
[3] Lester Smith, *Nature*, 1948, **162**, 144.
[4] Ellis, Petrow and Snook, *J. Pharm. Pharmacol.*, 1949, **1**, 287.
[5] Lester Smith, *J. Soc. Chem. Ind.*, 1946, **65**, 308.

with water or with a mild eluting agent (to remove weakly adsorbed impurities) than if it were stirred with the adsorbate. The main advantage of the shallow beds lies in their capacity for rapid treatment of very large volumes of liquor containing valuable but unstable solutes.

Precipitated silica is a weak adsorbent that has some value for further purifying material already containing a few per cent. of vitamin B_{12}. We were interested to see whether its adsorptive power could be increased usefully by the technique of Tiselius,[6] namely, the addition to the aqueous solution passed through the column, and to the developing liquid, of an inorganic salt at a concentration just insufficient to salt out the solute. On increasing the concentration of ammonium sulphate up to half-saturation, on a series of columns, a progressive sharpening of the red band was observed, and correspondingly an increase in the capacity of a column of given size. However, persistent impurities (mainly peptides) were not separated much more effectively than they were without the ammonium sulphate.

Partition chromatography on moist silica with butanol and other solvents has been one of the most valuable steps in concentrating vitamin B_{12}. By its means a dark brown (but already highly purified) liver extract could readily be resolved into yellow, pink and brown fractions, of which the pink one carried almost the whole of the anti-anæmic activity. Subsequent operations were then simpler because the active fractions could be located by their red colour. Early attempts to set up these partition chromatograms failed because the silica became gelatinous and impervious. The water-logged appearance of the silica suggested that it was abstracting additional water from the *n*-butanol used as mobile phase, which had been saturated with water as was customary. This proved to be so; equilibrium is rapidly established from either direction so that silica containing suitable amounts of water for partition chromatography (i.e., about 60 % of its own weight) is in equilibrium with *n*-butanol containing 11 %–12 % of water by volume (i.e., about two-thirds saturated), which accordingly gives very satisfactory partition chromatograms. Instead of grinding the silica with water, a suitable amount can be conveniently introduced by stirring the dry silica with water-saturated butanol (8 to 10 ml./g. silica) to give a slurry, which can be poured immediately into the tube. The mixture is preferably first degassed by putting it under reduced pressure for about a minute; development of the column can then safely be expedited by suction without air-bubbles appearing.

Propanol (*n*- or *iso*-), though completely miscible with water, behaves in a very similar fashion to butanol, in that liver extracts separate into the same system of coloured bands on silica columns developed with propanol containing 10 %–25 % of water, while the *R* values are also similar. It seems probable that water is redistributed between silica and propanol on contact, as with silica and butanol, so that two liquid phases are created, a water-rich one soaked up by the silica particles and a continuous alcohol-rich one.

Consden, Gordon and Martin[7] have commented that partition chromatograms can be run on filter-paper strips with some water-miscible solvents and have explained this as due to the creation of two liquid phases by a "salting-out" effect of the cellulose. The principle has recently been utilized by Patton and Foreman[8] for the separation of amino acids by partition chromatography on filter-paper with 77 % ethanol.

It is now well recognized that adsorption comes into play, for better or worse, in many of the separations effected by partition chromatography.

[6] Tiselius, *Arkiv. Kemi, Min. Geol. B*, 1948, **26**, 1.
[7] Consden, Gordon and Martin, *Biochem. J.*, 1944, **38**, 224.
[8] Patton and Foreman, *Science*, 1949, **209**, 339.

Martin and Synge,[9] its originators, found it necessary to add a trace of alcohol to the chloroform used to fractionate acetyl amino acids to prevent excessive adsorption. Another early example concerns Sanger's[10] use of fluorodinitrobenzene for end-group analysis of peptides. Attempts to repeat his successful separation of the resulting dinitrophenyl amino acids by partition chromatography on silica failed until a more adsorptive batch of silica was used. We quickly appreciated that this phenomenon was also operating in our fractionations of vitamin B_{12}. The pink band of active material travelled out from the almost stationary brown band at the top of the column and then hardly moved any further on continued development, although a fast-running yellow band could easily be developed right out of the column. Different lots of silica varied in their behaviour: with some, it was difficult to separate the narrow brown and pink bands, while others permitted fair separation and gave a more diffuse pink band. Attempts to use starch as a less adsorptive base soon showed the advantages of a mildly adsorptive silica. On starch the pink bands travelled faster, but were so diffuse as to be only just clearly visible, while the capacity of a column of given size was much reduced. Moreover, the purification effected was no better than on silica columns.

Some reviewers, for example, Strain,[11] have called into question the appropriateness of the term partition chromatography (which I was the first to apply to this technique), implying that it cannot properly be distinguished from the original technique of adsorption chromatography. Strain has accordingly proposed a new term, partition adsorbent; this suggestion introduces needless confusion, besides appearing to belittle the spectacular advance made by Martin and Synge, whose method is probably more widely used now than the classical one. Now although adsorption is often involved, the dominant factor is undoubtedly partition; when adsorption occurs, it has the effect of diminishing the concentration of solute in the static aqueous phase below what it would be in the absence of adsorption; the net effect is to diminish the rate of travel of the zone and to sharpen it, especially in front. There is a close analogy with the use of a buffer solution instead of water to moisten the silica in the partition chromatography of acids not very soluble in water, like penicillin,[12] the higher fatty acids[13] and recently dinitrophenyl amino acids.[14] In these instances salt formation instead of adsorption is superimposed on partition, but, unless the acids differ widely in strength, differences in partition coefficient are the main cause of their separation on the chromatograms.

The result is quite different if adsorption alone is relied upon. Two illustrative examples may be cited from the present work. A component that travels quickly on a partition chromatogram must be both readily extractable from water by the solvent and not strongly adsorbed by the supporting solid. A component that travels slowly may on the other hand owe its low R value either to a low partition coefficient and little or no adsorption, or to a higher partition coefficient counter-balanced by adsorption. It so happens that vitamin B_{12} falls into the second category and the accompanying brown pigments of liver extract into the first; these pigments are nearly insoluble in butanol and not appreciably adsorbed on silica.

A particular sample of this brown material was taken, which had twice been submitted to partition chromatography, each time remaining in the

[9] Martin and Synge, *Biochem. J.*, 1941, **35**, 1358.
[10] Sanger, *Biochem. J.*, 1945, **39**, 507.
[11] Strain, *Anal. Chem.*, 1949, **21**, 75.
[12] Levi, *Biochem. J.*, 1948, **43**, 257.
[13] Moyle, Baldwin and Scarisbrick, *Biochem. J.*, 1948, **43**, 308.
[14] Blackburn, *Nature*, 1949, **163**, 955.

top few mm. of the column despite prolonged development. This material was then submitted to adsorption chromatography on the same solid, silica, from aqueous solution. It now travelled with an R value of about 1·2, while just above this brown zone appeared a faint pink flush due to a trace of vitamin B_{12} that had not been completely separated from it by partition chromatography; the position of the vitamin B_{12} on the column was confirmed by microbiological assay of three adjacent sections. Thus the relative positions of the vitamin B_{12} and the brown pigments were reversed on changing from partition chromatography (assisted by adsorption) to adsorption chromatography on the same solid with one of the same liquids. Secondly, as mentioned above, propanol containing up to 25 % of water will give excellent partition chromatograms on silica, separating suitable liver extracts into at least three coloured bands. Yet if the water concentration of the developing solvent is increased appreciably above 25 % all these zones are washed quickly and indiscriminately from the column. Thus only when conditions permit the existence of two liquid phases does this system function as a useful chromatogram.

