An International Journal of Analytical Chemistry

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# Talanta

An International Journal of Analytical Chemistry

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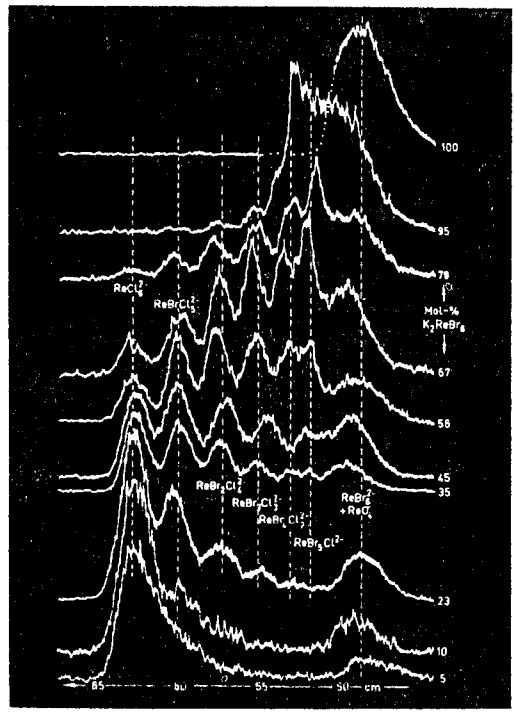


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V. V. SAVANT and C. C. PATEL Diphenyl sulphoxide complexes of some oxocations

E. E. FLAGG and D. L. SCHMIDT Some fluoroalanes and related intermediates

JUDITH KITCHENS and J. L. BEAR A study of some rhodium (II) acetate adducts

K. H. KHOO and J. D. MURRAY Saturated salt solutions–II. Ion pair formation between thallium (I) and nitrate in the presence of bromate and alkali metal ions

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Talanta, 1971, Vol. 18, pp. 365 to 394. Pergamon Press. Printed in Northern Ireland

# TALANTA REVIEW*

# ANALYTICAL AND EXPERIMENTAL ASPECTS OF MOLECULAR-SIEVE CHROMATOGRAPHY

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**Summary**—The object of this Review is to give analytical chemists a general introduction to molecular-sieve chromatography, a form of liquid chromatography in which molecular size forms the primary basis for separation, although other effects are also frequently involved. The technique can be used for inorganic and organic molecules, both monomeric and polymeric, in either aqueous or non-aqueous systems. The range of xerogel and aerogel molecular-sieves available at present is described, and the experimental techniques involved in their use are emphasized rather than mechanistic and theoretical considerations. The references cited have been selected critically to form a balanced, up-to-date review and also to indicate the general analytical potential and scope for future development of the technique. An Appendix lists the commercial sources of molecular-sieves and calibration standards.

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* For reprints of this review see Publisher's announcement near end of issue.
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## INTRODUCTION

This is one of a series of reviews of topics of current interest to analytical chemists, intended primarily to introduce the non-specialist to a new subject. The preparation of reviews has become a hazardous business, attempts to give the maximum useful information in the shortest space frequently being criticised in the words "the review reads like an Annual Report." In a subject as extensive as molecular-sieve chromatography has become, it is not always possible, if the review is to be completely up-to-date, to be critical or to be in a position to make constructive comment from direct personal experience. The limitation on length of this Review ensured that at least a critical selection of the references that form its basis had to be made. It also became necessary to restrict consideration of either the theoretical or the practical aspects of the subject: the emphasis is placed on the latter.

Molecular-sieve chromatography (MSC) is a form of liquid-partition chromatography based on the unique properties of aerogels (porous glass or silica) and xerogels (agarose, cross-linked dextrans, polyacrylamides, polystyrenes *etc*) to give a technique, used in either the column or thin-layer mode, for separating substances primarily on the basis of molecular size, although adsorption and other secondary effects influencing the basis of separation are also frequently involved.

The theoretical considerations are complex; conflicting views are held. In contrast, the experimental technique is rapid and effective, albeit somewhat empirical; the apparatus required is relatively simple and inexpensive.

This modern branch of chromatography has been applied predominantly, so far, to problems involving complex biochemical or highly polymerized molecules. MSC has, however, potential application to all branches of analytical chemistry since it

can be applied to inorganic or organic molecules of any molecular weight, in either aqueous or non-aqueous systems.

The main objects of this Review are to give analytical chemists an up-to-date picture of the present state of development of the technique, and to give an indication of its analytical potential, since it is still not being exploited fully in many fields of specialization.

