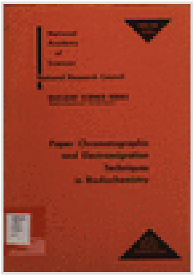

Paper Chromatographic and Electromigration Techniques in Radiochemistry (1962)

Pages
58

Size
6 x 9

ISBN
0309364965

Bailey, R. A.; Subcommittee on Radiochemistry; Committee on Nuclear Science; National Research Council

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Paper Chromatographic and Electromigration Techniques in Radiochemistry

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Troy, New York

Issuance Date: June 1962

Subcommittee on Radiochemistry
National Academy of Sciences—National Research Council

Printed in USA. Price $0.50. Available from the Office of Technical Services, Department of Commerce, Washington 25, D. C.

FOREWORD

The Subcommittee on Radiochemistry is one of a number of subcommittees working under the Committee on Nuclear Science within the National Academy of Sciences - National Research Council. Its members represent government, industrial, and university laboratories in the areas of nuclear chemistry and analytical chemistry

The Subcommittee has concerned itself with those areas of nuclear science which involve the chemist, such as the collection and distribution of radiochemical procedures, the establishment of specifications for radiochemically pure reagents, availability of cyclotron time for service irradiations, the place of radiochemistry in the undergraduate collége program, etc.

This series of monographs has grown out of the need for up-to-date compilations of radiochemical information and procedures. The Subcommittee has endeavored to present a series which will be of maximum use to the working scientist and which contains the latest available information. Each monograph collects in one volume the pertinent information required for radiochemical work with an individual element or a group of closely related elements.

An expert in the radiochemistry of the particular element has written the monograph, following a standard format developed by the Subcommittee. The Atomic Energy Commission has sponsored the printing of the series.

The Subcommittee is confident these publications will be useful not only to the radiochemist but also to the research worker in other fields such as physics, biochemistry or medicine who wishes to use radiochemical techniques to solve a specific problem.

W. Wayne Meinke, Chairman
Subcommittee on Radiochemistry

INTRODUCTION

This volume which deals with paper chromatographic and electromigration techniques is the sixth in a series of monographs on radiochemical techniques which will parallel the series on the radiochemistry of the elements. The same general style is used in both series of monographs, including general reviews of the technique, discussion of the principles involved, a survey of applications to different systems, and finally a collection of selected procedures which use this technique as reported in the literature.

This second series of techniques monographs will cover a number of radiochemical techniques which have not been reviewed elsewhere. Plans include revision of these monographs periodically as new information and procedures warrant. The reader is therefore encouraged to call to the attention of the author any published or unpublished material on paper chromatographic and electromigration techniques which might be included in a revised version of the monograph.

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Paper Chromatographic and Electromigration Techniques in Radiochemistry

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INTRODUCTION

Both paper chromatography and electromigration in paper (called variously paper electrophoresis or ionophoresis, ionography, electrochromatography) have been applied seriously to problems of inorganic separations only comparatively recently, and both are largely empirical. While they appear to have widespread utility, their practical employment in the inorganic field has not kept pace with that in some other branches of chemistry. Although superficially similar, these two techniques differ fundamentally. Paper chromatography is based on partition of the substances to be separated between a fixed and a mobile phase, and hence the separation depends upon different distribution coefficients. Electromigration depends on the different velocities of the substances under an electric field, so that in principle the stationary solid phase (paper) serves merely to prevent mixing by convection, while the liquid phase is also stationary and serves to keep the migrants in a mobile form with a suitable charge. In practice, adsorption effects and liquid flow may be important, but they are not essential to the success of the method generally. In certain arrangements, a combination of paper chromatography and electromigration has been realized; only these should receive the name "electrochromatography."

The succeeding discussion will emphasize technique and applications with regard only to the inorganic field. The use of tracers in problems of organic and biochemical interest is becoming widespread, but will not be dealt with specifically here. Many of the techniques described in the standard references to this subject may be applied directly in these cases.

PAPER CHROMATOGRAPHY

A. Introduction

The foundations of modern paper chromatography can be traced back to the work of Goepellsroder and Schoenbein who, in the 19th century, found that various substances rose to different heights when solutions were allowed to ascend in filter-paper. This was developed as Kapillar analyse. The method was used in its present form to give complete separations only much later, however, when it was taken up by protein chemists in the 1940's. Since that time, a very large amount of literature has appeared describing separations in all fields, including inorganic chemistry. A number of text-books are available on the subject, many of which give details of the history.

Most text-books emphasize organic or biochemical applications, often treating the inorganic field very superficially. The following books are of most value to inorganic workers; they contain more or less detailed descriptions of a number of separations as well as a thorough coverage of experimental methods and theory.

"Chromatographische Methoden in der Analytischen und Praparativen Anorganischen Chemie", E. Blasius, Ferdinand Enke Verlag, Stuttgart, 1958.

"Chromatography", E. Lederer and M. Lederer, Elsevier Publishing Co., Amsterdam, 2nd. ed., 1957.

"A Manual of Paper Chromatography and Paper Electrophoresis", R. J. Block, E. L. Durrum, and G. Zweig, Academic Press, New York, 2nd. ed., 1958.

"Chromatographic Methods of Inorganic Analysis", F. H. Pollard and J. F. W. McOmie, Butterworths Scientific Publications, London, 1953.

"Progrès Récents de la Chromatographie, Troisième partie; Chromatographie sur Papier des Radioelements", M. Lederer, Hermann et Cie., Paris, 1956.

Although primarily organic in outlook, a particularly good discussion of the paper and other factors is to be found in:

"Fundamentals of Chromatography", H. G. Cassidy, Interscience Publishers, Inc., New York, 1957.

A discussion of various Whitman filter papers can be found in:

"A Guide to Filter Paper and Cellulose Powder Chromatography", J. N. Balston and B. E. Talbot, H. Reeve Angel and Co. Ltd., London, 1952.

B. Theoretical Considerations

It is generally held that the process which is responsible for the success of paper chromatographic separations is partition between a mobile organic phase and a stationary aqueous phase supported by the hydrophilic paper. The latter may be adsorbed water, or more probably a water-cellulose "complex" (1,2,3); extraction of water by the paper effectively splits the solvent into phases. This mechanism is in keeping with the fact that the most successful solvents are wet organic liquids. Paper chromatography thus is analagous to separation by a multiple solvent-extraction process. However, other factors, such as ion exchange and true adsorption, frequently may play a part. For instance, paper possesses a small but definite ion exchange capacity due to the presence of carboxyl groups formed by oxidation of the cellulose. The actual importance of these other mechanisms is not certain.

The quantity usually stated when describing the behaviour of a substance in this process is the R_f factor, which is defined as

$$R_f = \frac{\text{distance moved by the spot of substance}}{\text{distance moved by the advancing front of the solvent}}$$

From analogy with fractional distillation at total reflux (4) or by a simple kinetic treatment (Lederer and Lederer, p. 105), it can be shown that

$$R_f = \frac{A_L}{A_L + \alpha A_S},$$

where A_L is the cross sectional area of the mobile phase, A_S that of the stationary active phase, and α is the partition coefficient;

$$\alpha = \frac{\text{concentration in the stationary phase}}{\text{concentration in the mobile phase}} .$$

Values of R_f are useful in comparing the behaviour of various ions provided the measurements are carried out under strictly identical conditions, but the actual values obtained are quite sensitive to minor changes in experimental technique. In addition to tabulations given in the aforementioned texts, the section on chromatographic data of the Journal of Chromatography also includes R_f values for many systems.

Wilson (5), DeVault (6), and Weiss (7,8) have dealt with the theory of chromatography mathematically. Although developed for columns, the results are equally applicable to paper strips. The book by Blasius gives a complete summary of this work.

C. Techniques

Three basic types of procedures are in common use in paper chromatography; these are ascending, descending, and circular chromatography. In the ascending technique, the solvent is allowed to rise in the paper by capillary action. It may be carried out with extremely simple apparatus. Small scale work may be done in large stoppered test tubes with the paper suspended from the stopper, or cut in a wedge-shaped form such that the widest part is at the top and holds the rest of the strip in position with the lower end dipping into the solvent. A glass rod may be used as a simple means of supporting one or more strips in a larger cylinder; rubber stoppers on each end wedged against the wall hold the rod at the desired height. The paper may be clipped to the rod, or hung across it with both ends dipping into the solvent so that the two chromatograms may be run at once. Work on a still larger scale may be carried out in a suitable vessel by rolling a paper sheet into a cylinder and sewing or stapling the edges together. In this way, the paper stands upright on the bottom of the container, and, if a sufficiently strong grade is used, it may be self-supporting. Alternatively, a glass supporting frame may be introduced. This approach is suitable when several runs are to be made side by side. If a tank is available, square or rectangular sheets may be used in place of strips.

In the descending technique, the upper end of the paper is dipped into a trough of solvent, which then flows down the strip. The upper end is held in place by the weight of a glass rod, and the paper is usually hung over a so-called anti-syphon rod, rather than directly over the edge of the trough, to give a better flow. This method has the advantage of being somewhat faster than the ascending technique, and longer strips can be used if necessary. In the latter, the useful solvent rise is limited to 30 - 40 cm.

Horizontal development is also possible with strips or sheets, but it is not used commonly.

With both of the above methods, the mixture to be separated may be started at one corner of a sheet of paper so that after chromatographing in one direction the sheet may be dried and chromatographed again with a different solvent at right angles to give a two dimentional process.

It has sometimes been found useful for material with small R_f values to use multiple development - the strip is dried and then re-chromatographed in the same direction with the same solvent. A zone containing two or more unseparated substances may be cut out of the rest of the chromatogram, sewed to another strip, and re-chromatographed with a different solvent.

With both of the above techniques the material to be separated is applied as a spot or streak of solution near one end of the strip of paper, and dried. This end is then dipped into the developing solvent so that the spot is a centimetre or so above the solvent. The initial spot may be kept small by applying the solution in small amounts and drying between each addition, but too high a concentration in the spot must be avoided for this leads to trailing. (Trailing means the formation of very long zones, usually because of a region of low concentration extending back towards the starting point.) A large initial zone is better than a small one which is too concentrated.