In the course of chromatographic fractionations of liver extract we have had repeated evidence of the separation of two red pigments. Two pink zones have often separated on silica partition chromatograms, especially on rechromatographing a fairly clean fraction. Reproducible separation can, however, be achieved more reliably by using as base starch equilibrated with moist air, with butanol 9/10 saturated with water as mobile phase. The two bands then separate well apart, even though they are rather diffuse, with R_F values of about 0·5 and 1·2. The faster component corresponds with the crystalline vitamin B_{12}, the other has not yet been obtained pure.

It is worth noting that this separation fails if the starch is allowed to become too dry, or if development is hastened by applying pressure or suction to the top or bottom of the column respectively. It may be presumed that the rather large molecule of vitamin B_{12} (molecular weight about 1600 in the hydrated state) requires appreciable time for the successive redistributions between the two phases to approach equilibrium as development proceeds.

The relationship between these two red pigments is not yet established, but it is probably close; solutions of the two pigments of equal colour intensity are also of approximately equal potency, both microbiologically and clinically. A curious feature of their chromatography is the incompleteness of the separation. Thus on a silica partition column two bright pink zones appeared, separated by several cm. of white silica. Yet on eluting and rechromatographing, the major fast zone again splits into fast- and slow-moving components. The fast component had to be rechromatographed a third time to free it completely from the other. These experiments were carried out by Dr. K. Fantes. The incompleteness of the original separation in this and in other similar experiments was confirmed by chromatographic analysis of eluates from the two pink zones on filter-paper strips.[15] The components separated on the paper strips were located and approximately assayed by placing the strips on large plates of suitable nutrient agar inoculated with *L. lactis* Dorner and measuring the maximum diameter of the zones of growth after incubation. It was clear from the results that each pink zone on the silica column contained 20 % or more of the "wrong" red factor. The probable explanation is loose association between the two factors.

The technique described above, of using a sensitive microbiological assay to locate the spots on a paper chromatogram, is interesting in that it probably represents the limit of sensitivity yet achieved. The separation of amounts

[15] Cuthbertson and Lester Smith, *Biochem. J.*, 1949, **44**, P. 5.

of vitamin B_{12} and the related factor down to about 10^{-9} g. can readily be demonstrated. Possibly even smaller amounts of "carrier-free" radioactive compounds could be separated and could then be located by radio-autography, but such separations do not appear to have been reported except in presence of relatively large amounts of carrier.

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HIGH MOLECULAR POLYMERS SEPARATION

BY STIG CLAESSON

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The difficulties associated with chromatographic separation of high molecular substances are discussed. Normally, the rate of adsorption is low requiring an extremely low flow-rate in order to reach equilibrium. The curves obtained by frontal analysis provide information about the composition of high polymer solutions, in special cases, the agreement with other methods of determining molecular weight distribution is good.

It is rather remarkable that very few papers [1-5] dealing with chromatography of high molecular weight substances have been published. If one considers the fact that almost all other types of compounds have been very thoroughly investigated by many different authors, it is even more striking that these of high molecular weight hardly have been studied at all, especially as most of our knowledge about such substances is based upon the use of new and powerful analytical methods.

There are several reasons why we know so little about the chromatography of high molecular substances. It is generally difficult to observe these colourless substances on the column or in the effluent and as they have a continuous distribution of molecular weights no discrete zones will be obtained and it is therefore not profitable to study a limited number of fractions in the effluent. Due to this continuous distribution of material on the column it is also necessary to have very well-packed columns as uneven fronts will cause great errors in the observed distribution of material.

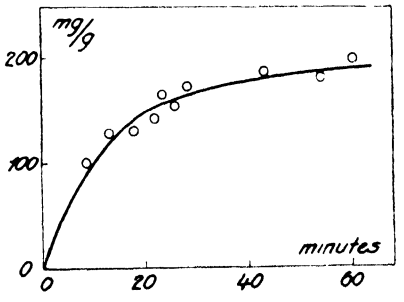

FIG. 1.

It is also mostly impossible to elute the substance quantitatively from the column getting good separation at the same time. Furthermore very little is known about the very complicated theory for chromatography of substances

[1] Mark and Saito, *Monatsh.*, 1936, **68**, 239.
[2] Levi and Giera, *Gazz. chim. ital.*, 1937, **67**, 719.
[3] Landler, *Compt. rend.*, 1947, **225**, 234.
[4] Claesson and Claesson, *Physic. Rev.*, 1948, **73**, 1221.
[5] Claesson, *Arkiv. Kemi, Min. Geol. A*, 1949, **26**, No. 24.

with continuous distribution of molecular species. However, even if very little quantitative work has been done in this field it seems of interest to discuss the little that is known at present as it may initiate further research.

The most important fairly well-established facts are the following. As can be expected the rate of adsorption is low when the molecular weight is high. The reason for that is certainly the low diffusion constant for such substances. A typical curve [6] showing the low rate of adsorption is shown in Fig. 1, where the amount adsorbed (mg./g.) is given as a function of time for the adsorption of a polyvinyl acetate of molecular weight 22,000 on activated carbon. That causes extra difficulties, as the rate of flow through

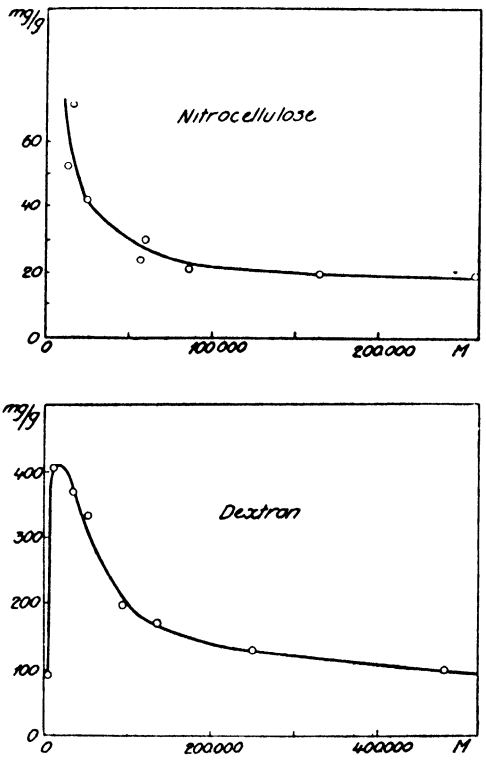

FIG. 2.

the column must be extremely low in order to reach adsorption equilibrium. Otherwise instantaneous equilibrium cannot be assumed in the theoretical treatment and the mathematical difficulties will then be unsurmountable.

The amount adsorbed per gram adsorbent is also generally small, but of course not lower than for many low molecular compounds without polar groups.

For a high polymer homologous series the amount adsorbed usually decreases with increasing molecular weight. That has proved to be the case for many different high polymers.[4 6] That is in marked contrast to the case of low molecular substances where the reverse is true. There must consequently exist a maximum adsorbability for an intermediate molecular

[6] Claesson and Claesson, *Arkiv Kemi, Min. Geol. A*, 1944, **19**, No. 5.

weight (most probably around $M = 10{,}000$). Two curves [4 7] showing the amount adsorbed (in mg./g. adsorbent) as a function of the molecular weight M for 1·5 % solutions of nitrocellulose in acetone and dextran in water on activated carbon are given in Fig. 2.