## HISTORICAL

Analytical chemistry, for all the concern it should have with technique, has always been astonishingly slow to spot the winners. In retrospect, the use of all the powerful modern methods started off much more slowly than their potential warranted. As examples, infrared spectroscopy and chromatography compare strikingly: after the pioneer work of Coblenz (1905) and Tswett (1906) respectively, both techniques were not developed significantly until after 1940. Then, even after such a gestation period, many of us—perhaps insufficiently gifted with originality or foresight, perhaps denied the luxury of the freedom of time necessary for fundamental thinking—were slow to assist in the act of development. Consequently, important techniques have expanded only slowly during some 5–10 years before the eruption of more appropriate activity, as the following illustrations from chromatography show. James and Martin[1] described the use of GLC in 1952, but the technique was not applied to derivatives of carbohydrates[2] until 1958, nor to steroids[3] until 1959—with successes that led quickly to revolutionary advances in these fields. Thin-layer chromatography, first described[4] in 1938, was used[5] in 1949; but not until 1956 did Stahl publish the work that attracted the attention for the technique to become popular.

Similar stories hold for molecular-sieve chromatography. Porous zeolites with the property of acting as differential molecular-sieves were described by McBain[6] in 1926, and the subsequent studies of these materials, natural and synthetic, by Barrer has led to the association of his name with the unit of permeability.[7] In 1930, Friedman[8] observed, from the rates of diffusion of urea and glycerine, that the pore-size in agar gels was inversely proportional to the agar concentration in the gel. In the period 1944–49, there were reports, reviewed by Deuel *et al.*,[9] of chromatographic separations in which differences in molecular size were involved in addition to the other properties of the molecules intended to form the basis of the desired separation. (Barker *et al.*[10] have recently modified molecular-sieves to give them ion-exchange properties as well.)

Not until 1952, however, did the pace of development quicken. Reviewers have differed in their assessment of the most significant developments; Porath, whose name came to be identified with the term "gel filtration," has given[11] his account of the development of molecular-sieve chromatography. In our assessment, the following contributions deserve recognition. In 1953, Wheaton and Baumann[12] observed that some ion-exchangers sorbed non-ionic water-soluble molecules to an extent inversely proportional to their molecular weights; in the following year, Deuel and Neukom[13] cross-linked a galactomannan polysaccharide and obtained a gel useful for desalting solutions of macromolecules. In 1955, Lindqvist and Storgårds[14] fractionated peptides and amino-acids by molecular-sieving on starch. Polson[15] observed in 1956 that the penetration of proteins into agar gels was a function of the gel concentration and of the size of the protein molecules, although this appears, in effect, to be what Friedman[8] had reported.

It is generally agreed by reviewers,[11,16–18] however, that a report by Lathe and Ruthven[19] in 1956 did not attract the attention it deserved. Although these authors are reported[11] to have believed that molecular-sieving properties were unique to starch, they controlled undesirable ion-exchange and other effects by using saline solutions, separated neutral molecules of molecular weight less than 1000, and altered the ranges of effective molecular-sieving by swelling starch granules in water and in thiocyanate solutions, achieving separations of polysaccharides and proteins of molecular weights up to $1{\cdot}5 \times 10^5$. As Determann[17] has pointed out, Lathe and Ruthven developed a concept that is still valid by suggesting that "columns of starch in water form a new type of partition system in which the volume of the stationary phase is determined for each substance by the depth to which it can penetrate the starch granules."

Interest in this new chromatographic principle then developed slowly but steadily, with significant developments occurring in 1959 (the commerical availability of the cross-linked dextran gels[20]); 1961–62 (agar and agarose gels[21–23] and cross-linked polyacrylamide gels[24,25]); 1964 (cross-linked polystyrene gels for non-aqueous systems[26]); 1965 (porous glass,[27] methylated derivatives of dextrans[28] for use with organic solvents, and hydroxyalkyl ethers of dextrans[29] for use with both aqueous and organic solvent systems).

In our view, it was only thereafter—10 years later than Lathe and Ruthven's work—that the growth of molecular-sieve chromatography became really rapid. By 1969 it had become difficult to find time for even superficial scanning of the published work involving MSC (we estimate 1200 papers in 1970). We believe that the peak still lies ahead, with advances in theory, technique, and the development of new molecular sieves yet to be made.