It is often useful to run several spots side by side for purposes of comparison. These can be separated by slots in the paper if necessary. Known substances run as standards in this way may be helpful in identifying the separated zones.

In the circular technique, the substances to be chromatographed are placed at the centre of a circle of filter paper and the developing solvent spreads horizontally from this point. The resulting zones form a series of concentric circles. Solvent flow may be brought about by means of a wick dipping into a dish of liquid below the paper, or by flow through a capillary from a reservoir above. By rotating the paper at high speed, the flow of solvent may be accelerated to increase the speed of a separation.

The circular technique has advantages from the point of view of speed, even when not centrifugally accelerated. It also yields very narrow zones. However, from a radiochemical stand-point, detection of the zones becomes a problem if counting techniques are used. Isolation of a single zone is also more difficult. Two-dimensional techniques and other procedures utilizing odd shapes of paper offer similar problems; they may be quite satisfactory if chemical tests or autoradiography are used for detection, but simple scanning cannot be employed, and even "counting" segments becomes inconvenient. Therefore, procedures involving strips are to be preferred.

In paper chromatography the solvent moves through the paper by capillary action to bring about the movement of the applied materials. This step is termed the development of the chromatogram, and should not be confused with the detection step which is also sometimes called "development" when colour tests are employed.

Whichever technique is used, the paper must be kept in an atmosphere saturated with vapour from the solvent, or at a particular humidity, and time must be allowed for the paper to come to equilibrium with its surroundings before development is started. The importance of this depends on the solvent system used. In some cases it is not necessary, but in others - particularly with water-miscible solvents such as acetone - it seems to be vital if satisfactory results are to be obtained. The initial spot must be dried fully. Proper conditions are set up by having excess solvent present in the chromatographic chamber, and it is helpful to line the walls with thick paper which dips into excess solvent. Since the system is always disturbed when the vessel is opened, it is advantageous to introduce the developing solvent through a small aperture after equilibrium has been reached. With small volumes of solvent and a large chamber, the possibility of changing the composition through evaporation must be kept in mind.

A large selection of chromatographic apparatus is available commercially. However, perfectly suitable set-ups for most purposes can be made from equipment usually present in a laboratory.

D. Experimental Considerations

1. Temperature. Temperature should be kept reasonably constant, especially when organic solvents saturated with water are used. Changes in temperature from that at which the solution was prepared can then alter the solubility relationships very markedly, giving rise to anomalous results. In particular, a decrease in temperature may lead to "waterlogging" of the paper. Higher temperatures lead to an increased rate of solvent flow and also to increased diffusion with resultant spreading of the zones. In special cases, where it can influence the solubility of a migrant or hasten the attainment of chemical equilibrium, an increase in temperature may be beneficial. Muller and Clegg (9) have studied the influence of temperature, as well as other factors, in paper chromatography.

2. The Paper. A large number of different kinds of filter paper are available which may be used for chromatography, although only a few types have received widespread application. Most of these are available especially prepared "for chromatography." It has not yet been determined what distinguishes a good chromatographic paper from a poor one, but in choosing a

type for a separation, a number of obvious factors, such as uniformity, the presence of impurities, the wet strength, the thickness, and the flow rate, should be considered.

While it is advisable to inspect the paper used for thick or thin regions, most chromatography paper is extremely uniform.

The amount of inorganic impurities may be appreciable, particularly in some qualitative papers. These consist chiefly of alkali metals, calcium, magnesium, iron, and silicates. Acid washed papers are appreciably lower in mineral content; iron and silicate make up the largest part. The importance of such impurities depends on the nature of the work. With carrier- free materials they may be quite troublesome since in certain cases they may interact to precipitate traces of the migrants. Various means of purifying the paper have been used. The wash process employed may involve elution over a long period of time, or a batch process with frequent changes of wash liquid. It is often advisable at least to wash the paper thoroughly with the solvent to be used, followed by careful drying. Treatment with dilute HCl, followed by distilled water, removes most inorganic impurities, but a more thorough purification can be obtained with solutions of complexing agents such as alcoholic 8-hydroxyquinoline. The complexing agent must be carefully washed out as well. Contamination from laboratory vapours may be avoided by storing the paper in a 50-50 mixture of acetone and alcohol and drying just before use. Such washing procedures may alter the fibres physically, and often result in higher flow rates than are found with the original paper.

A high wet strength is a desirable feature of the paper used, but not a necessary one unless a good deal of handling of the wet chromatogram is required. The various hardened filter papers are good in this respect, and are usually low in impurities besides.

The thickness of the paper largely determines the amount of material which can be handled in this process. A thick paper obviously will hold more material than a thin one for a spot of equal size.

The flow rate varies greatly with the type of paper and, in some cases, is faster parallel to the machine direction than across it. (The machine direction is usually indicated on chromatography paper). A fast flow rate results in a more rapid separation, but, if it is too fast, equilibrium of the solute between the fixed and the moving phases may not be set up and the zones may be poorly defined.

Properties of various filter papers are collected in the books by Blasius and Balston and Talbot, while that by Cassidy deals extensively with the factors influencing the choice of paper.

The paper may be impregnated with other adsorbents such as alumina (10), or with reagents such as 8-hydroxyquinoline (11). Papers impregnated with cation or anion exchange resins, as well as those chemically modified to have a large ion exchange capacity, have been prepared and are now available commercially. (The H. Reeve Angel Co., for instance, supplies a complete line). Blasius (p. 228) gives references to a large amount of work using impregnated and chemically treated paper. Other porous materials may also be used in this process; glass fibre paper and asbestos paper seem particularly promising. The latter has given good results with the alkali metals, for example (12,13).

The above materials usually function by adsorption or ion exchange, and permit these types of chromatography to be carried out with the convenience of using a paper strip. In general, these aspects of inorganic paper chromatography have not been investigated extensively.

3. <u>The Developing Solvent</u>. The selection of a solvent material for a new separation is almost entirely an empirical procedure. Some guidance may be obtained from the known behaviour of given complexes, and perhaps from solvent extraction data, but analogy with the latter procedure must not be carried too far. In many cases small variations in the concentration or acidity of the solvent mixture can have a marked effect on the results. This is probably due largely to the influence on complex formation and stability. In most instances, the solvent consists of an organic liquid containing some water (often saturated) and, in many cases, also an acid such as HCl or a salt. Butanol saturated with various concentrations of HCl is a widely used

example. Organic bases are also useful. Often a complexing agent is present, although frequently the organic solvent or the added electrolyte takes this role. Some success has been obtained with non-aqueous solutions, mineral acids, pure liquids, and even fused salts, but these are all unusual solvents so far, at least.

A gradient elution technique (continuous change of solvent composition during development) has been used by Lederer (14) in conjunction with an ascending technique. The solvent was stirred magnetically while acid was added to it from a buret at a rate determined by the rate of rise in the paper.

There is an almost unlimited variety of solvent systems which could be useful, and, in view of the present theoretical knowledge of the process, very little generalization or prediction of behaviour can be made. The book by Pollard and McOmie gives a list of many of the solvents used up to September, 1952, while later books, such as Blasius, and Lederer and Lederer, include more recent work.

4. The Migrant Solution. The behaviour of a given substance may be dependent on the other substances present. In particular, acids or complex forming agents in the original mixture may influence the chromatogram markedly. Materials which are not considered to react with one another in other circumstances may effect each other, presumably by changing the solubilities in the two phases, but this is not often important.

Also important is the concentration of material in the initial spot. If this is too great the spot "trails" or streaks. The final size of the zone (as distinct from shape) depends on the amount of material present, and, in some cases, it has been proved possible to use this fact for quantitative estimations. This has been reviewed by Giddings and Keller (15). The extreme sensitivity of radiochemical methods of detection sometimes shows trailing of small amounts of material which is not revealed by ordinary chemical tests. In work with "carrier-free" substances this may become so extreme that no movement occurs at all, while with larger quantities, using chemical means of detection, motion appears to take place normally. Examples of this have been given by Lederer in "Progrès Récents de la Chromatographie, III me partie," and other examples are given in Lederer and Lederer, p. 528. Trailing or lack of motion of small amounts of material may be due to some irreversible adsorption process by the paper, or precipitation by traces of impurities. Reduction by aldehyde or other groups in the paper may also take place in some cases. This difference in behaviour between tracer and larger amounts of material has seldom been encountered in strong acid solutions, but chiefly with weak acids and complexing agents. Fortunately it does not seem to be widespread, but it must be kept in mind if separations of "carrier-free" amounts are based on work with inactive substances.

5. Double Fronts. With some solvent mixtures two liquid fronts are observed. This results from take-up of water from the solution by the cellulose. Then, the leading front is formed by the dry organic phase, while the second, which may be an appreciable distance behind, is formed by the real developing mixture. Adsorption of other components can give rise to other fronts similarly, but in a properly equilibrated system it is only adsorption of non-volatile components that is likely to result in this phenomenon. Such fronts are often visible on the dry chromatogram as dark bands caused by impurities in the paper.

6. Multiple and Distorted Zones. It is found occasionally that one ion will yield two or more distinct and well separated zones on chromatographing. Such behaviour can be explained by the existence in solution of more than one species which are not undergoing rapid chemical exchange with one another, and occurs in some complexing systems. Other causes of two zones where one remains stationary are partial precipitation of the migrant at the starting point by other substances or by hydrolysis, or partial conversion to an insoluble form on drying of the original spot, but these are the result of faulty technique rather than inherent in the system.

The usual form of a spot in paper chromatography is circular or elliptical, but distortions often occur. Theoretically, the distribution of substance in the direction of motion is expected to be gaussian about the centre of the spot. Excessive elongation or trailing may be due to too much material present or to reaction with the paper or impurities as discussed above. Flattening often occurs if the R_f value is large. Other forms such as crescents or shapeless smears are also encountered, but the causes are not well understood.