Some experiments have been carried out by the present author in order to investigate the possibilities of using chromatography for a determination of the composition of solutions of high polymers. It is evident that such an investigation has to be arranged with care in order to get useful results in spite of the difficulties mentioned above. In order to avoid the trouble with incomplete elution only frontal analysis was used. The concentration of the solution leaving the filter with adsorbent was followed by measuring

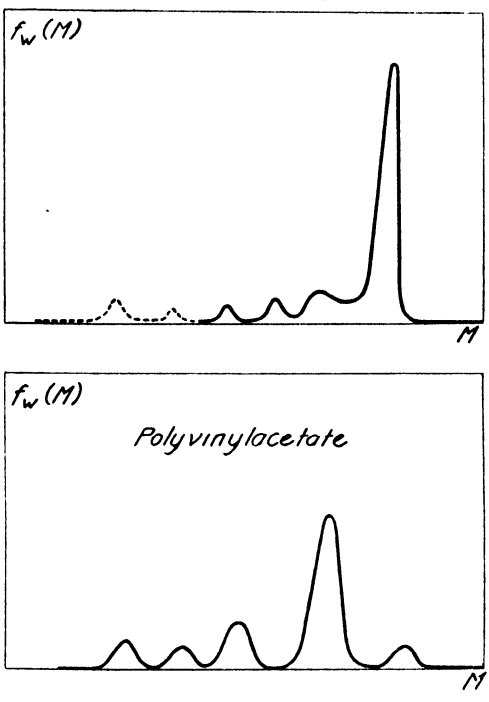

FIG. 3.

the refractive index by a micro-interferometer in the way described by Tiselius and the present author.[8] In order to reach sufficient accuracy (better than 10^{-6} in refractive index) a new improved interferometer was constructed which had a 400 mm. long cuvette. Activated carbon (usually about 1 g. Carboraffin) mixed with three parts by weight of Supercel was used as adsorbent. The system with coupled filters [9] (the solution passes from a filter with a large to a filter with a smaller diameter through a mixing chamber) was used to get good fronts. The rate of flow was kept very low (0·1–0·2 ml./min.) in order to be close to adsorption equilibrium. The most difficult part of the problem is, however, to avoid the use of a complicated

[7] Ingelman and Halling, *Arkiv Kemi*, 1949, **1**, No. 10
[8] Tiselius and Claesson, *Arkiv Kemi, Min Geol B*, 1942, **15**, No 18.
[9] Claesson, *Arkiv. Kemi, Min. Geol. A*, 1947, **24**, No. 16.

theory to compensate for the fact that the different molecular species may displace each other. It has in fact been demonstrated by separate experiments with fractions of high polymers that this effect is fairly large. An attempt to avoid this difficulty was made by using very dilute solutions where such effects are smaller. The diagrams were then extrapolated to zero concentration. This procedure seems to be very useful in general; there is, however, no guarantee that it must give a correct result if it cannot be verified by other means. As very little is known about the adsorption isotherms of high polymer fractions at the present time it was thought that this procedure was the best possible way of getting any results at all. Experiments have therefore been carried out at a series of total concentrations down to 0·02 % and extrapolated to zero concentration. It was found that very reproducible results could be obtained in this way which showed very little variation with changes of rate of flow up to 0·3 ml./min. It is quite clear that a diagram from a frontal analysis under such conditions where displacement effects and non-equilibrium are disregarded directly will give information about the composition of the high polymer solution. If a curve of the type given in Fig. 2 is used to correlate molecular weight and retention volume, the result can easily be expressed as the molecular weight distribution function.

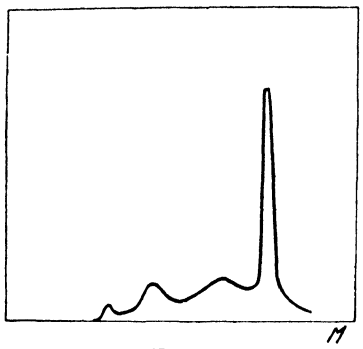

FIG. 4.

Two typical curves obtained in this way are given in Fig. 3 showing the extrapolated curves for a solution of polymethyl methacrylate in acetone and a solution of polyvinyl acetate in acetone. The curves are given as frequency distribution functions.

The great number of marked features on these curves is very remarkable. It is hard to believe that this should be caused only by a very complicated molecular weight distribution. It is more probable that other factors come into play—e.g., different branching of the chain, nature of end-groups, the order of the groups in a copolymerizate and so on. Neither can it be excluded that some components in the diagram are low molecular weight impurities. However, it is obvious that chromatography will give such very detailed information about the composition of high polymer solutions that almost no other analytical method for high polymers can compare with it.

Much still remains to be done in this field of chromatography. One of the most obvious things to try is to use mixed solvents [3 5] (a solvent and a precipitant) to increase the adsorption. This may cause a decrease in selectivity as the larger molecules will be more affected than the smaller ones, but it will be compensated for if such weak adsorbents can be used in this way that quantitative elution is possible. A study of the properties of different adsorbents and particularly the use of partition chromatography will certainly bring forward new results. It will also be very interesting to carry out more experiments with well-fractionated materials of different kinds to get more detailed information about the adsorption properties of such substances.

We do not know to-day if chromatography can give information about molecular weight distribution in general. The agreement with other methods is, however, very good in special cases. Fig. 4 gives the molecular weight

distribution determined [10] from fractionated precipitation and viscosity measurements for the same polymethyl methacrylate as in Fig. 3 and the general shape of these two curves is very similar. Chromatography therefore seems to be an extremely powerful method also for the study of high molecular weight substances, so promising indeed that it may very well happen that this new branch of chromatography in the near future will prove to be one of the most important applications of Tswett's original brilliant idea.

Fysikalisk-Kemiska Institutionen,
Uppsala.

[10] Kinell, *Acta Chem Scand.*, 1947, **1**, 749

GENERAL DISCUSSION

Dr. G. H. Beaven (*Thornton Research Centre*)* said: Work carried out in collaboration with C. N. Thompson and L. F. Foster, which has been submitted elsewhere for publication, has shown that the high affinity of activated silica gel for aromatic hydrocarbons generally can be utilized for the efficient separation of heavy petroleum fractions into total saturate and total aromatic fractions. The technique which we have devised is very similar to that described by Smit [1] and uses *iso*pentane as the initial solvent which conveys the total saturates through the column, followed by, e.g., ether to desorb the total aromatic fraction. Working on an " analytical " scale with sample weights of *ca.* 20 g. and a 30 in. × 1 in. column of silica gel (100–200 mesh), essentially complete recoveries can be obtained from distillates and semi- or completely-refined products, and their total saturates and total aromatics estimated to ± 1 % or better. This method has been most used in the spindle-oil range, which contains material in the molecular weight range *ca* 200–300, but it is also applicable to petroleum fractions extending from gas oils to heavy lubricating oils.[2 3 4] The adsorption analysis of gasolines has been described by several American workers,[5] while Lawrence and Barby [6] have shown that, using alumina as the adsorbent, the method is also applicable to residual oils containing asphaltic material, so that the entire range of liquid petroleum fractions is susceptible to adsorption analysis.