## LITERATURE AND ABSTRACTING DIFFICULTIES

Useful information services have been a commendable feature of the part played by some of the chemical companies interested commercially in MSC, and these will serve to keep many chemists reasonably abreast of developments. But for those who require to cover the literature thoroughly and promptly, MSC has always been a difficult field to master. The breadth of its applicability to problems in organic and inorganic chemistry, ranging from physical measurements to biochemical interactions, has resulted in the publication of reports in a vast array of specialized and, frequently, rather obscure journals, as indicated by some of those cited at the end of this Review.

There has been for some time more than sufficient work to support a journal catering solely for MSC; a central focus for all the major contributions would simplify the task of those intent on keeping completely up to date with its development.

## NOMENCLATURE

No other technique in chemistry has ever been called so many names. At least 11 terms have been proposed since 1959, *viz.* Gel Filtration,[20] Molecular-Sieve Filtration,[30] Exclusion Chromatography,[31] Restricted Diffusion Chromatography,[23] Molecular-Sieve Chromatography,[24] Gel Permeation Chromatography,[26] Gel Chromatography,[32] Gel Diffusion Filtration,[33] Molecular Exclusion Chromatography,[34] Steric Chromatography,[35] and Gel Exclusion Chromatography.[36] This is, of course, needlessly confusing: all these terms refer essentially to the same basic

process—a chromatographic separation by size facilitated by porous solid and gel-type molecular-sieves, although in such a complex process, involving complex molecules, separation mechanisms of other types must almost always be expected to become involved. Their participation is not always detrimental, however; such effects have frequently been exploited (see below).

This situation has not developed without controversy. In our view, the most unfortunate aspect has been the early use[20] of the word "filtration." This was quickly criticised[31] by Pedersen, but in the next year Tiselius, Porath and Albertsson[37] maintained that the essential process "differed basically from common chromatography," and the nomenclature was later defended by Gelotte and Porath.[38] The fact remains that the technique, as it has always been practised, has always been without doubt a form of chromatography, and Porath himself has used the terms "chromatography on molecular sieves"[20] and molecular-sieve chromatography.[39] The booklet "Sephadex LH-20 ® for gel filtration in organic solvents" (Pharmacia Ltd., Uppsala) contains the statements (*a*) "Gel filtration with Sephadex ®—fractionation according to molecular size—is a well-established *chromatographic technique*" and (*b*) "Sephadex ® is a cross-linked dextran—on swelling it gives a three-dimensional network which acts as a *molecular-sieve.*" This gives us confidence to believe that "molecular-sieve chromatography" is the appropriate term.

Porath[11] has, however, explained that "gel filtration" was first used because "that expression would not put too strong an emphasis on the mechanism," even although it had been realized that molecular sieving was, in most cases, the main physical factor responsible for the differential migration. Nevertheless, Porath suggested that the terms "exclusion chromatography and "molecular-sieve chromatography" should be used only in cases where it is evident that the molecular-sieve effect is acting alone and is not superimposed on some other physical or chemical separation factor. This does not appear to be entirely reasonable. In all chromatographic processes, the basis of separation can rarely, if ever, be totally assigned to one physical effect. The term molecular-sieve chromatography is reasonable as a description when the sieving effect is predominant amongst those that can be distinguished.

"Gel Chromatography," proposed by Determann[32] as a distinction from "Gel Filtration," was later used[17] to embrace the other separation effects that are not based solely on steric factors, and it has also been favoured by Heitz and Coupek[40] and by Fischer.[41]

The short-sighted limitation imposed by the use of the word "gel" in a general term must be criticised. Rigid-type (aerogel) molecular-sieves have been used in this technique for at least 7 years; their particular properties and advantages (see below) suggest that future advances will result from their development rather than that of xerogels.

Pedersen's term "Exclusion Chromatography" has been supported by Giddings,[42] who sees the term as covering both Gel Filtration and Gel Permeation Chromatography, and also by Cassasa and Tagami.[43]

Perhaps the other main contention has involved Moore's introduction[26] of the term "Gel Permeation Chromatography" (GPC). Moore claimed that his extension of the technique, involving what is apparently the same process, to the use of non-aqueous solvents by the introduction of hydrophobic cross-linked polystyrene gels was "a new method." Although Moore's claim appears to us and to other reviewers[44]

to have been excessive, some authors[45] prefer the term GPC to the alternatives.

It has, however, become fairly common to attempt to differentiate between the terms Gel Filtration and GPC on the basis that the former is usually applied to work carried out in aqueous solutions, with the latter restricted to separations in organic solvents.[46] Indeed, the differentiation has been taken further by Wolf[47] who has proposed that "gel filtration, the group separation of molecules with large differences in size, and gel permeation chromatography, the fractionation of molecules of similar size, are fundamentally quite different. Since column operating parameters have different optima it is desirable to consider the two cases separately." In our view this does little to clarify the situation, although the first sentence of the quotation has in effect also been suggested by Determann (ref. 17, p. 87).