E. Detection of Separated Zones

Detection of the separated zones has been accomplished in most instances by spraying the dried chromatogram with reagents giving suitable colour tests; occasionally the paper has been exposed to a gas or dusted with a solid chemical. Many detection agents are given in the books by Pollard and McOmie, and by Blasius. Other methods which may be used particularly for quantitative measurements depend upon the change in capacitance of two plates as the ionic zone of the strip is brought between them, resistance changes, photometry, etc. The more recent text-books, such as Lederer and Lederer, should be consulted for details and references.

For radiochemical purposes, however, it is likely that radioactivity would be used to detect the zones, since this is most sensitive and also can be used to identify the zone by its nature, energy, decay period, etc. Most of the usual methods of detecting radioactivity can be used. Autoradiography has been employed, and is probably most useful with circular or two-dimensional work, but normally a counting technique is much preferable. The simplest approach is to cut the strip into segments, say one centimetre broad, and measure each with any instrument suitable for the radiation emitted. This is tedious when many strips are involved, even if the zones can be roughly localized beforehand, and a scanning device is very useful. Many designs have been proposed and several are available commercially. Detectors may be Geiger counters, thin window or windowless gas-flow proportional counters, or scintillation counters. The principle in all is the same: the paper chromato gram is moved slowly past a slit in front of the detector, the output of which is recorded on a chart. The speeds of the chromatogram and chart are synchronized, and if there is some reference point on each, peaks on the chart can be related to areas of activity on the paper. Winteringham et al discuss detection and other problems in radioactive chromatography (16), and also quantitative scanning (17); a review on quantitative radio-paper chromatography has been published by Pocchiari and Rossi (18). Numerous references to various designs of scanners are included in the book by Lederer and Lederer.

Inactive materials have been detected by neutron activation of the entire chromatogram and subsequent use of radiochemical techniques.

F. Measurement and Recovery of Separated Material

For many radiochemical purposes the separated substances need not be removed from the paper, but may be measured in situ after cutting away the unwanted regions. This is certainly the case if gamma rays are measured, or if only half-life determinations are required. Reasonably accurate quantitative measurements of beta emitters are possible with a properly calibrated technique (19). Quantitative scanning has already been mentioned (16,17).

If removal of the material from the paper is required, the paper may be ashed, or an elution technique adopted. Lederer has used both methods in the paper chromatographic isolation and purification of several "carrier-free" activities (Progrès Récents de la Chromatographie, IIIme partie). The elution procedure is undoubtedly best, although some materials are very strongly retained by the paper. The region of paper containing the wanted substance is cut out and eluted by descending chromatography with a suitable solvent - perhaps water. The eluant is collected as it drips from a point at the lower end. Another procedure is to form a loose roll from the desired portion of the paper and extract in a soxhlet type of apparatus (20). Svendsen (21) has described a method of eluting material directly from the paper to a planchet for "counting." If the required substance moves with the solvent front, it may be collected directly by elution with the descending technique. The strip need only be long enough to ensure separation, and may be quite wide at the upper end to keep the slower moving constituents in a narrow band, but tapering sharply to a point at the lower end (22). In principle, all moving zones could be eluted successively this way as in column work, but this usually is not practical.

G. Large Scale Work

In order to increase the amount of material which can be handled, the "chromatopack" (23) and the "chromatopile" (24) have been devised. The former consists of a number of sheets of paper clamped together; the latter, of a stack of paper discs in a suitable holder. These are usually developed by ascending chromatography.

A more convenient way of working with larger quantities is to use a column of cellulose powder. The technique is then the same as for ordinary columnar chromatographic methods, using the solvents of paper chromatography. Several grades of cellulose powder are available commerically for this purpose in both qualitative and "ashless" forms, although filter paper can be converted to a suitable form by boiling with dilute HNO_3. Cellulose powder tends to show greater adsorption powers than does filter paper itself, so that it may not always be possible to apply a paper chromatographic solvent system to column work without modifications. The book by Lederer and Lederer deals with a number of inorganic separations by this method (p. 521).

H. Examples of Radiochemical Separations

Hundreds of separations of inorganic ions by paper chromatography have been reported, and while most of them have used inactive material, they are all potentially applicable to radiochemical problems. For the latter it may be necessary to remove the bulk of the target material by some simple chemical means before chromatography in order to avoid over-loading the paper. Most of the books listed in the introduction include detailed descriptions of some inorganic procedures and references to many more; Blasius in particular gives a good tabulation. Frierson and Jones (25) have discussed the use of tracers in paper chromatography.

A few illustrative radiochemical separations are described briefly below, and some in more detail in Section J.

1. Purification of Tracers and "Carrier-free" Material. Lederer (Progrès Récents de la Chromatographie, III me partie) has used paper chromatography to separate a large number of isotopes in "carrier-free" form from targets. Most of them were the products of deuteron bombardment. Substances isolated include (with solvent system in brackets) Na^{22} from Mg (ethanol-20% H_2O), Pd^{103} from Rh (butanol-HCl), Rh^{102} from Ru (butanol-HCl), Fe^{55} from Mn (acetone-HCl-water), Zn^{65} from Cu (butanol-HCl), and U^{233} from Pa^{233} (ether-HNO_3). Ascending and descending techniques were used, and the times required ranged from 10 to 20 hours. Recovery was by elution or by ashing. In addition, purification of Sn^{113}, Au^{108}, Fe^{55}, and Co^{58} was also reported by the same technique.

In the same publication, procedures for the separation of a number of naturally occurring radioactive mixtures are described. These are U and Th; Th and Pa; Ba and Ra; and Pb, Bi, and Po.

A number of workers have reported paper chromatographic separations of RaD, RaE, and RaF (the elements Pb, Bi, and Po). Most successful as a solvent seems to be butanol saturated with 1N HCl (26, Lederer above), or with 3N HCl (27). Here, RaF has the largest R_f value, followed closely by RaE. RaF moves fairly slowly. Also successful has been a solvent composed of 80% acetone, 10% 1N HCl, and 10% 1N HNO_3 (28), and a solution made up of a mixture of 1:1 butanol-n-propanol shaken with 7N $LiNO_3$-2N HNO_3 (29).

Perey and Adloff have used this method to separate AcK from AcX and AcB (30), AcC" from AcB (31), and AcK from Ac (32). In each case, 10% $(NH_4)_2CO_3$ was used for the elution. The products were described as very pure.

Kiba et al (33) have used ethanol-10% aqueous NH_4SCN (5:2) in the separation of "carrier-free" Y^{90} from Sr^{90}. Appreciable amounts of Y interfere.

A related use of this technique has been in the identification of radiochemical impurities in pile produced activities (25,34).

2. Spallation and Fission Products. Carleson has used the technique

of paper chromatography to separate products of the deuteron spallation of copper (35,36,37), and found it to be very suitable. Separations were accomplished in 3 - 4 hours, using methyl n-propyl ketone - HCl as solvent. The separated zones were ashed for recovery and chemical yields determined colourimetrically.

Fission products have been studied with the help of this technique by a number of workers. Gotte and Patze (38,39) dealt with those in the group not precipitated by H_2S, and were able to separate various combinations with ethanol - HCl, ethanol - methanol - HCl, and ethanol - butanol - HCl. Matsuura (40) also investigated the fission products, using methanol - ethanol - ammonium thiocyanate, acetic acid - HCl, and methanol - HCl for different groups. A number of uranium fission products were separated with methylethyl ketone - HF as solvent by Crouthamel and Fudge (41). Raaen and Thomason (42) have made a detailed study of the separation of U^{233} from 32 other radioisotopes with 2-methyltetrahydrofuran containing 2.5% conc. HNO_3 and saturated with water.

For a review of separations of isotopes by chromatographic and electromigration techniques, see Chemla (43).

J. Some Procedures for Radiochemical Separations by Paper Chromatography

1. Spallation Products from Proton Bombardment of Copper Metal.

G. Carleson, Acta Chim. Scand., 8, 1697 (1954).

Experimental: Descending technique using Munktell O. B. paper purified to remove copper impurities. Solvents: methyl-n-propyl ketone + 15% 8N HCl for Co, Zn, Fe, and Cu; methyl-n-propyl ketone + 15% 10N HCl for Mn and Ni. Development time: 3 - 4 hours.

Procedure: The metal target was dissolved in HCl + H_2O_2, and the excess Cu removed by H_2S precipitation. The filtrate was evaporated to a small volume and equal amounts were placed on two paper strips with a micropipette. After equilibration for 15 min. in their respective chambers, they were developed. The separated zones were located by scanning, cut out, and ashed in a Pt dish for counting.

Relative movement: Mn > Ni; Fe > Zn > Cu > Co > Mn + Ni.

2. Separation of Zn^{65} from Copper Targets. M. Lederer, Anal. Chim. Acta, 8, 134 (1953).

Experimental: Ascending technique. Solvent: Butanol shaken with 1N HCl. Development time: 20 hours.

Procedure: Zn^{65} was produced by deuteron bombardment of copper. The target was dissolved in aqua regia, evaporated to dryness, and finally taken up in dilute HCl. Excess copper was precipitated with H_2S. The supernatant liquid was then applied to the paper and developed. Zn moved ahead of the copper.

3. Separation of Cs from Uranium Oxide Fuel. C. A. Crouthamel and A. J. Fudge, J. Inorg. Nuclear Chem. 5, 240 (1958); and C. A. Crouthamel, R. Heinrich, and C. Gatrousis, Talanta, 1, 396 (1956).

Experimental: Ascending technique, using 1/2" wide strips of Whatman No. 1, 2, or 3 MM. paper. Chambers were 29 mm. x 30 cm. polyethylene cylinders. Solvent: 60 g. of 49% HF per 100 ml. dry methyl-ethyl ketone. Development time: 3 - 5 hours. Yield of Cs was 100%.