The practical problem of determining the point at which all the saturates have been eluted is best solved by the empirical method, described by Smit [1] and by Reichstein and Shoppee,[7] of continuing elution with a given solvent until the solute content of the eluate becomes negligible. This is conveniently done by using a suitable eluant sampling schedule (or an automatic fraction-cutter) together with some form of rapid solvent-removal device. Refractive index and specific dispersion measurements are also of great value in distinguishing between saturate and aromatic fractions, but it is often difficult to select definite values of these characteristics to indicate the transition point, owing partly to some degree of fractionation within the saturate fraction itself (cf. Smit [1]), and also because of the anomalous refractive indices of some hydrocarbons of mixed structural type. The freedom from aromatics of the total saturate fraction obtained in the manner described above may be confirmed by fluorescence and ultra-violet absorption tests.

[1] This Discussion.
[2] Dinneen *et al.*, *Anal. Chem.*, 1947, **19**, 992.
[3] Lipkin *et al.*, *Anal. Chem.*, 1948, **20**, 130.
[4] Fred and Putscher, *Anal. Chem.*, 1949, **21**, 903.
[5] Cf. Mair *et al.*, *J. Res. Nat. Bur. Stand.*, 1945, **34**, 435; 1944, **32**, 165.
[6] This Discussion.
[7] This Discussion.

* Present address: M.R.C. Spectrographic Unit, The London Hospital, E.1.

For the separation of the aromatics into several fractions rather than a single bulk fraction, our work has fully confirmed the value of fractional elution recommended by Smit and other contributors to this Discussion. We have used up to five solvents, e.g., *iso*pentane, carbon tetrachloride, ether, acetone and benzene (in this order), but carbon tetrachloride followed only by ether is found to give almost complete elution of the aromatics of light lubricating oils and related products. By fractional elution some separation of the total aromatic fraction

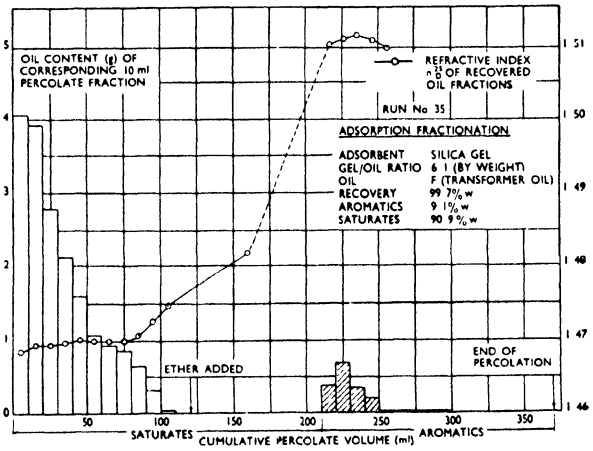

FIG. 1.—Adsorption fractionation.

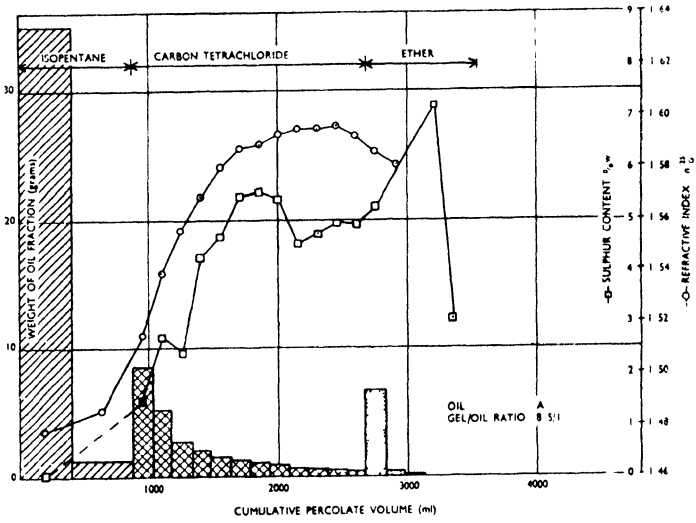

FIG. 2 —Three-solvent desorption analysis.

into different aromatic types can be achieved, as shown by the progressive increases in the refractive indices and specific dispersions of the successive subfractions, but the complete separation of, e.g., monocyclic and dicyclic aromatics, which is possible with pure hydrocarbons,[1] has not yet been definitely observed with petroleum products. The problem is made more difficult by the simultaneous segregation of the sulphur compounds, which are mainly associated with the aromatic fractions during adsorption analysis.[6]

Fig. 1 shows a typical separation of a transformer oil into a large total saturate fraction and a small total aromatic fraction; the small transition fraction of intermediate refractive index may be noted. Fig. 2 shows a 3-solvent preparative-scale separation of a crude spindle-oil distillate into a bulk saturate and two aromatic fractions; the variation in sulphur content of the individual aromatic sub-fractions is also indicated. These two examples serve to show the value of adsorption analysis, even in a simple form, for the study of petroleum fractions.

Our work has also confirmed the observation by Smit [1] regarding the photosensitivity of some oil fractions when adsorbed on silica gel. In this connection it may be noted that Ewles [8] has found that water and several polar organic liquids show unexpected fluorescence and phosphorescence characteristics when adsorbed on silica gel and other inorganic solids. These characteristics must presumably be associated with alterations in the light-absorbing properties of the compounds to the extent that in the adsorbed state they can be excited by radiation of much longer wavelength than that required to excite the same compounds in bulk. A similar alteration in light-absorbing properties may be responsible for the photosensitivity of adsorbed petroleum hydrocarbons to visible light.

Dr. A. S. C. Lawrence (*Sheffield*) said: Black oils are, as their name suggests, black and include residua from the distillation of petroleum and mixtures of such residua with heavy distillates. The black colour is due to the fraction which we isolate as ω. Since the paper was written, ultimate analyses have been made of fraction 57 with the following results:

Fraction	C	H	H/C	O	S	N	*Ash*
α	84·3	12·7	1·79	3·0	—	—	—
β	83·3	11·9	1·72	4·5	—	—	0·3
γ	82·9	9·3	1·345	4·05	2·15	—	0·2
δ_1	82·5	8·2	1·195	6·2	1·9	—	1·2
δ_2	80·7	8·1	1·275	8·9	1·7	—	0·1

These results suggest the rather startling conclusion that the higher fractions of petroleum oil contain no hydrocarbon. The literature is extraordinarily lacking in results of ultimate analyses of all petroleum products. It is considered that the easy elution of fractions α and β indicates that the oxygen is present in non-polar form and it is obviously not very important from the point of view of fractionation of hydrocarbon types if these very large molecules contain a few –C–O–C– or –C–S–C– links in place of –C–C–C–. However, the higher resinous fractions show signs of polar properties such as their stronger adsorption on alumina, spreading on water, efficiency as water in oil emulsifying agents and flocculation changes with pH. These properties all suggest that some at least of the oxygen is present in polar groups.

The hydrogen/carbon atomic ratios show considerable divergence from the theoretical value of nearly 2 for the paraffin and cycloparaffin types which should be α and β fractions, and from 1 for the γ aromatic one. The results of the chromatographic fractionation suggest that these divergencies are due to mixing of types in the molecules rather than to any limitations of the method.

It may be noted that the oxygen-containing resins appear to be quite different from the coal tar pitch resins which are also distinguished by high oxygen content. These are easily oxidized by alkaline permanganate to colourless water-soluble products but our γ fraction is not so oxidized.

Although our products are considerably unsaturated, no sign of irreversible adsorption was observed. The literature contains numerous references to the irreversible adsorption of olefins. It is suggested that the preliminary wetting of the alumina by pure solvent, which gives remarkable evolution of heat, so reduces the heat of adsorption of olefins that polymerization does not occur; that is, that irreversible polymerization, when it does occur, is a thermal effect. A number of patents for improving oils by filtration through alumina (bauxite) include wetting of the alumina by water.