Agreement has, however, been reached on at least one point—that the technique involves a form of chromatography. In 1966, Anderson and Stoddart[48] proposed that MSC was the most appropriate of the terms introduced, with the sole requirement that the term "molecular-sieve" must be extended from its narrower connection with zeolites (pore diameters 0·5–2·0 nm) to include all those types of molecules, containing pores of much larger dimensions (up to 200 nm), that allow large molecules to be eluted faster than smaller molecules in a chromatographic process. The term MSC was, in effect, adopted at an international meeting in Bruges in 1966, and there has been a strengthening of support for the term by prominent workers[49–52] particularly with a view to the future development of porous materials other than gels. Determann,[17] however, retained the term gel chromatography on the grounds that "the following terms are too closely linked with restricted areas and are not generally applicable: 'molecular-sieve with zeolites, 'gel filtration with dextran gels, and 'gel permeation chromatography' with porous polystyrene gels." Determann[52] subsequently pointed out the association of these terms with particular industrial products. In a review of the utilization of molecular-sieve effects, Flodin[53] elected to retain the term gel filtration, on the justification that it is "most widely used, despite the inaccuracy of the description!" In an excellent recent review dealing with the estimation of molecular size and weight of biological compounds, Andrews[44] more reasonably uses the term gel filtration since his discussion is confined to processes involving hydrophilic gels in aqueous solutions.

In all probability, the technique will become so ubiquitous that, as suggested by Porath,[11] it may be more informative to use terms that describe the bed material, *e.g.*, dextran gel chromatography, porous glass chromatography, *etc.* In the meantime, authors[54] continue to describe GPC or gel chromatography as "new methods." Determann[52] has pointed out that, for cases where exactly the same experimental procedure is employed, it is illogical that the established separation procedure should suddenly require a new name when a development is devised.

## REVIEWS AND BOOKS

### *Reviews*

Many reviews and introductory or general articles have appeared since 1961. Merely to review these would now be a task: only a selection of these published since 1966 need be mentioned here.

1966: Andrews;[55] Anderson and Stoddart.[48]

1967: Porath; Anderson and Stoddart; Maitland; Andrews; Kellett; Male (all in ref. 49); Gelotte and Porath;[38] Altgelt and Moore;[56] Flodin;[53] Moore.[57]
1968: Wieland;[58] Tiselius;[59] Johnson and Porter.[60]
1969; Determann;[52,61] Fischer;[41] Winzor;[18] Joustra;[62] Peeters, Brisbois and Gillo.[63]
1970: Wood and Cooper;[64] Andrews;[44] Curling.[65]

*Books*

Fischer's book[41] is also published as a contribution to *Laboratory Techniques in Biochemistry and Molecular Biology*, Vol. 1 (Eds. T. S. Work and E. Work). Determann's book[17] has also appeared as *Gel Chromatography*, Second Edition (1969) but it is important to note that this is in effect a second edition of the English translation of his *Gel Chromatographie* (1967); the text and references are unchanged from the first English translation (1968) except for improvements in the technical translation and proof-reading. This book is useful for earlier work, but the number of references to work published in 1967 can be counted on one's fingers and there appear to be only two references for 1968—one a misprint for 1958, the other a reference to Determann's own work. The book is based essentially on the literature of the 1962–66 period, as is Determann's other major review,[52] dated 1969.

## RIGID-TYPE MOLECULAR-SIEVES (AEROGELS)

This Section and the next give an account of the main types of molecular-sieve available at present. Chromatographic separations involving molecular size were first observed in studies with certain porous ion-exchangers; the historical sequence of events has been summarized above.

For chromatographic purposes, rigid, porous solids (aerogels) have several advantages as molecular-sieves over the comparatively soft xerogels. The properties and applications of the latter have already been reviewed and given rather disproportionate space elsewhere;[17,41] to correct this imbalance we deal with the aerogels first of all, particularly since they appear to offer the best scope for future advances.