Procedure: The uranium oxide was dissolved in conc. HNO_3 containing 1µ g. of Cs carrier/ml. The solution was evaporated to dryness with HF several times and finally taken up in 1:3 HF. Samples of 2 - 100 µ g. were placed on a 5 - 10 mm. diameter spot on the paper, air-dried, and developed. Detection was by means of the activity of the spot.

4. Separation of Carrier-Free Cs^{131} from $BaCO_3$. M. Lederer, Anal. Chim. Acta, 11, 528 (1954).

Experimental: Presumably ascending technique with Whatman No. 1 paper. Solvent: phenol saturated with 2N HCl. Development lasted over night.

Procedure: Barium carbonate was irradiated in a reactor. Removal of excess barium was achieved by dissolution in HCl, and precipitation of $BaCl_2$ with conc. HCl at 0°C. The mother liquor was twice concentrated until a volume of 0.2 ml. remained. (Leaching of the carbonate with H_2O removed only 4% of the activity.) This was applied to the paper and developed. Barium (as well as any strontium impurity) remained at the start. The Cs^{131} could be recovered by ashing the paper - very little residue remained.

5. Separation of Carrier-Free Na^{22} from Mg. M. Lederer, Anal. Chim. Acta, 8, 134 (1953).

Experimental: Ascending technique. Whatman No. 1 paper. Solvent: ethanol - 20% H_2O. Development time: 10 - 20 hours.

Procedure: Na^{22} was produced by deuteron bombardment of magnesium, which was dissolved as the chloride. The solution was evaporated to dryness and heated to convert most of the magnesium to MgO. This residue was then leached with water. (No Na activity remained in the insoluble matter.) The water solution was concentrated and about 0.2 ml. was streaked on the paper and developed. Detection was by scanning. Most of the Na^{22} travelled behind the Mg, and could be recovered pure.

6. Separation of Nb^{95} from Fission Products. A. J. Fudge, UKAEA Report AERE C/R 1502 (1954).

Experimental: Ascending technique, using Whatman No. 1 paper. Solvent: 100 ml. of 40% HF - 40 ml. methylethyl ketone. Development time: 20 min.

Procedure: A suitable aliquot of fission product solution was evaporated to dryness in a Pt basin with 1 ml. 40% HF, and dissolved in 1 ml. of 1:3 HF. A sample of 0.01 ml. was then placed as a narrow band on a strip of paper, with Nb^{95} standard on an adjacent strip. These were developed simultaneously.

The strips were exposed to NH_3 in a dessicator, dried, and sprayed with 0.2% tannin solution for detection. They were then re-dried and the coloured portions cut out and placed between 'Sellotape' for counting.

7. Preparation of Carrier-Free Rh^{102} from Ruthenium. M. Lederer, Anal. Chim. Acta, 8, 134 (1953).

This procedure was developed as an alternative final step to a standard procedure. It has the advantage of eliminating traces of iron carrier.

Experimental: Ascending technique, using Whatman No. 1 paper. Solvent: 20 ml. butanol + 10 ml. conc. HCl.

Procedure: The ruthenium target was dissolved by fusion in NaO_2, and RuO_4 was removed by volatilization. The Rh^{102} was isolated by co-precipitation with $Fe(OH)_3$. The precipitate was dissolved in HCl, then applied to the paper and developed. Rhodium exhibited an R_f value of about 0.1, while the iron moved with the solvent front. The former could be extracted with 2N HCl.

8. Separation of Carrier-Free Tellurium from Zirconium and (macro amounts of) Copper. A. S. Ghosh-Mazumdar and M. Lederer, J. Inorg. Nuclear Chem., 3, 379 (1957).

This procedure was developed: (a) to eliminate Zr^{95} contamination from carrier-free Te isolated from fission product mixtures, and (b) to separate the Te from inactive copper used in the preliminary steps of separation.

Experimental: Ascending technique, using Whatman No. 1 paper. Solvent: butanol saturated with 20% HBr.

Procedure: The solution containing Te, Cu (inactive), and Zr contaminant was evaporated to dryness and taken up in dilute HBr. It was twice evaporated to dryness to ensure the conversion of all tellurium to Te^{+4}. The final solution in HBr was applied as a streak on a 25 cm. wide strip of paper, and developed. The maximum amount of copper was 2 mg. since some streaking forward occurred. Te^{+4} moved near the solvent front (R_f = 0.96) with Cu close behind (R_f 0.69, but streaking forward). Zirconium (and any Te^{+6}) remained at the starting point. Tellurium could be recovered from the paper by extraction with 1:1 HCl.

9. Separation of Polonium from Bismuth, Platinum, Gold, Mercury, and Lead. G. W. Warren and R W. Fink; procedure taken from "The Radiochemistry of Polonium" by P. E. Figgins (NAS - NS - 3037 (1961)).

Experimental: Ascending technique, using Eaton - Dikeman 320 paper suspended in a tube from the stopper. The paper was purified by downward percolation of 1N HCl followed by water. Solvent: 20% n-butylphosphate - 80% acetone. Development of 5 - 6 inches (10 - 15 min.).

Procedure: The sample was evaporated to dryness and dissolved in H_2O. A maximum of 1 ml. of solution was distributed from a micro - pipette over a minimum of 10 cm. width of paper a distance 3 cm. from the end. After development, the polonium was extracted from the cut-out zone with aqua regia or with an organic solvent. The decontamination factor was 10^6.

R_f values: Au^{+3}, 1.0; Hg^{+2}, 0.90; Po^{+4}, 0.80; Pt^{+4}, 0.5; Tl^{+3}, 0.05; Bi^{+3}, 0.60; Pb^{+2}, 0.00.

10. Separation of Polonium from Bismuth Targets and from Selenium and Tellurium. C. E. Crouthamel and C. Gatrousis, Talanta, 1, 39 (1958).

This method was developed to separate Po^{210} produced by neutron bombardment of bismuth. In practice it may be necessary to remove the bulk of the bismuth by chemical means, and therefore tellurium is present as carrier.

Experimental: Ascending technique, using Whatman 3 MM paper in strips 2 cm. wide. A polyethylene cylinder was used as the chamber. Solvent: 15 ml. tert-butanol + 7.5 ml. isopropyl ether + 1.0 ml. conc. HCl if only Bi and Po present, or 60 g. 49% HF per 100 ml. methylethyl ketone for all four elements.

Procedure: The bismuth target was dissolved in aqua regia, and a sample applied to the paper strip; this was then air-dried before development.

Detection was by autoradiography or counting. Quantities of up to 0.3 mg. Bi and 0.3 mg. Te could be handled. (Po and Se were studied only in tracer amounts.)

In the HCl-based solvent, Bi remained at the start but formed a comet, while Po was much faster. In the HF-MEK solvent, Bi remained at the start, Po was about half-way along the strip, while Te was ahead of it and Se was near the solvent front.

11. Separation of Polonium, Bismuth and Lead; and Selenium, Tellurium and Polonium. M. C. Levi and J. Danon, J. Chromatog., 3, 584 (1960).

Experimental: Presumably ascending technique, using Whatman No. 1 paper. Solvent: 50% butanol - 50% propanol shaken with a solution of 7M $LiNO_3$ - 2M HNO_3. Development time: 18 hours (20 - 25 cm.).

This method gives a good separation of Po, Bi and Pb, and of Se, Te and Po. All five elements may be separated, but the Bi and Se zones overlap. (R_f values are: Pb, 0.27; Te, 0.47; Bi, 0.62; Se, 0.72; Po, 0.97.) Larger amounts of Pb and Bi do not interfere. A possible disadvantage is that if it is desired to extract any element, an appreciable amount of $LiNO_3$ may be mixed with it.

12. Separation of Actinium K (Fr^{223}) from Actinium and its Decay Products. J. P. Adloff, Thèsis, Université de Strasbourg, 1958. Taken from E. K. Hyde, "The Radiochemistry of Francium", (NAS - NS - 3003 (1960)).

Experimental: Descending development, using 1 cm. x 15 cm. strips of Schleicher filter paper. Solvent: 10% ammonium carbonate solution. Development was carried out in a chamber saturated with water vapour at 60°C. Development time: 15 min. (solvent movement ca. 8 cm.) from sources containing appreciable amounts of rare earths (total processing time ca. 30 min.), or as little as 6 - 8 min. if no rare earths are present.

Procedure: (a) If the source material is an actiniferous lanthanum solution, the AcK must be partially purified.

(1) $(NH_4)_2CO_3$ is added to precipitate Ac and rare earths.

(2) To the filtrate from step (1), Pb, Ba, and La carriers are added to strip the residual Ac, AcX, and RdAc as insoluble carbonates. This step is repeated once or twice.

(3) The filtrate is evaporated to a small volume and placed on the paper 2 - 5 cm. from the upper end; the chromatogram is then developed. AcK and AcC" move down the strip about 8 cm. in 15 min., while the remaining Ac, AcX, RdAc, etc., are insoluble in $(NH_4)_2CO_3$ and remain near the starting point.

(b) If the source contains little rare earth contamination, the following steps may be used:

(1) Ce(IV) carrier is added and precipitated with a minimum of NH_4OH under oxidizing conditions. This removes RdAc. (This step may require repetition.)

(2) A few mg. of $BaCl_2$ are added to the filtrate from step (1) and NH_4OH is added to precipitate actinium - rare earth hydroxides. The precipitate may be contaminated with AcX.

(3) The actinium is dissolved, the solution concentrated and applied to the paper. After AcK has grown back to near its equilibrium concentration (1 - 2 hours), it is developed.

Neither of the above variations will separate AcK - AcC"; if this is required, a precipitation step immediately before chromatography is necessary. This can be done by precipitating the AcC" with added Pb carrier as the sulfide in acetic acid medium. Ammonium carbonate remaining from steps (1) and (2) must first be destroyed by heating.

The AcK is easily washed from the paper, and the $(NH_4)_2CO_3$ may be destroyed by heating.

The position of the AcK can be determined by scanning, or a calibration using a cesium tracer may be carried out.