[8] Ewles and Farnell, *Proc. Physic. Soc. A*, 1949, **62**, 216.

Prof. A. Tiselius (*Uppsala*) said : In Dr. Cassidy's paper, as well as in several other contributions to this Discussion, the use of active carbon in adsorption chromatography has been exemplified. We have had considerable experience with this adsorbent in my own laboratory, and I would like to point out that, although it has great advantages in its specificity and large adsorption capacity, it has the disadvantage of showing some irreversible adsorption (or slow desorption), particularly with strongly adsorbed substances. In his summing-up of the first section of this Discussion, Dr. R. L. M. Synge mentioned some experiments in which this difficulty had been partially overcome by pretreating the carbon with stearic acid, which blocks the most active patches on the carbon surface. Nevertheless, losses often occur on account of this irreversible adsorption, particularly in elution, and also to a certain extent in displacement analysis.

Dr. A. J. P. Martin (*Medical Research Council Unit, London*) said: Displacement partition columns for acids or bases, the column being loaded with a base or acids, have potentially very large capacities. Probably concentrations in the range of 0·5 to 2 N can be used on the support. A theory for such columns has been given.[9]

The scope of partition chromatography can also be extended to substances with partition coefficients in favour of the non-polar phase by treating silica, precipitated or in the form of kieselguhr (Celite or Hyflo Supercel), with dimethyl dichlorosilane. The silica will then hold the less polar of a given phase pair. With a hydrocarbon on the silica, e.g., medicinal paraffin or octane, and an aqueous solution of acetone or methanol as the mobile phase, it is possible to separate fatty acids differing by two carbon atoms, e g., lauric, myristic, palmitic and stearic acids.[10]

Dr. E. Lederer (*Paris*) said : Cassidy and Nestler have rightly stressed the difficulties encountered in the purification of carboxylic acids by adsorption chromatography. I wish to report a case of the successful application of chromatography to the purification of high-molecular biologically important carboxylic acids

TABLE I

SEPARATION OF α- AND β-MYCOLIC ACIDS (1585 MG. ON 32 G. ALUMINA ACTIVITY II) [11]

Eluting Solvents	Eluate (mg)	M.p. (° C)
a 150 ml. benzene	14·5	—
b, *c* 300 ml. dry ether	4	—
d 150 ml. ether	8	—
e 150 ml. ether 0·1 % acetic acid	322·5	55–56
f 200 ,, ,, 0·1 % ,, ,, ...	571	55–56
g 200 ,, ,, 0·1 % ,, ,, ...	268	55–56
h 200 ,, ,, 0·3 % ,, ,, ...	194	57–58·5
i 200 ,, ,, 0·5 % ,, ,, ...	74	—
k 200 ,, ,, 1·0 % ,, ,, ...	63	62–64
	1519 (96 %)	

Mycolic acid is a branched hydroxy-methoxy acid isolated by Stodola and Anderson [12] from the wax of a human strain of *Mycobacterium tuberculosis* and to which the authors assigned the formula $C_{88}H_{176}O_4$ (mol. wt. 1298) ; this is the only acid-fast substance found in the tubercle bacillus. The salts of mycolic acid are soluble in organic solvents, even in petroleum ether, so that it cannot be separated from neutral substances by the usual extraction with aqueous alkali from an organic solvent.

[9] Martin, *Biochem. Soc. Symp.*, No. 3, 1949.
[10] Howard and Martin (unpublished).
[11] Asselineau and Lederer, *Compt. rend.*, 1949, **228**, 1892.
[12] Stodola, Lesuk and Anderson, *J. Biol. Chem.*, 1938, **126**, 505.

In a recent paper, Asselineau and Lederer [13] have described the chromatography of mycolic acid on alumina. Mycolic acid is strongly adsorbed and can be quantitatively eluted by ether containing 5 %–10 % of acetic acid. By this method it was easy to show that different strains of mycobacteria contain free mycolic acid (up to 4 %).

Thus purified, mycolic acid can then be split into two isomeric components $C_{88}H_{176}O_4$, which we have called α- and β-mycolic acids. Table I shows such a separation which uses increasing amounts of acetic acid in ether to elute the two isomers separately. This may be regarded as a displacement development.

We have isolated isomeric forms of mycolic acid from a bovine strain of *Mycobacterium tuberculosis*. The mycolic acid of the B.C.G. strain which was said to have a molecular weight of only 770 can be easily split by chromatography into a neutral part (m.p. 75°) and an acid (m p. 54°–58°) having a molecular weight of about 1000. (Unpublished results.)

After a second, similar chromatography, fractions *e*, *f*, *g* give α-mycolic acid, m p., 55°–56°, and fractions *i*, *k* give β-mycolic acid, m.p. 71°–73°.

Prof. L. Zechmeister (*California*) said: I agree with Prof. Tiselius that the main difficulty in protein chromatography is the recovery of the protein from its adsorbate and the reliable check of its unaltered state. However, this difficulty can be overcome when the protein studied shows well-defined enzymatic (or serological ?) properties. Then an unchanged enzyme potency offers sufficient proof in the direction mentioned. In pertinent experiments carried out by Dr. Tóth and others in our laboratory some unexpected variations in the adsorption behaviour of enzymes were observed upon the repetition of the chromatographic experiment. On the basis of the important paper under discussion, it seems possible that these variations were caused by an alteration of the salt content in the initial, crude enzyme extracts during the first adsorption process.

Dr. R. Consden (*Maidenhead*) said: One of the most important applications of paper chromatography is in the separation of peptides from partial hydrolyzates of proteins. The number of peptides may be so large that preliminary separations are essential before paper chromatography can be used to the best advantage. Anion-exchange resins have been used for the separation of acidic from non-acidic material, and ionophoresis has separated the acidic fraction into groups of similar mobilities, each group then being examined by paper chromatography. In this way, some twenty dipeptides of glutamic and aspartic acids have been identified in partial hydrolyzates of wool, and twelve dipeptides of cystine, after converting them to cysteic acid peptides. Only a few lower peptides of neutral or basic amino acids have been identified. These are more difficult to investigate than acidic peptides. The neutral peptide fraction may contain many constituents, which are not easily separated in ionophoresis. Many of them may be too fast running on paper chromatograms with the usual solvents. Basic peptides often do not behave satisfactorily on paper chromatograms and often do not deaminate readily. The problem of adequately separating and analyzing basic and neutral peptides is therefore one which requires further attention.

Dr. E. Lederer (*Paris*) said: Dr. Jones mentions his attempts to isolate γ-aminobutyric acid from molasses; Dr. Dent (London) has told me that he also has difficulties in isolating this substance from potato juice. I wish to report a method of isolating ω-amino acids which may be of interest in this respect (to be published with Pavlov).

Some years ago, I have shown with Tchen [14] and then with Jutisz [15] that β-alanine is adsorbed on acid alumina in presence of 10 % formol, just as are glycine, serine and threonine.[16] We had explained the preferential adsorption of glycine by sterical considerations and had written "Si vraiment l'effet du formol sur l'acidité du glycocolle est dûe à la présence d'un $-NH_2$ lié à un carbone primaire

[13] Asselineau and Lederer, *Bull. Soc. Chim. biol.*, 1949, **31**, 492.
[14] Lederer and Tchen, *Biochim. Biophys. Acta*, 1947, **1**. 35.
[15] Jutisz and Lederer, *Biologie et Médecine*, *No.* 5 (*Symposium sur les protéines*), p 239
[16] See Schramm and Primosigh, *Ber.*, 1943, **76**, 373.

nous devons trouver le même phénomène chez tous les acides aminés en ω." The first example was β-alanine and we have now extended these experiments to γ-aminobutyric, δ-aminovaleric and ε-aminocaproic acids Adsorption of these ω-amino acids on acid alumina, in presence of 10 % formol, is very dependent on pH. On using columns washed with water until the effluent has a pH of 4·0 (± 0·1), up to 95 % of the above-mentioned amino acids are adsorbed on the column and can be quantitatively eluted by hot water which dissociates the formol-amino acid complex.