The ideal properties of a molecular-sieve acting as the solid support in a chromatographic column are: high chemical, mechanical, and (to a lesser extent) thermal stability; high permeability to fluid flow; absence of ionic groups; uniform particle-size; and small pore-size distribution. It has been proposed[66] that these properties will be satisfied most readily by an inorganic substance. In addition to the usual distinction between *xerogels* (organic polymers that swell in suitable solvents to give particles containing a three-dimensional network of polymer chains[67]) and *aerogels* (which do not shrink on drying; air penetrates[41] into the pores) Kun and Kunin[68] distinguished between *macroreticular* and *microreticular* gels. Aerogels are normally macroreticular, *i.e.*, have some regions where the matrix material is aggregated, and other regions where there is little matrix present, *i.e.*, there are large spaces virtually free from gel matrix, into which large molecules can penetrate; the high density regions form a skeleton that rigidly stabilizes the aerogels.[41]

*Porous glass*

The preparation and application of porous glass in molecular-sieve chromatography is popularly credited to Haller (see below) but, just as in the case of thin-layer

chromatography described above, precedence must be credited to earlier workers.

In 1943 a U.S. Patent for treating borosilicate glass was issued to Hood and Nordberg,[69] and the latter[69] described the preparation of a porous glass (Corning Code No. 7930), the properties of which could be altered by treatment with hydrofluoric acid, boiling methanol, or alkali. The glass was heat-treated at 530–700° for various times to develop a leachable sodium borate phase, and, after crushing and sieving, the particles were leached with 3*M* hydrochloric acid to give a porous glass with a narrow distribution of pore sizes of about 5 nm diameter. Treatment with 0·5*M* sodium hydroxide enlarged these by removal of siliceous residues from the pore interiors, and it was possible to obtain pore diameters in the range 4–400 nm.

In 1960 Vaughan[70] recognized that porous glass would be an ideal chromatographic support, and in the following year MacDonell[71] suggested that this glass would be useful in chromatography and applied it to the separation of printing inks. In 1962–3, Russian workers[72,73] prepared and used porous glass, and in 1965 Haller[74] described the preparation of a high silicate glass with pores of larger, closely controllable diameters and pointed out[27] the advantages of this glass in molecular-sieve chromatography.

Haller[74] described a rearrangement process at elevated temperature within the microphases of a segregated alkali borosilicate glass. Governed by an activation energy of 167 kJ/mole, a diffusive growth of the diameters of the microheterogeneous regions, proportional to the square root of the time of the process, takes place. Heat treatment schedules, resulting in the formation of microheterogeneous glasses with certain desired microphase dimensions, were established. These heterogeneous phases are completely continuous in nature, and one of the phases can be isolated by a series of acid-leaching and washing processes. A rigid, high silica glass, with a network of interconnected pores of narrow pore-size distribution, can be produced.

Haller's process[74] involved initial heat treatment of the bulk glass to give microheterogeneous regions of the desired dimensions. Crushing and screening gave a non-porous glass powder having the external particle size of the required product. In a series of leaching stages, the solid microphase within the grains could be removed and the micropores of the desired diameter were developed.

At present, the pore-diameters available commercially (See Appendix I) range from about 7 to 300 nm, giving a potential for MSC separation of compounds in the molecular-weight range of approx. $10^3$–$10^7$, although the exclusion limits vary with the nature of the material under study.

Barrall and Cain[75] made an electron-microscope study of the shape and average size of the pore channels, and it is important to note that "Controlled Pore Glass" ® and "Bio-Glas" ® are not made by exactly the same process; they are not identical, and, in our experience, they differ in performance. This is readily understandable in terms of the different compositions, methods of manufacture, and operating parameters of these two types of glass. The borosilicate glass composition is critical; traces of certain materials affect the nucleation process responsible for the pore size.[76]

The ion-exclusion properties of porous glass were reported in 1966, and, two years after Haller's reports, Cantow and Johnson[78] described the use of porous glass and determined its pore-size distributions by gas desorption and mercury porosimeter measurements. This permitted the calculation of elution volumes as a function of pore diameters; the elution volumes in toluene for polystyrenes of known coil

dimensions could then be calculated, and good agreement with experimental results was found. Haller[79] correlated the chromatographic and diffusional behaviour of molecules in beds of controlled-pore glass and was able to offer evidence regarding the validity of earlier theories.

Ross and Casto[80] have pointed out the advantage of porous glass for separations at elevated temperatures (>100°). Hair and Filbert[81] have described the surface chemistry of glass and the chromatographic properties of porous glass which has cation-exchange properties[82] and adsorption tendencies for polystyrenes[81,83] and polyisobutene.[83] Such effects may also occur with other types of molecule, resulting in retardations or possibly even total adsorption. Such effects can be minimized by operating at high ionic strength and/or high pH, or by blocking or deactivating the undesired active sites (usually hydroxyl groups) by passing a dyestuff down the column or presaturating the column with the substance to be examined chromatographically. Silicones, or highly polar compounds, can be injected at intervals to maintain the level of deactivation.[81]

The preferred method, however, is to deactivate by esterification of the active hydroxyl groups with 3-hydroxypropionitrile,[84] or, more commonly, by silanation—as has long been advised in column chromatography for the elimination of "wall-effects."