13. Separation of Protactinium from Ta, Nb, Ti, Bi, Fe, and Po.

J. Vernois, J. Chromatog., 1, 52 (1958).

Experimental: Ascending technique, using Whatman No. 1 paper, 22 x 22 cm., rolled into a cylinder. The apparatus was a cylinder 17 x 27 cm. in size. Polyethylene apparatus was used throughout. Systems studied (R_f values in brackets):

Pa (0.50) - Ta (1.0) - Nb (0.82): developed with 25 ml. 12N HCL - 50 ml. butanol - 1 ml. 20N HF - 24 ml. H_2O.

Pa (0.45) - Ti (0.66) - Bi (0.66): developed with 25 ml. 12N HCl - 50 ml. butanol - 5 ml. 20N HF - 20 ml. H_2O.

Pa (0.46) - Fe (1.0): developed with 33 ml. 12N HCl - 50 ml. butanol - 1 ml. 20N HF, made up to 100 ml. with water.

Pa - Po: developed with an unspecified mixture of the solvents used above. Po moved with the solvent front; Pa, with an R_f value of about 0.5.

Development required about 10 hours.

14. Separation of Carrier - Free $(S^{35}O_4)^{-2}$ and $(P^{32}O_4)^{-3}$ from One Another. M. Lederer, Anal. Chim. Acta, 12, 14 (1955).

Experimental: Ascending technique, presumably with Whatman No. 1 paper. Solvent: butanol saturated with 1N HCl. The phosphate ion is faster and a clean separation is obtained.

PAPER ELECTROMIGRATION

A. Introduction

Although most of the interest in this technique has been very recent, the basic ideas go back to the turn of the century. Several interesting inorganic separations were carried out by Kendall in the 1920's. Among these was the first application to a radiochemical problem - separation of Ra from Ba (44). Although using agar gel, the principle was similar to that of modern work. The method has received its chief development since 1948, when workers in the biochemical field took it up, and further applications were made in inorganic chemistry soon after. Although gels, etc., have received some use, filter paper has proved to be most convenient.

At present, electromigration in supporting media is a well developed tool in organic and biochemistry, but it has not received a great deal of practical use in inorganic separations although a large amount of work has been published illustrating its power. It may be noted that most of the inorganic work which uses it as a tool rather than an end in itself involves radiochemical problems. It is also useful in studying complexes in solution.

As with paper chromatography, most text-books emphasize the biochemical applications. Most useful to inorganic chemists are:

"Chromatographische Methoden in der Analytischen und Praparativen Anorganischen Chemie", E. Blasius, Ferdinand Enke Verlag, Stuttgart, 1958.

"Paper Electrophoresis", M. Lederer, Elsevier Publishing Co., Amsterdam, 1955.

B. Terminology

It may be useful here to add a few words about terminology, which is in a rather confused state. The technique itself is called variously by the names paper electrophoresis or ionophoresis, ionography, electrochromatography, and others, but electromigration as used here is in some ways most reasonable. Electrochromatography is reserved for procedures employing both chromatography and electromigration, usually in a two-dimensional separation. The porous material used to prevent convection is called the supporting medium, while the electrolyte solution with which it is moistened is best referred to as the background electrolyte, although the terms carrier electrolyte and buffer solution are also used. The zone mobility of a substance is defined as the velocity under unit potential gradient of the moving spot, or zone, in the paper.

C. Theoretical Considerations

Like paper chromatography, electromigration in paper is an empirical technique. Attempts have been made to relate theoretically the observed zone mobility to the mobility of the ion in free solution (45,46), but with limited success. The equations proposed have been tested for inorganic ions by Bailey and Yaffe (47) who find that there is often a specific interaction between the ions and the paper which is not accounted for in the theory.

The most successful approach assumes that the ions travel through the paper along winding channels, and hence the zone mobility is less than the free solution mobility because the ion really travels a longer distance. The zone mobility U_Z is given by

$$U_Z = \rho U_F ,$$

where U_F is the free solution mobility of the ion in the background electrolyte solution used, and ρ is the tortuousity or obstructive factor. The latter may be calculated from the relationship (47,48)

$$\rho = 0.96 \left(\frac{a}{a + v_s} \right),$$

where v_s is the specific volume of the fibres and a is the absorbance - the volume of background electrolyte per unit weight of paper. For fibres such as cellulose which swell in solution, the relation becomes

$$\rho = 0.96 \left(\frac{a - 2v_s X}{a + v_s} \right),$$

where X is the fractional increase in volume of the fibres which is effective in hindering the movement of the ions. The value of this factor is not known, but experimental results fit an equation of this form if X is suitably chosen. Ion-fibre interactions can be accounted for by X which then becomes an adsorption, rather than a swelling, factor. It may be looked upon as the fractional volume occupied by adsorption forces. No independent means of estimating the extent of ion-fibre interaction has been successful. It varies with the migrant, the electrolyte, and the type of paper.

A rough estimate of an upper limit for U_Z can be obtained if X is given some reasonable value (ca. 0.2, for many metal cations), and this may be useful in estimating the maximum distance which an ion would be expected to travel in a given experiment. Unfortunately, in many complex-forming systems which are most useful in this work, U_F cannot be estimated since it depends on the mobilities and stabilities of all the species in equilibrium (49).

Most values of zone mobility given in the literature have no real meaning because various factors which affect it were not defined, but relative values are useful in indicating possible separations.

Weber (50) has considered mathematically the effects of adsorption on the shape of the zone, and Edward (51) has dealt with conductance factors.

D. Experimental Techniques

Numerous designs of apparatus have been used, but they may be classified into two basic types - these are the open strip, or moist chamber, and the closed strip, forms. In either case, the mixture to be separated is applied as a spot or streak across a strip of filter paper previously moistened with the chosen background electrolyte solution. Usually, the ends of the strip dip into reservoirs of excess electrolyte solution; these serve to dilute the products of electrolysis and prevent their interfering with the process by changing the pH, or in other ways. The electrodes, which are connected to a source of D.C. potential, also dip into these reservoirs, and are usually of carbon or platinum. In some circumstances, as when weak electrolytes are used and when the ends of the strip are suitably buffered, the electrodes may be attached directly to the paper and the reservoirs discarded.

In the open strip technique, the moist paper is suspended in a chamber which is saturated with vapour from the electrolyte solution used. In one popular and simple form, the strip hangs with its mid-point over a glass rod, thus forming an inverted "V" shape (52). In another popular arrangement, the strip is supported horizontally on a plastic frame (53).

With this type of operation, only comparatively low voltage gradients may be used, since the heat developed in the paper is dissipated only by conduction through the air-space, and by evaporation of liquid from the strip. The latter process, if too extensive, leads to drying of the strip, and in any event to a concentration of non-volatile electrolyte in the strip, and an increase in solvent flow.

The most convenient form of closed-strip apparatus has the strip placed between two plates of glass or insulated metal. If good contact is maintained between the paper and the plates, evaporation is negligible and large amounts of heat may be carried off. Very high voltage gradients may be used, particularly with water-cooled plates. Voltage gradients of up to 200 volts per cm. have been used, and a total applied potential of 10,000 volts with a migration path of about 100 cm. is not uncommon. Since the speed of a separation depends directly on the voltage gradient, high voltages are useful, especially when long migration distances are necessary. Michl describes a number of high-voltage arrangements (54).

In the closed-strip technique, the strip must not be too wet; otherwise the migrants will travel partly in a film of liquid between the strip and the plates, resulting in large smeared zones. The cooling surface should be hydrophobic; treatment of glass with 'Desicote' is advantageous.

Another form of closed-strip apparatus has the moist paper suspended in an organic liquid such as hexane or carbon tetrachloride (54). Arrangements of this sort can dissipate large amounts of heat, but are hazardous and inconvenient in operation, and offer the danger that some migrants may be extracted from the strip. In radiochemical work the risk of contaminating the organic phase thus arises. This arrangement therefore cannot be recommended.

E. Experimental Considerations

A number of factors which influence electromigration in paper must be considered (49).

1. Temperature. The speed of an ion moving in an electric field increases with increasing temperature by about 2% per degree C. This is true in paper as well as in free solution. For inorganic separations the actual temperature is of little importance as long as it is uniform and does not lead to drying and charring. In the closed-strip technique where water is circulated through the plates, temperatures well above that of the room can be used if the proper care is taken with the experimental arrangements. This can give an

appreciable reduction in separation time. The increase in size of the zones which might be expected from enhanced diffusion does not seem to be important; in some cases the zones are sharper due to reduced adsorption. In certain instances it is possible that the influence of temperature on the stability of the complex species present may result in anomalous effects.

2. Absorbance. The zone mobility increases with the absorbance (the volume of liquid present per gram of paper). This is predicted by theory (48). At the same time, the zones tend to become larger, but this is important only at high absorbances when the paper is "flooded." The practical limit depends on the type of paper used.

3. Concentration of the Migrant. It has been predicted theoretically that if the ionic strength of the initial zone is greater than that of the background solution in the rest of the strip, then on electromigration the zone will elongate until the ionic strengths become equal (51). Experimentally it is found that when the migrant concentration is larger than that of the background solution, the resulting zones are very large, and "tailing" is extensive. It is clear that the migrant concentration must be kept low for satisfactory separations. A larger volume of comparatively dilute solution is better than a small volume that is more concentrated, and can be handled as a streak across a wide strip, rather than a single spot.

If the ionic strength of the initial zone is high because of foreign ions, the migrant zone of interest may be elongated even if the other ions are separated rapidly from it, as by migration in the opposite direction. The concentration and volumes which can be handled without undue enlargement of the final zone depend on the conditions chosen for the experiment as well as on the particular ion. As a rough guide it may be assumed that a maximum of about 10μ l. of 0.01N solution can be applied as a single spot to a paper such as Whatman No. 1 to give a reasonably compact zone.

As with paper chromatography, carrier-free material may show trailing or no motion, whereas similar experiments with inactive substance indicate normal motion. The causes are the same.