Dr. Jones mentions a paper of Proom and Woiwod [17] in which the authors have come to the " pessimistic " conclusion that in a great number of bacteria examined no new amino acid has been found. We wish to report that, on chromatographing on paper a hydrolyzate of the hydrosoluble portion of a lipo-polysaccharide isolated from *Mycobacterium tuberculosis var. hominis*, we got three spots, two of which are due to glutamic acid and alanine, the third being chromatographically identical with the spot No. 17 which Work [18] has isolated from the proteins of *Corynebacterium diphtheria* It seems that Dr Synge has isolated the same substance from the bacteriæ of the rumen of sheep (private communication). This new compound seems to be a neutral, aliphatic amino acid. We have only found it in the above-mentioned lipo-polysaccharide and not in the proteins of the tubercle bacillus.[19]

There are now available different methods of group separations of amino acids.[20] In a paper with Fromageot and Jutisz [21] we have described in detail such a method, using silica gel for adsorbing the basic amino acids, acid alumina for adsorbing the acidic ones and a special carbon (Activit 50 XP) for the aromatic amino acids. We have checked that this method gives quantitative results also for simple synthetic di- and tripeptides.

On studying recently the behaviour of a synthetic tetrapeptide, kindly put at our disposal by Prof. Prelog (Zurich), we found a somewhat unexpected result. The peptide in question is lysyl-glycyl-glycyl-glutamic acid and thus has a positive charge on one end and a negative charge on the other end of the chain. This substance would probably prove electrophoretically neutral, but it is adsorbed on silica gel as well as on acid alumina. It thus behaves in these group separations as if both charges were distinctly separated.[22]

Dr. J. A. V. Butler (*London*) said: I should like to supplement Dr. Tudor Jones's examples by referring to two uses of chromatography in separating peptides with which I have been concerned. The first was the separation of the smaller peptides produced by the action of chymotrypsin. This was a complex mixture [23] of about 15 peptides of molecular weight of the order of 600. Paper chromatography with phenol and collidine gave, on developing with ninhydrin, a series of coloured bands which merged into each other. When material eluted from different parts of these bands was run again it gave rise to a similar series of coloured bands, so that it seemed that, for example, the portion which ran rapidly at first was distributed between slow- and fast-running fractions in the second run. However, using butanol-acetic acid mixtures two-dimensionally, a separation of about 17 spots with distinctive colours was observed [24] and the separation of this rather complex mixture now looks rather promising with this solvent.

The second example is the concentration of the toxic substance produced by the action of nitrogen trichloride (agene) on flour. It has been shown that the toxic substance is produced by action on the protein constituent of flour. A number of groups have been working [25] on the isolation of the toxic substance

[17] Proom and Woiwod, *J. Gen. Microbiol.*, 1949, **8**, 319.
[18] Work, *Biochim. Biophys. Acta*, 1949, **3**, 400.
[19] Asselineau, Choucroun and Lederer, *Biochim. Biophys. Acta* (in press).
[20] See the paper of Dr. Jones.
[21] Fromageot, Jutisz and Lederer, *Biochim. Biophys. Acta*, 1948, **2**, 487.
[22] Jutisz (unpublished results).
[23] Butler, Dodds, Phillips and Stephen, *Biochem. J.*, 1948, **42**, 116.
[24] Phillips, *Biochim. Biophys. Acta*, 1949, **3**.
[25] Reiner *et al.*, *Fed. Proc.*, 1949, **8**, 230. Butler and Mills, *Nature*, 1949, **163**, 835. Bentley, Moran *et al.*, *Nature*, 1949, **163**, 675; 1949, **164**, 438.

and their methods, so far as they have been published, involve the digestion of the protein with pancreatin, followed by a chromatographic fractionation of the products. Acid- and base-exchange resins permitted the separation of the fractions rich in acid and basic amino acids, and activated alumina was used to remove some of the neutral amino acids. The selectivity of chromatographic methods is very strikingly illustrated by this work, in which gallons of digest are passed through large columns, in order to obtain finally a very small quantity of the active substance.

Dr. A. J. Woiwod (*Beckenham, Kent*) said: The factors responsible for the progressive loss of amino nitrogen as an amino acid passes down the paper must be determined if the colorimetric copper method is to be of use for more than comparative work. If failure of the amino acid to reach equilibrium with the mobile phase is the reason for the loss, then slowing up the rate of running by suitable choice of paper and solvent may effect an improvement. Miettinen [26] reports that very satisfactory separations are obtained by such slow running.

Gordon suggests that a more likely explanation of the loss is reaction with material in the paper. Fowden [27] has found, using the elution technique of Consden, Gordon and Martin,[28] that the longer a paper strip with an amino acid on it is washed the less the apparent recovery as measured by the colorimetric copper method. Using glycine the recovery fell to 51 % after 24-hr. washing. Washings from paper blanks did not affect the reaction between copper and sodium diethyldithiocarbamate and presumably the reaction between copper and amino acid was the one affected The material responsible may perhaps be a metal forming complexes with amino acids of such stability that it cannot be displaced by copper.

Estimates of the sensitivity of the fluorescent detection technique should be accepted with caution as paper has been found to vary markedly in its ability to permit fluorescence of amino acids. With good papers fluorescence appears to rival ninhydrin in sensitivity in detecting amino acids and peptides.

Dr. K. W. Pepper (*Teddington*) said: I should like to amplify the reference made by Dr. Partridge to the use of sulphonated polystyrene resins of different degrees of cross-linking. The results obtained emphasize the importance of polymer structure as a parameter in ion-exchange chromatography and thereby confirm the findings of Kunin and Myers with anion-exchange resins (this Discussion).

Cation-exchange resins were prepared by sulphonating the products obtained from the copolymerization of styrene with different proportions of divinyl benzene. The decrease in swelling resulting from increased cross-linking is shown in the Table.

TABLE I

C.R.L. Reference no.	Nominal DVB Content	Capacity (m.mole/g. dry H^+ resin)	Swelling (g. water/g. dry H^+ resin)	% Volume shrinkage of H^+ form in water to		
				1 N HCl	2 N HCl	5 N HCl
2088	4 %	5·30	1·80	10	15	25
2168	5 %	5·30	1·47	5	12	20
2169	10 %	5·25	0·82	0	4	10

The performance of the above resins in the attempted separation of the more basic amino acids was compared directly, using similar conditions for each run. (Full details of the procedure will appear elsewhere.) After regeneration, the columns were first saturated with 0·1 M glycine hydrochloride in order to minimize changes in ionic strength, which cause shrinkage of the resins and disturbance

[26] Miettinen, *Proc. 1st Int. Congr. Biochem.*, 1949, 229.
[27] Fowden (personal communication).
[28] Consden, Gordon and Martin, *Biochem. J.*, 1947, **40**, 590.

of the column. The test solution containing leucine, histidine, lysine and arginine (0·15–0·2 M concentration) was added and subsequently displaced with 0·15 N NaOH (rate of progression of Na^+ boundary, 5 cm./hr.).