The achievement of effective silanation is frequently a form of art. Hexamethyldisilazane, trimethylchlorosilane, or dimethyldichlorosilane[81] may be used. Attention to detail is necessary; more complete silanation appears to result if the material is acid-washed, then very carefully dried at temperatures >200°. After treatment, all free chloride must be removed; otherwise hydrolysis will restore free hydroxyl groups. Cooper and Johnson[83] have reported a successful procedure for porous glass with hexamethyldisilazane at 250° for 2 hr under vacuum. Treatment at 130° for several hr was ineffective, and silanation *in situ* was only partially successful [six successive injections (1 ml) of hexamethyldisilazane carried by dry nitrogen at 0·5 ml/min through a packed column wound with heating tape].

The introduction of porous glass has been a most important advance in MSC. Columns are easily packed; no swelling or conditioning time is required. Their rigid structure and narrow pore-size distribution gives a high permeability, a constant bed-volume, and constant pore-size regardless of the temperature or composition of the eluent, its flow-rate, ionic strength, or applied pressure. The use of gradient elution is possible. Good resolution and high capacity are given. There is no organic polymer "bleed" and no tendency to biodegradation. Packed columns can be regenerated, cleaned or sterilized by heat treatment or by hot nitric acid.

Karger, Conroe and Engelhart[85] have described the use of "surface-textured" glass beads for high-speed liquid chromatography.

*Porous silica beads*

Porous silica beads can be produced in a range of carefully controlled pore-diameters, and have similar advantages to porous glass. They were introduced[86] by de Vries *et al.* in 1966; evaluations of their use and studies of the operative processes followed.[87] The beads developed by de Vries are available commercially in 6 different types (see Appendix I) as Porasil ®.

Porous silica, like glass, may require deactivation. Pyridine and valeric acid are

strongly adsorbed on untreated Porasil but give only slight adsorption effects on the deactivated beads; polyvinyl alcohol is permanently adsorbed on untreated Porasil, but it can be fractionated reproducibly,[88] with no evidence of adsorption, after the Porasil has been deactivated. Barker *et al.*[89] have developed an automatic procedure for the fractionation of dextrans on porous silica beads, and have also evaluated their advantages in the fractionation of dextran and of hyaluronic acid. They found that porous silica beads have distinct advantages over organic xerogels; after a column had been calibrated, it could be used indefinitely, as a routine analytical and preparative device, with no possibility of sample contamination. The rigidity of the beads gives a constant total bed-volume, even when the column is operated at high back-pressures, which can arise from high flow-rates or from the use of finemesh particles.

Silicones can be synthesized on, or chemically bonded to, silicic surfaces, *e.g.*, with octadecyltrichlorosilane, giving non-extractable, thermally stable coatings.[90]

Kirkland[91] has described spherical siliceous particles that have a porous surface of controlled thickness and pore-size, for which several chromatographic advantages have been claimed.

*Inorganic oxides*

Meyerhoff[92] has used a standard, highly cross-linked polystyrene to test the resolving power of porous glass, silica, alumina, and mixed silicon-aluminium oxide beds. He concluded that, for low molecular weight substances, organic and inorganic molecular-sieves were equally effective, but inorganic sieves were preferable for high molecular weight materials.

*Porous ceramic (illite)*

The use of illite was investigated by Eriksson *et al.*[93] in 1968 for use with the strongly alkaline solvent triethylenediaminecadmium hydroxide (Cadoxen), for which porous glass was unsuitable.

*Microporous carbon*

The formation of microporous carbon with molecular-sieve properties has been studied by Russian workers.[94]

*Inorganic "quasi-matrix gels"*

The properties of silica and alumina gels can be modified[95] with polyvinyl alcohol, and the resulting "quasi-matrix gels" can be use for the fractionation of high polymers, *e.g.*, polystyrene and styrene–butadiene block co-polymers.[96]

## XEROGEL MOLECULAR-SIEVES

In contrast to the aerogels, xerogels have been extensively reviewed elsewhere.[17,41,67]

*Cross-linked dextran gels*

The cross-linked dextran gels were described[20] in 1959; they were the first gels to have their behaviour systematically investigated,[41] and the first xerogels to become available commercially. Dextran, an α-1,6 glucan with 1,3-linked side-chains derived from fermentation of sucrose by *Leuconostoc mesenteroides*, is fractionated by alcoholic precipitation to yield a fraction of appropriate molecular weight; this is emulsified in

a non-aqueous medium and treated with epichlorhydrin, which forms glyceryl cross-links between the original dextran chains. The extent of cross-linking can be controlled within limits; 8 types that swell reversibly in aqueous solution to give effective molecular-sieves are available commercially (see Appendix II).