4. Composition of the Migrant Solution. The composition, as distinct from the concentration, of the applied migrant solution may be important. It is usually true that the ions in a mixture migrate independantly (provided the overall ionic strength is low enough), but this will not be so if interactions, such as the formation of stable complexes, is possible. Complexes formed in the applied solution may migrate as such even in non-complexing media, and so give rise to entirely unexpected behaviour.

On the other hand, desired complexes with the background solution may not form if a complex destroying agent, such as a strong acid, is present in the initial zone. Then, for example, a cation may start to migrate as such until freed from the interfering substance, when it forms an anionic complex and reverses its direction (the "umkehreffekt"). In his book, Lederer gives an example of this in the separation of Bi^{+++} as the anionic chloride from Cu^{++} Nitric acid in the applied solution prevented the initial formation of the bismuth-chloride complex.

In some cases interfering substances in the applied solution may have to be removed by some chemical means. It is always desirable to apply the migrants as a solution in the background electrolyte used, although for most metals a neutral, or only weakly acidic, nitrate solution is satisfactory.

Reactions of the migrants with one another or with the background electrolyte to yield insoluble substances obviously will lead to motionless zones, and sometimes to streaking.

5. The Supporting Medium. Interactions between the migrant and the supporting medium may amount to considerably more than just mechanical obstruction, but the nature of the specific interactions is not well understood. Cellulose is known to have some ion-exchange groups, and these may be important. Another possible form of interaction is complex-formation, particularly chelation by two adjacent hydroxyl groups (55). At any rate, many ions are "adsorbed" by some mechanism. This may be irreversible, with the migrant reamining at the starting point or trailing back to it. In other cases, the zone remains compact, but moves with a mobility much lower than expected. Weber has shown

theoretically that some types of reversible adsorption can lead to "comet-shaped" zones (50), and most examples of streaking, when "overloading" is not the cause, are attributable to this effect.

Reducing action of the paper may also take place, as can interaction with impurities in it.

In some cases, at least, irreversible adsorption can be eliminated by soaking the paper in an inactive solution of the species which is adsorbed, and washing out the excess. A radioactive zone of the same ion may then move without trailing. This procedure was first used by Harbottle (56). The only other usual means of eliminating adsorption is by use of a background solution which forms a different complex species, or of a stronger acid if this does not result in too high a conductance.

Other porous supporting media may be used, and, while these are usually less convenient than filter paper, they may have advantages in particular cases. Glass fibre paper is an obvious possibility. Adsorption on this material is usually greater than on cellulose papers, but this may not be the case when ion-cellulose interaction is one of chemical reduction or complex formation. Another possibility is the cellulose acetate strips now used for some organic purposes. Ion-exchange resin loaded papers, and chemically modified ion-exchange papers may also be used, although little work has been done to indicate their value.

Zone mobilities vary with paper type, and this variation is different for different ions, so that relative, as well as absolute, values are changed.

6. The Background Electrolyte Solution. The background electrolyte is the most important factor governing the behaviour of an inorganic ion in this process. This is especially true for inorganic cations, which may be made to have an effective positive, negative, or neutral charge by the choice of suitable complexing solutions. Ions with disimilar complex-forming properties are often readily separated by causing one to form an anionic complex while the other remains cationic. This technique, then, becomes most powerful with inorganic

cations when used with complex-forming agents. The effect of pH is only important in so far as it influences complex formation, and with amphoteric ions.

The fact that the ionic strength of the background solution should be greater than that of the migrant solution has been mentioned. For small, well-shaped zones it is advantageous to use a background solution which is as concentrated as possible without drawing an excessively high current. In many cases a compromise must be reached between a highly concentrated electrolyte which gives good complexing power and sharp zones, and a more dilute solution which enables a higher voltage gradient to be used to give a faster separation.

As with paper chromatography, there seems no limit to the number of solutions which can be used as background electrolytes. These include organic as well as the usual aqueous solutions, although the former usually lead to lower mobilities. Fused salts have also been used (See section I.).

7. Other Factors. A number of extraneous factors arising from the experimental arrangement can have an important influence on the mobilities of the migrants. Normally, two are of most importance. Electroosmotic flow is almost always encountered when electrolyte reservoirs are used. Ordinarily its direction is toward the cathode, since cellulose tends to take on a negative charge, so that cations are helped, and anions hindered in their movement. An approximate measure of this effect is given by the motion of uncharged molecules - H_2O_2 is an example of one most readily detected.

If evaporation occurs the concentration of non-volatile electrolyte in the strip will increase. At the same time, liquid will tend to flow from the electrolyte reservoirs at the ends toward the centre of the strip. The latter may occur also if the initial wetness of the paper was too low (the "wick" effect). The velocity of this flow is greatest towards the ends of the strip. While the above factors may not be serious in many procedures, they must be taken into account if the results of several runs are to be compared.

F. The "Focussing" Technique

If, in a complex-forming system, a gradient of concentration or pH exists along the strip such that at the anode end the migrants exist as cations, while at the cathode end they act as anions, at some point along the gradient they will behave as neutral particles. Each migrant zone will travel to this point and will form a narrow band here. The position of this band will depend on the stability of the complex formed, and so may be different for various ions. Many inorganic cations are well adapted to this approach. This technique may be extremely fast since the migrants may not have to move far to reach their equilibrium positions. Moreover, the zones are exceedingly narrow - often only a few millimetres wide. Schumacher (57-64) has studied this method in detail, and his work should be consulted. The theoretical basis is also covered. Many of his separations are of interest radiochemically.

G. Counter - Current Electromigration

If a flow of electrolyte is set up which opposes the electromigration of the ions, and is just large enough to maintain the slower migrant motionless, then it is possible to carry out difficult separations with quite short migration distances. Various designs of apparatus have been proposed, using paper strips (65 as well as packed tubes (66,67). Although it has yielded separations of similar ions and enrichment of isotopes, it has not been used to a great extent, in part because of the difficulty in maintaining the desired solvent flow. Moreover, as the zones tend to become quite large, complete separations may not be possible.

H. Two-Dimensional Techniques and Electrochromatography

By using square sheets of paper, two-dimensional separations may be carried out as in two-dimensional paper chromatography. In addition, chromatography and electromigration may be combined by setting up a solvent flow at right angles to the field. This is ordinarily done simultaneously with application of the potential, but they can be applied successively, and with different solvents, in a discontinuous technique. Such procedures are properly termed "electrochromatography."

This process is readily adapted to continuous operation, and is normally used in this way. It permits large amounts of migrants to be handled. The importance of chromatographic adsorption depends on the particular migrants and electrolyte involved, but the method will work equally well if the migrants are adsorbed strongly or not at all, provided their movements under the electric field are sufficiently different. The electrolyte solution now has the additional task of carrying the migrants uniformly across the paper.

In operation, the migrant solution is allowed to flow continuously at a point onto the upper edge of a sheet of paper, while a flow of background electrolyte is maintained down the strip. The potential is applied at right angles to this flow, and fractions are collected from a number of "drip-points" along the bottom of the sheet. If a steady state is maintained, each migrant follows its own particular path diagonally across the sheet, and is collected in one or a few fractions.

A moist chamber procedure is usually employed. The electrodes may be attached along the sides of the sheet, or tabs on its lower end may simply dip into electrolyte reservoirs. In the former case interference from electrolysis products must be eliminated by a very rapid solvent flow along the electrode regions, or by means of semipermeable membranes, while in the latter case a much less effective field is produced. Various designs of apparatus are described in the texts listed. Strain has described the use of this method in a number of inorganic separations (68,69). McDonald has developed a method of speeding up the solvent flow by centrifugal force (70). Schumacher has also adapted the continuous approach to the "focussing" technique (62).

I. Column and Gel Techniques

Some early electromigration experiments utilized electrodes at the ends of chromatographic columns, but this technique is of little interest to inorganic chemistry. Some pieces of apparatus are available commercially, and some designs are described in the book by Blasius. Tubes filled with agar gel, or ion-exchange resins, and slabs of agar, starch, or silica gel, have also been used, but again only rarely in inorganic chemistry. While such techniques may permit larger quantities to be handled, they are difficult to cool uniformly at high voltage gradients, and are not at all as convenient as paper.

Continuous electrochromatography has been carried out in cells packed with powders such as quartz, ground glass, or cellulose, and these seem to be quite effective. In operation, they are quite analagous to the paper sheet technique. The thickness made possible this way is an advantage. Again, very little has been done in the inorganic field, but Shvedov and Stepanov have obtained very promising separations of some rare earths in such a cell packed with powdered quartz (71).

While paper chromatography may be scaled up to handle larger amounts of material without loss of speed, electromigration cannot; continuous separations, which have no limit as to quantity, tend to be slow, and thick slabs, etc., operated in the discontinuous manner require low voltage gradients and hence are also slow. Some scale-up without accompanying loss of speed may be obtained by using very wide sheets of paper, and as long as they are uniformly moistened this is satisfactory, but a limit is soon reached. The inconvenience is obvious.

J. Fused Salts

Fused salts have been used as background electrolytes with asbestos paper as supporting medium. The salt (usually a mixture of KNO_3 - $NaNO_3$) is kept molten by placing the strip in a tube heated with resistance wire. Presumably glass paper could be used as well. Most work along these lines has been done by Arnikar and Chemla, who obtained good results with alkali metals (72), as well as enrichment of isotopes (73,74). From the limited amount of data available, fused salts seem to be quite effective for certain types of separations where complexing is not effective (as for the alkali metals). Experimental difficulties which include high conductance as well as a need for high temperatures are a major drawback.

K. Detection and Recovery

The discussion of the detection and recovery of separated zones given in the section on paper chromatography applies here equally, and need not be repeated.

L. Radiochemical Separations

Many separatory procedures developed for inactive mixtures may be applied to radiochemical problems directly, or with slight modifications. The situation in this respect is similar to that in paper chromatography. Lists of inorganic separations by this technique have been published in the book by Blasius, and by Bailey and Yaffe (75).