The flowing chromatograms obtained by Dr. Partridge are shown diagrammatically in Fig. 1. The influence of polymer structure is strikingly demonstrated

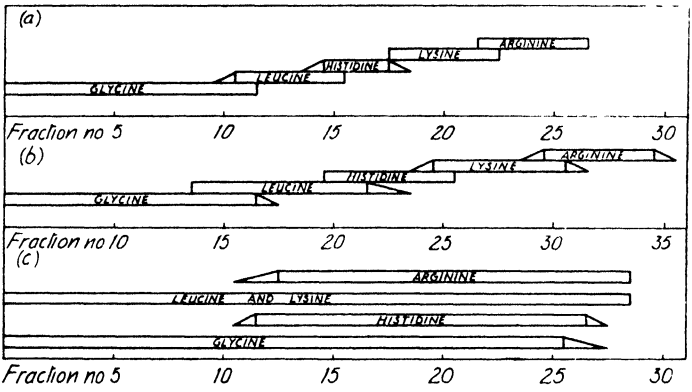

FIG. 1.—Flowing chromatograms obtained by displacing the more basic amino acids from sulphonated polystyrene resins of different degrees of cross-linking.

(*a*) Sample No. 2088 (4 % DVB).
(*b*) Sample No. 2168 (5 % DVB).
(*c*) Sample No 2169 (10 % DVB).

in that good separations were obtained with the lightly cross-linked resins, whilst the resin containing 10 % DVB was ineffective. A highly cross-linked commercial resin of this type (swelling, 0·75 g. water/g. dry H^+ resin) also proved to be ineffective.

SUMMARIZING PAPER

BY A. J. P. MARTIN

Received 19th October, 1949

General.—The renaissance of chromatography that has occurred during the last decade has been principally in its extension to water-soluble substances; most of the earlier successful applications being of relatively non-polar substances which were eluted from the adsorbent by such solvents as alcohols. It is noteworthy that in the papers above concerned with the separation of hydrocarbons, carotenoids, steroids and fatty oils, the classical type of adsorbent and solvent is still found to be the most suitable. In the same period, understanding of the ion-exchange properties of variously treated alumina has permitted the separation of many inorganic ions and of various amino acids and their derivatives. Absorbent clays and natural zeolites have also found employment, but the greatest extension has been with synthetic resins deliberately made for their ion-exchange properties. Tiselius and his school have shown how use can be made of the curved adsorption isotherms characteristic of active charcoal in frontal analysis and displacement chromatograms; and so catholic is the adsorption affinity of carbon that, at least in frontal analysis, the method seems applicable

to all classes of soluble substances. Partition chromatography has shown itself capable of separating an extremely wide range of substances, particularly those of biochemical interest. It is perhaps not fanciful to suggest that the success of, say, the butanol-water system for many metabolic products is because they are transported to the cell, and within it, in an aqueous medium, and they have also to pass lipid membranes.

Specificity.—The question of specificity of the adsorbent for particular substances or classes of substances has been raised several times, but it is clear that work and ideas on this subject are still in their infancy. So far as metals are concerned a number of more or less specific reagents which form complexes are available, and these can be used directly as in the 8-hydroxyquinoline columns, or as an adjunct in partition or ion-exchange columns. That such molecular groupings should be incorporated in synthetic resins is an attractive idea, though perhaps one not easy to achieve.

An interesting method, not mentioned in the Discussion, is that of Hamoir.[1] Using Ag_2S treated so that the surface was covered with Ag^+ ions, he found that amino acids which formed insoluble silver salts, or complexes with silver, were preferentially adsorbed; similar results could be shown with $BaSO_4$. The ideal of preparing an adsorbent specific for a particular substance has been attempted by Dickey [2] by precipitation of silica in the presence of the molecule to be preferentially adsorbed, but it is as yet too early to show how widely this can be applied. The high specificity shown by enzymes and antibodies in nature, which indeed inspired the method, is an example of what should ultimately be possible. As a converse of this argument it would seem to be reasonable to attempt to purify enzymes upon columns of the substrate, or some appropriately modified substrate, which should of course for preference be insoluble. The writer has no concrete example except perhaps starch, cellulose or other insoluble polysaccharide, or indeed protein in mind. But no doubt if it could be shown to work in any individual case, other more speculative examples could be tried.

Crystallization as a Method of Purification.—It seems relevant at this point to recall that the process of crystallization can be regarded as a special case of adsorption, where the adsorbent remains unaltered, no matter how much solute is adsorbed. In very many cases the specificity is extraordinarily high; classical chemistry has used this method of purification more than any other. No doubt the specificity of the outside unimolecular layer is not as high as within the crystal, since only one side has to fit, and as the crystal grows many of the "wrong" molecules are displaced, since their fit will become progressively worse as they become more nearly enclosed; the slowness of crystallization in complicated mixtures may well be attributed to this necessity for displacement. It is curious that though fractional crystallization has been a long-practised method, no one has published a fractional crystallization method employing a column arrangement. The writer wishes to suggest the following scheme;[3] it cannot perhaps be called a chromatogram though some resemblance is obvious. The apparatus would consist of a long tube packed with some filter aid material, kieselguhr, paper fibres, glass wool, asbestos, etc. Over a fraction of the length of the tube a temperature gradient would be imposed, as by a metal block, hotter at one end than the other. A solvent, in which some at least of the solutes had a high temperature coefficient of solubility, would flow down the tube in the direction hot to cold, and the tube itself be moved through the

[1] Hamoir, *Biochem. J.*, 1945, **39**, 485.
[2] Dickey, *Proc. Nat. Acad. Sci.*, 1949, **35**, 227.
[3] The writer has since heard that Dr. J. Bowman, of the Mellon Institute, has used essentially this method for the separation of waxes.

temperature gradient in the opposite direction. Solid would be placed originally at the hot end of the tube and would be carried to the colder part by movement of the solvent and deposited. The filter aid material would prevent its being carried along the tube as solid. As the tube moved through the temperature gradient the solid would redissolve to reprecipitate again, in approximately the same position relative to the temperature gradient. Continuous recrystallization should thereby be possible with minimal losses in solvent and vessels. Where a number of substances were present, which did not form mixed crystals, only the least soluble would be obtained pure. By a curious paradox where mixed crystals were formed, substantial amounts of each substance should be pure, with regions of overlap as in displacement chromatography. Where temperature coefficients are too small to make this method useful, conceivably gradients of salt concentrations or pH could be devised.

Using paper strips in place of the packed tube, an essentially similar method utilizing evaporation of the solvent suggests itself. A long dry strip of paper would pass through a region where the vapour pressure of the solvent gradually rose to a reservoir of solvent, the flow of solvent in the opposite direction being due to capillarity, with or against gravity. A steady state should eventually be established where substances put on to the paper would collect at characteristic places in the region of the gradient. A similar distillation analogue of the gas-phase chromatogram is obvious.