*Substituted dextran gels*

Methylated dextran gels have been prepared,[28] and the hydroxypropyl ether of the Sephadex ® G-25 dextran, available commercially as Sephadex LH-20 ®, can be used with either aqueous systems or polar organic solvents.[29] The methylated dextran gels have had relatively little application,[97] but the LH-20 product has been widely used as the following selection of recent applications indicates: the separation of prostaglandins;[98] studies of cholesterol autoxidation products;[99] the fractionation of complex mixtures of reducing sugars and glycosides;[100] the separation of non-ionic detergents from proteins;[101] studies of protected peptides in attempted peptide syntheses,[102] and of thyroid iodoamino acids;[103] the purification of heptafluorobutyrate esters of steroids;[104] the separation of fat-soluble vitamins,[105] plant growth regulators,[106] alkanes,[107] fatty acids,[108] phospholipids,[108] and of neutral hydroxy lipids.[109]

Gradient-elution has been useful[110] with Dextran LH-20 ®; column and detection techniques for use with this molecular-sieve have been reviewed.[111] Horler[112] found LH-20 useful for the clean-up of pesticides extracted from grain, but Ruzicka *et al.*[113] found it of little use for this purpose although it was potentially useful for the identification of pesticides because of the constancy of elution volumes on columns over long periods and of the almost invariable quantitative recoveries obtained. Chromatography on Dextran LH-20 is a useful adjunct in the identification of organophosphorus pesticide residues in river waters and effluents.[114]

In experiments with lignin, fractionation on LH-20 was observed[115] to be based on molecular size. Adsorption effects on Sephadex LH-20 columns have, however, occurred with oligomers of diphenylcarbonate and 4,4-dihydroxydiphenyl-2,2′-propane,[116] with phenolic glucosides,[117] and with porphyrin esters.[118] Brooks and Keates[119] have made a careful study of liquid chromatography on lipophilic dextran derivatives. They found the molecular size of alcohols, alkanes and steroids to be the major factor determining elution volumes, but indicated the need to match the chromatographic "polarity" of the gel and the solvent to ensure that separation on the basis of size takes place.

The preparation of substitution products of Sephadex-LH-20 has been described;[120] the diethylaminoethyl derivative is effective for the fractionation of acidic lipids.[121] Oelert[122] has described some anomalous effects on LH-20 columns.

Cross-linked dextrans contain a small proportion of carbonyl groups, and that deviations occur from ideal molecular-sieving on dextran gels, as a result of ion-exclusion, adsorption, and other interactions, has been known since 1960, when Gelotte[123] reported the now well-known "aromatic effect." The anomalous effects given by inorganic ions,[124] aliphatic alcohols, alkane diols, aromatic and heterocyclic compounds, conjugated polymers, proteins, and low molecular weight compounds with extended coplanar systems of $\pi$-electrons have been reviewed, together with the methods of overcoming such experimental difficulties.[41,44] Nevertheless, anomalous effects with oestrogens,[125] biologically important phosphate esters,[126] and phenols,

organic acids and bases[127] continue to be reported. These effects, which are not exclusive to the dextran gels, can be exploited with some systems in order to achieve difficult separations. New methods of improving the chromatographic properties of dextran gels have been described,[128] and it has been suggested[129] that their internal pressure is an additional characteristic.

Recently, cyclodextrin gels (cyclohepta-amylose) have given[130] useful separations of nucleic acid components, probably on the basis of inclusion complex formation.

*Rubber gels*

Brewer[131] used swollen rubber gels in 1960, but little further use has been made of these.

*Agar and agarose gels*

The early recognition[8] of the porous character of agar gels, and Polson's utilization of agar gel[15] and of 7% cross-linked granular agar[21] has been described. Advances came when the possibility of increasing agar[132,133] to obtain the uncharged agarose component was exploited;[22–24] a further development was the introduction of the "bead-gelled,"[134] "pearl-condensed"[135] and "sphere-condensed"[136] products.