A number of separations have been developed for radiochemical use. Among the earliest of these is the separation of "carrier-free" activities from precipitates of other elements in a stack of filter paper discs moistened with a suitable electrolyte as carried out by Garrison et al. (76). Elements separated include Nb from MnO_2, and Nb and Zr from Y and the rare earths in ammonium oxalate solution, and As from $Cu(OH)_2$ in HCl. Other separations of radiochemical interest include that of radium and its decay products by Sato et al. (68,77), and of Ra D, E, and F by Bailey and Yaffe (78); the separation of "carrier-free" $P^{32}O_4^{-3}$ and $S^{35}O_4^{-2}$ from one another by Lederer (79), and the separation of Ca^{45} and $P^{32}O_4^{-32}$ from each other and from other radioactive contaminants by Sato et al. (80). Bailey and Yaffe have described the separation of Tc^{99} from its parent Mo^{99}, and also of Sr^{90} - Y^{90}, and Ba^{140} - La^{140} (78). Schumacher has used his focussing technique to separate a number of "carrier-free" radioactive materials (59,64).

This method should hold many possibilities for separating the products of Szilard - Chalmers reactions, and it has been used most successfully in a number of cases. Rauscher and Harbottle (81), for example, have used it to separate several species produced from this reaction in potassium hexacyanocobaltate (III); these included $[Co(CN)_6]^{-3}$, $[Co(CN)_5H_2O]^{-2}$, $[Co(CN)_4(H_2O)_2]^-$, $[Co(CN)_4(NH_3)_2]^-$, and Co^{+2}. Electromigration can aid in identifying the products as well as in estimating their relative amounts. Similar work with alkali bromates and iodates, and with arsenic pentoxide, have been reported by Jach, Kawahara, and Harbottle (82), while Sato et al. (83) have applied this technique to phosphates.

Mach has used electromigration in paper to estimate the radiochemical purity of samples of P^{32}, S^{35}, Na^{22}, K^{42}, Rb^{86}, I^{131}, and Sr^{90}, and recommends it as a routine method for such purposes (84).

Finally, Zimakov et al. (19) have used this method to determine quantitatively all of the elements Ce, Y, Sr, Zr, Nb, Ru, and Cs in a solution of fission products. By carrying out electromigration in 0.1 N HCl, 0.1 N NaOH, and 2% $K_4Fe(CN)_6$ electrolyte solutions, and using standardized techniques for measuring various sections of the strips, one can obtain quantitative estimates of each of these substances rapidly.

M. Some Radiochemical Separations by Electromigration in Paper

1. Determination of Radiochemical Purity of Isotopes; S^{35}, Cl^{36}, irradiated alkali chlorides, I^{131}, and Sr^{90}.

M. Mach, 2nd Intern. Conf. Peaceful Uses Atomic Energy, Geneva, 1958. A/CONF 15/P2109. Also, Chem. prumysl., 8, 236, 303 (1958).

The following procedures were developed for the determination of systematically occurring impurities found in these isotopes. All experiments were carried out with the paper strips placed between glass plates, and using electrolyte reservoirs.

(a) S^{35}, Cl^{36}, Na^{24}, K^{42}, Rb^{86} (and p^{32}). Irradiation of alkali chlorides with neutrons can produce the isotopes P^{32}, S^{35}, and Cl^{36}, as well as the alkali metal activity. In compounds of S^{35} made from an irradiated chloride, Cl^{36} and P^{32} may be contaminants, while similarly P^{32} and S^{35} may contaminate Cl^{36} preparations from such sources. The methods of isolation of S^{35} and Cl^{36} ensure that the species concerned are present as chloride, phosphate, or sulfate, and no particular treatment or modification is neccessary for separation by electromigration. For analysis of irradiated chlorides, 10 - 13% solutions were made by dissolving the solids in distilled water.

Whatman 3 MM paper, 1.5 x 76 cm., was used between glass plates 9 x 60 cm. Background electrolyte was 0.25 N $HClO_4$ or HNO_3. (With the former, it was neccessary to neutralize the moist strip immediately upon completion of the separation in order to prevent damage to the paper.) A volume of 5μ 1. of active solution was applied at a point 10 cm. from the centre of the strip in the direction of the anode. Two hours with a voltage gradient of 6V/cm. was used. Chloride, sulfate, and phosphate all moved toward the anode. The first of these was fastest, while phosphate showed only a small movement.

(b) I^{131}. The contaminant of importance in this case is radiotellurium. The irradiated substance (NaI or KI) must be treated to obtain both I and Te each in one chemical form. For this, a volume of 15μ 1. of carrier solution composed of 13 g. KI and 0.01 g. $NaHCO_3$ in 1 litre H_2O was added to 0.25 ml. of the original solution; this was oxidized in a test-tube with 0.3 ml. of 1.5 - 2% sodium hypochlorite while heated for 15 minutes in boiling water. This yields periodate and tellurate ions.

Whatman No. 1 paper, 1.5 x 56 cm., between glass plates 9 x 40 cm., was used. Background electrolyte was 0.1 N H_2SO_4 with 2% (vol.) glycerine at the cathode, and 0.4 N Na_2SO_4, also with 2% glycerine (vol.), at the anode and throughout the strip itself. A volume of 1μ 1. of solution was applied at a point 10 cm. from the centre of the strip in the direction of the anode. Separation was accomplished in 3 hrs. at a voltage gradient of 10V/cm. (The separated solution contained 0.02 mg. iodine and 0.3 mg. tellurium.) The periodate moved toward the anode, while the tellurate moved very little.

(c) Determination of Sr^{89} in Compounds of Sr^{90}. Separation of Sr^{90} from Y^{90} was carried out in strips of Whatman No. 1 paper, 1.5 x 56 cm., between glass platee 9 x 40 cm., with 2% $K_4Fe(CN)_6$ as background electrolyte. The solution to be analysed (1μ 1.) was applied at the centre of the strip. Separation was accomplished in 2 hrs. at a potential gradient of 10V/cm. (Sr moved toward the cathode, Y was nearly motionless.)

The separated radiostrontium is essentially free from daughter Y^{90} at the end of the separation, and contains no other impurities. Since the Y^{90} builds up only slowly (0.02% of the equilibrium value after 3 hrs.), the Sr^{89} content can be found by absorption analysis. (The maximum energy of its β-radiation is 1.48 Mev as compared to 0.54 Mev for Sr^{90}.) An aluminum absorber of 182 mg./cm.^{2} absorbs all of the Sr^{90} β-radiation, but transmits 15% of that from Sr^{89}. Determination of less than 1% Sr^{89} is then possible.

The fraction of impurities separated in these procedures may be found by measuring the activity in the various separated zones with a suitable counter. The absorption of the activity by the paper must be determined in each case by calibration experiments.

2. Separation of Tc^{99} from Mo^{99} Parent. R. A. Bailey and L. Yaffe, Can. J. Chem., 38, 1871 (1960).

A high voltage technique using water-cooled metal plates insulated with mica was employed. Platinum electrodes were in direct contact with the paper - Whatman 3 MM. Background electrolyte was either acetic acid (1M) or ammonium acetate (0.15%). The molybdenum-99 was separated from fission products by a standard chemical method, and was present in solution as the molybdate ion. The technetium formed from the decay of the molybdenum was apparently all present in one chemical form, i.e., as the pertechnetate ion.

Separations could be made in either of the above electrolytes; 25 minutes at 100V/cm., 30°C., was adequate to give a perfectly radiochemically pure zone of technetium when acetic acid was used. In this period, $MoO_4^{=}$ remained essentially at the starting point, while TcO_4^- moved about 12 cm. Behaviour in ammonium acetate was similar, with the TcO_4^- zone being somewhat more compact. A lower voltage gradient was neccessary, but a satisfactory separation could be accomplished in about the same time.

3. Separation of Carrier-Free Activities from Scavenger Precipitates: (a) Nb from MnO_2; (b) Nb and Zr from Y and Rare Earth Oxalates or Hydroxides; (c) As from $Cu(OH)_2$. W. Garrison, H. Haymond, and R. Maxwell, J. Chem. Phys., 17, 665 (1949).

Separations were carried out in a stack of 30 filter paper discs, 2.6 cm. in diameter. A current of 0.2 amp. was drawn. The time for a separation was 1/2 hr. The sample was applied to a disc which was placed at the centre of the stack, and the whole was moistened with the background electrolye. Separated material could be found in not more than 5 discs. Electrodes were of platinum.

(a) Nb from MnO_2. Two procedures are available. The MnO_2 precipitate could be dissolved in 5% oxalic acid, and the separation carried out in 1 M ammonium oxalate. The Nb moved toward the anode, Mn toward the cathode. Alternatively, the MnO_2 could be dissolved in conc. HCl and 3N HCl used as background electrolyte. In this case, Nb remained at the initial point, while Mn moved toward the cathode.

(b) Nb, Zr from Y and Rare Earths. In this separation, 1 M ammonium oxalate was used as background electrolyte so that the Y and the rare earths remained at the starting point as insoluble substances. The Nb and Zr could be recovered from the anodic discs.

(c) As from $Cu(OH)_2$. The $Cu(OH)_2$, present in milligram amounts, was dissolved in 3N HCl. The latter was also used as background electrolyte. Arsenic remained at the origin while copper moved toward the cathode.

This technique allows samples containing large amounts of at least one component to be handled, but it would appear limited to low voltage gradients and simple separations.

4. Separation of Bismuth, Lead, Polonium, and Radium. T. R. Sato, W. P. Norris, and H. H. Strain, Anal. Chem., 27, 521 (1955).

A large scale separation (50μ l. of solution) was carried out in Eaton-Dikeman 301 paper strips, 1 meter long and 30 cm. broad. The strip was cooled by a "thermopane" window, and was wrapped in polyethylene. The background electrolyte was 0.1 M lactic acid, and the potential gradient, 7V/cm. Development time was 24 hrs.