Application of the Chromatogram to Large-scale Separations.—The quantity of material handled on a chromatogram is usually but a small fraction of the mass of the chromatogram itself, and with elution development solutions are normally very dilute. As a result the application of the chromatogram for industrial-scale separation has been limited to special cases, as in water softening where high dilutions are no disadvantage, or where valuable products are concerned. However, with displacement chromatograms relatively high concentrations can be used, and it can be predicted confidently that they will ultimately find wide application. For many industrial processes continuous operation is very desirable. A continuous chromatogram can be envisaged where an adsorbent is forced up a tube counter-current to the solvent with the substances to be separated introduced somewhere between the ends of the tube. The difficulties of obtaining uniform packing and flow of materials are, however, so formidable that no practical arrangement of this type has yet been published. Perhaps if the adsorbent were made in the form of long fibres, e.g., by incorporation in a viscose thread, an endless rope, untwisted or lightly twisted, could be drawn with acceptable uniformity of packing through a column and through appropriate eluting baths to return the rope to its starting condition to re-enter the column.

An idea rising from a discussion with Prof. Tiselius and Dr. Synge during this Discussion can also enable chromatography to be a continuous process, provided that the developing solvent returns the adsorbent to its initial state within a reasonable period. Imagine the chromatogram to be packed within a narrow annular space between two concentric cylinders. The upper surface of the chromatogram is flooded with solvent and at one point the solution to be separated is fed on slowly. The annular chromatogram is slowly and uniformly rotated with the result that different zones will form helices of characteristic angle which can be collected at various fixed points around the bottom of the chromatogram.[4] The annular chromatogram

[4] The writer has since heard that this scheme is already being tried out by Dr. Wadman, of the University of Bristol.

could of course be replaced by a circular array of chromatogram tubes without essential differences, and perhaps less practical difficulty.

Application of the Chromatogram to Separation of Micro-quantities.—Scaling down the chromatogram should also be considered. There is perhaps no essentially chromatographic problem here. It is primarily a matter of detection of the substances separated. With radioactive substances this offers little difficulty, and biological methods can detect as little as 10^{-10} g. vitamin B_{12} after separation on chromatograms. Nevertheless, using the colour reactions conventional in paper chromatography, replacement of the paper strip by a single yarn of cotton (or perhaps viscose) should result in the reduction of scale by probably a factor of one hundred in the amount of solute required. For ease of observation it would probably be convenient to wrap the yarn round a rod about 1 mm. diam. before or after development of the colour. Another possibility of detection lies in collecting the effluent from the thread in short lengths of thermometer tubing, and determining the amount, after freeze-drying within the tube by absorption of soft X-rays. It should be possible to detect at any rate 10^{-9} g. per sample, and by the use of characteristic wavelengths, following Engstrom,[5] to do an approximate elementary analysis. Observation of the refractive index, so convenient in larger columns, is difficult if the column diameter is reduced to a fraction of a millimetre, but a total reflection method may be applicable since only a thin layer of liquid is required. It should be noted that there is a peculiar gain obtained with very narrow columns, with frontal analysis or displacement development, that lateral diffusion will permit no irregularity of flow to upset the sharpness of front. Hence, provided the observational difficulties could be overcome, micro-columns might well give better analytical results than larger columns.

Chromatographic Separation of Large Molecules.—So few studies of chromatographic separations of large molecules have as yet been made that difficulties tend perhaps to be overestimated. Claesson has shown that in spite of slow adsorption and difficulty of elution characteristic of large molecules on carbon, impressive results can be obtained by frontal analysis of high polymers. So far as partition chromatography is concerned, the principal difficulty is likely to be in finding solvent systems in which partition coefficients are not extremely large or small, since as a general rule, for molecules of the same type, the higher the polymer the more it will favour a given phase. Where suitable solvents exist, and kieselguhr can be used as a support for the stationary phase, there is every reason to believe that very large molecules could be successfully separated. It is improbable that cellulose, starch or even precipitated silica would be as effective as kieselguhr as a support, owing to molecular-sieve effects. For these may be expected, not only to reduce adsorption capacity of the column, but also, particularly in the region where the sieve effect is becoming important, to slow down the attainment of equilibrium, and thus reduce the resolving power of the column. On the other hand, with materials such as starch and cellulose, solvents consisting of mixtures of two miscible components may be used and the difference between the stationary and mobile " phases " will more often be small enough to permit of " partition coefficients " of convenient values even for large molecules.

The separation of proteins by chromatography is greatly complicated by the instability of many native proteins. Denaturation seems to occur on most of the common adsorbents though perhaps the use of low tempera-

[5] Engstrom, *Acta Radiol.*, Suppl. LXIII, 1946.

tures to prevent this has not yet been fully explored. There do not appear to be any immiscible phase pairs in which hæmoglobin possesses both stability and a convenient partition coefficient. Even a small percentage of denaturation on passage from one phase to another would quickly, in a chromatogram, result in total destruction, and in a partition chromatogram accumulation of denatured protein at the surface of one phase would probably render this surface impermeable to native protein. The success with which proteins can be crystallized, however, suggests that other more insoluble proteins might be non-denaturing adsorbents.

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AUTHOR INDEX *

Angell, F. G., 47.
Barby, D., 255.
Barker, K. H., 199.
Barrer, R. M., 135, 162.
Beaven, G. H., 325.
Boldingh, J., 162
Brockmann, H., 58.
Burstall, F. H., 179.
Butler, J. A. V., 330
Cassidy, Harold G., 259.
Claesson, Stig, 11, 34, 321.
Consden, R., 329
Cook, C. D., 153, 240.
Crisp, D. J., 161.
Davies, C. W., 158, 241.
Davies, G. R., 179.
Davison, B., 45, 49
Duncan, J. F., 52, **104,** 154, 156, 158.
Elbeih, I. I. M., 183.
Flood, H., 190.
Glueckauf, E., 12, 47, 50, 53, 154, **199,** 238, 239, 240
Gordon, A. H., 128.
Hale, D. K., 79, 160
Heymann, E., 161.
Hirst, E. L., 268.
Jones, J. K. N., 268.
Jones, Tudor S. G., 285.
Kitchener, J. A., 90, 155, 159.
Kitt, G. P., 199.
Klinkenberg, A., 151.
Kressman, T. R. E., 90.
Kunin, Robert, 114.
Lawrence, A. S. C., 255, 327.
Lederer, E., 328, 329.
Leigh, T., 311.
Lessing, R., 240.
Lester Smith, E., 317.
Levi, Alfred A., 124.
Lister, B. A. J., 104, 154, 156, 237.
Martin, A. J. P., 151, 328, **332.**
McOmie, J. F. W., 183.
Mongar, J. L., 118.
Myers, Robert J., 114.
Nestler, F. H. Max, 259.
Offord, A. C., 26, 51.
Partridge, S. M., 296.
Pepper, K. W., 331.
Phillips, C. S. G., 241.
Pollard, F. H., 183, 238.
Reichenberg, D., 79, 160.
Reichstein, T., 305.
Richardson, R. W., 159.
Robertson, R. H. S., 163.
Robinson, F. A., 168.
Robinson, G., 195, 238, 239.
Sacconi, Luigi, 173, 238.
Schwab, George-Maria, 170.
Shepard, Charles C., 275.
Shoppee, C. W., 305.
Smit, W. M., 38, 48, 240, **248.**
Spedding, Frank H., 214.
Stewart, A., 65, 163
Strickland, J. D. H., 157.
Synge, R. L. M., 163, **164.**
Tiselius, Arne, 7, 151, 162, 239, **275,** 328.
Tompkins, Edward R., 232.
Wassermann, A., 118, 160.
Weiss, D. E., 142.
Weiss, Joseph, 26, 52, 53.
Wells, R. A., 179, 238
Williams, K. A., 264.
Wolwod, A. J., 331.
Zechmeister, L., 54, 151, 239, 329.

* The references in heavy type indicate papers submitted for discussion.