A faster separation (1 hr.) was carried out with 1μ l. of solution in a hardened filter paper, but no further details are available. Separation was said to be complete, but with the large scale runs, Po and Bi formed overlapping zones.

All of these elements move as cations with radium fastest and lead next. Polonium remained at the starting point with bismuth migrating slowly away, but trailing and probably always contaminating the former zone. Radium recovery on elution with lactic acid solution was > 97%.

5. Separation of Polonium from Lead and Bismuth. R. A. Bailey and L. Yaffe, Can. J. Chem., 38, 1871 (1960).

Although lead is readily separated from its decay products in the mixture Ra D, E, and F, it is more difficult to obtain pure polonium. The latter and bismuth do not give rapidly moving zones in paper electromigration under most circumstances. If a good complexing agent is used, the movement of bismuth as an anion may be increased, leaving pure polonium behind.

This separation was carried out in Whatman 3 MM paper between metal plates insulated with polyethylene. With a background electrolyte composed of a saturated solution of ethylenediaminetetraacetic acid in water, a good separation could be achieved in 30 min. at 90V/cm., 41°C. Lead and bismuth moved more or less together, leaving the polonium behind in a large but pure zone. It was readily eluted with HCl.

6. Separation of Carrier-Free Sulfate and Phosphate. M. Lederer, Chem. and Ind. (London), 1954, 1481.

Whatman No. 1 paper in strips 30 x 3 cm. was used. It was sandwiched between glass plates with its ends dipping into beakers of background electrolyte. The latter was 0.5 N HCl. Separation required 2 hrs. at 150V. potential. The sample to be separated was applied at the centre of the strip. Phosphate remained near the starting point, while sulfate moved rapidly toward the anode.

7. Separation of Nb^{95} from Zr^{95}. J. Vernois, J Chromatog., 2, 155 (1959).

Separation of Nb^{95} from its parent Zr^{95} was carried out with 0.06 N HCl - 1 N HF as background electrolyte. Arches No. 302 paper, clamped between plexiglass plates, was used. At an applied potential of 150V., separation was achieved in 4 hrs. The zirconium had moved about 21.5 cm., and the niobium about 15 cm., toward the anode.

8. Analysis of Fission Product Solutions for Ce, Y, Sr, Zr, Nb, Ru, and Cs. P. V. Zimakov, A. G. Bykov, and I. A. Usacheva, Isotopes and Radiation Chemistry, Moscow, 1957, p. 303.

Fission product solutions containing radioactive isotopes of the elements Ce, Y, Sr, Zr, Nb, Ru, and Cs were quantitatively analysed by electromigration in paper. The mixture was acidified, any precipitate dissolved, and the solution diluted with 0.1 N HCl to a specific activity of about 10^{-3} millicuries/gram. A drop was then placed at the centre of a strip of chromatography paper of dimensions 2 x 24 cm.; this was moistened with background electrolyte. The paper was placed between glass plates with its ends dipping into beakers of excess electrolyte. A voltage of 350 - 500V. was applied. After separation, the dried paper was cut into 11 parts and each measured with an end-window counter. Distribution of isotopes, counting efficiency, etc., should be determined with known samples.

Separations were made in 3 electrolyte solutions.

(a) 0.1 N HCl. After migration for 20 min. in this solution, the fission products were divided into three groups; Ce, Y, Sr, and Cs which were cationic, Zr and Nb which did not migrate, and Ru which moved primarily toward the anode. The Zr and Ce content of the solution could be found by absorption analysis with aluminum filters of thickness 24 mg./cm.^{2} and 710 mg./cm.^{2} respectively. If the content of Ru exceeded 10% of the total, this was not satisfactory and NaOH was a better electrolyte.

(b) 0.1 N NaOH. After migration for 1 hr. in this electrolyte, Cs had left the strip completely; Ce, Y, Zr, and Nb remained in the starting area; Sr formed a cationic zone, and Ru an anionic one. With a 20 min. migration, Cs was separated from the others. Those elements remaining at the starting point could be re-migrated by acidifying that portion of the strip, and placing it at the centre of a second strip moistened with 0.1 N HCl. After 30 min., these separated as described in part (a). Thus, all isotopes may be measured. (c) 2% $K_3[Fe(CN)_6]$, pH9. After 45 min. migration time, Sr formed a cationic zone well separated from the rest. The latter were spread in a zone extending in both directions from the starting point, but mostly toward the anode. Ruthenium moved farthest in this direction, and could also be determined here.

The above procedures were claimed to be more rapid than classical procedures for the analyses required. The accuracy obtained was 10% for those isotopes present in an amount of 10% or more, but less if the content was less. This was primarily dependent on radioactive "counting" errors.

9. Preparation of Carrier-Free Activities: (a) Y^{90} from Sr^{90}; (b) La^{140} from Ba^{140}; (c) Pr^{144} from Ce^{144}. E. Schumacher and W. Friedli, Helv. Chim. Acta, 43, 1706 (1960).

These separations were carried out with material obtained from fission sources. The separation technique was based on "focussing" using a medium producing a gradient of complex-forming ability. It is very fast, and the products were obtained in yields of > 99% and with high radiochemical purity.

(a) Y^{90} from Sr^{90}. The solution of Sr^{90} containing its daughter Y^{90} (with a content of not more than 10 mg. total alkaline earths, and as little foreign electrolyte as possible) was placed in a zone, 8 - 12 mm. long, on an ash-free paper strip 10 - 20 mm. broad and 12 cm. long. For production of a complexing gradient, the electrolyte solutions used were the following: at the cathode, 40 ml. of 0.3 M $(NH_4)_2H_2EDTA$ + 10 ml. Of 0.3 M CH_3COONa, or 0.1 M $(NH_4)_2HNTA$; at the anode, 0.1 M HCl with EDTA, or 0.05 - 0.1 M HCl with NTA. (EDTA = ethylenediaminetetraacetate; NTA = nitrilotriacetate). Voltage applied was 700 - 1000 V. (current < 20 mA.). The separation was complete in 3 - 4 min. Radiochemical purity of Y^{90} was > 99.99%.

The Y^{90} could be eluted from its zone (5 mm. broad) with 0.1 M HCl. The Sr^{90} could be eluted from a zone also 5 mm. broad with 0.1 M acetic acid.

This could be done directly onto a second strip after further daughter activity had built up so that further sources of Y^{90} could be obtained.

(b) La^{140} from Ba^{140}. The procedure was essentially the same as in part (a) above. The radiochemical purity of La^{140} after a 3 min. separation was > 99.95%

It was also possible to separate simultaneously Y^{90} and La^{140} from a source containing both Sr^{90} and Ba^{140}.

(c) Pr^{144} from Ce^{144}. A volume of 0.01 ml. of the solution of Ce^{144} - Pr^{144} was treated with 0.01 ml. of a solution of SO_2 which was 0.05 M with respect to Ce^{+3}. Electrolyte solutions were: at the cathode, 50 ml. 0.3 M $(NH_4)_2HNTA$ + 20 ml. 0.3 M CH_3COONa + 20 ml. CCl_3COONa; at the anode, 0.1 M HCl. Experimental procedure was as in part (a). Time for the separation was 10 - 12 min., yielding Pr^{144} with a radiochemical purity of 98.6%.

In all cases, a second separation can be used to obtain higher purity.

An Overall Evaluation of Paper Chromatography and Paper Electromigration

On considering the number and kinds of separations reported by the above techniques, there can be no doubt of their effectiveness. Unfortunately, their empirical nature makes selection of conditions for a new application largely a matter of trial and error. All too often separations described in the literature deal with combinations of elements that appear to have been chosen simply because they could be detected readily. Enough work has now been done in paper chromatography to show the behaviour of most elements in some solvent systems such as butanol - HCl, and this could serve as a guide for many uses. Less work has been done in electromigration, but choice of a suitable background electrolyte is probably less difficult.

Both techniques are very well suited to dealing with small amounts of material - indeed, this is one of their chief advantages. Larger amounts, even of just one component, may cause trouble. Paper chromatography may most readily be scaled up without much loss of speed, and can be changed to work with cellulose columns if required. Paper electromigration poses a larger problem if scale-up is necessary, but if speed is not important a continuous process may be used to separate any quantities. In general, however, this is an inconvenient, as well as slow, technique.

As a rule, paper chromatography is slow, although this is not always the case. Paper electromigration may be extremely rapid if high voltage gradients are used, but then the apparatus and technique become more complicated. Paper chromatography may be carried out with much simpler equipment. Additional important advantages of electromigration are the longer migration distances which can be used with reasonably simple equipment, and the fact that the components of a mixture may be made to travel in two directions depending on the sign of their charges. This can lead to very clean separations.

With either technique, the use of linear strips of paper makes detection of radioactivity by an automatic scanning device fairly easy. Relative amounts of material in the various zones may be determined directly from the area of the scan, or these sections may be measured more carefully by cutting them from the strip and using another "counting" technique. This is particularly simple if the same isotope in different chemical forms is being investigated, as with the products of Szilard - Chalmers reactions. For different isotopes or for absolute measurements, calibration may be necessary, but good accuracy can still be achieved fairly readily. The separated material on the dry paper is in a very convenient form for handling.

If it is necessary to remove the separated activity from the paper, difficulty may be encountered. Some substances are absorbed very strongly, and, in addition, other impurities from the paper may be undesirable contaminants. This will not be too important in general, perhaps, but in any event removal from the paper introduces another time-consuming step.

Although neither paper chromatography nor electromigration is likely to displace methods such as ion exchange for general radio-chemical separations, they do hold advantages for certain purposes, and could well be used to a much greater extent than heretofore. By using radioactive tracers to eliminate detection difficulties, these techniques should prove to be more useful in inorganic chemistry in general. They have already been used in the study of complex species in solution; electromigration, for instance, will give the sign of the charge on an ion directly. It is hoped that further work in these techniques will make it possible for the non-specialist to use them much more readily than at present.

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