The agar and agarose gels differ from the dextran gels fundamentally; agarose gels were found by Laurent [137] to be random three-dimensional networks of long fibres, approx. 5 nm in diameter, held together by hydrogen bonds. The concentration of agarose in the gel largely determines its fractionation range as a molecule-sieve. In contrast, the preparation of agarose cross-linked with epichlorohydrin has also been described.[138] Commercial products based on agarose concentrations from 0·5–10% are available from several sources (see Appendix II). A good agarose preparation will form gels at concentrations of approx. 0·1%.[139] Because of the complex nature of agarose, its variation from one seaweed species to another, the seasonal variations that occur, and the fairly complex fractionation and bead-gelling manufacturing processes involved, there is, in our experience, considerable variation in the properties and performance of the different commercial agarose gels. How and Long[140] have also reported this.

Renn and Mueller[139] have stated that dehydrated agarose products can be prepared; when rehydrated, they are essentially indistinguishable from freshly prepared gels. Such dehydrated products ("Sea-Sep") are now available commercially (see Appendix II). In this respect, recent reviews[41,44,67] are not entirely correct. At the present time agarose gels offer the possibility of sieving molecules of molecular weight up to $150 \times 10^6$ and a suitable void-volume marker (bacterial lipopolysaccharide) to extend the range of calibration offered by "blue dextran" (Pharmacia Ltd., Uppsala) has been described.[34] The softer agarose gels of lower concentration do, however, have disadvantages; they compact and have poor permeability, especially in long columns; they are subject to "bleed", and are not too stable chemically, thermally, or mechanically.

An interesting distinction has to be made between the physical structure of the commercially available "beads" and the form of agarose described as "crushed granules." It has been reported[141] that the beads and crushed granules do not have the same internal geometry, and, although the beads are more uniform and easier to handle, the crushed granules appeared to be preferable in terms of performance.

In terms of the "skin" theory,[142] some or all of the pore entrances are believed to become fractionally restricted during the bead emulsion polymerization process.

Composite acrylamide–agarose gels were described by Uriel[143] in 1966. The composition 2·25% acrylamide + 0·5% agarose gives pores sufficiently large for the examination of polyribosomes; 3% acrylamide + 0·5% agarose gives gels useful for smaller molecules.[144]

Hayes and Mitchell[145] have studied the chromatographic characteristics of polydeoxynucleotides on agarose columns, using different temperatures, eluents and agarose pore-sizes. The addition of urea to the solvent for part of the elution minimized unwanted hydrogen-bonding co-chromatography, and the polydeoxynucleotides were considered to chromatograph as elongated structures since they gave elution volumes greater than those of globular proteins of the same molecular weight. Other recent applications of agarose gels have included the detection of artifacts of high molecular weight in acidic polysaccharides,[146] the resolution[147] and preparation[148] of DNA, the separation and fractionation of hyaluronic acid from synovial fluid,[140] the determination of the molecular weight of ribonucleic acid polymerase[149] and of other proteins,[150] the purification of lipopolysaccharides,[151] and the fractionation of endotoxins of gram-negative bacteria.[152]

*Acrylamide gels*

These were first described by Hjertén and Mosbach[24] and by Lea and Sehon.[25] The polymerization of acrylamide in aqueous solution in the presence of *N,N*-methylenebisacrylamide, with ammonium persulphate as a redox catalyst and *β*-dimethylaminopropionitrile as regulator, leads to the formation of gels, the pore-diameters of which depend on the proportions of the reagents taken. Fawcett and Morris[153] observed that more porous gels of a different structural type result when relatively high concentrations of the cross-linking reagent are used. Ethylene diacrylate can also be used for the cross-linking of acrylamide.[154]

Polyacrylamide gels are commercially available in 10 different pore-sizes (Appendix II), each of which is available in different particle-sizes. In our experience with the chromatography of polysaccharides, polyacrylamide gels are preferable to dextran gels since they appear to be more stable biologically and chemically, and do not in any event give a carbohydrate "bleed." At extremes of pH, hydrolysis of the amide groups in the polyacrylamide gels to give carboxyl groups can increase the possibility of ion-exchange side-effects.[41] Adsorption effects for both organic and inorganic molecules have been reviewed;[44,155] "Bio-Gel P2" has been used[156] for the adsorption chromatography of lysozyme.

*Silica gels*

In 1962, Vaughan[157] reported a fractionation of polystyrene on a silica gel preparation, "Santocel A." Little interest was shown until a report by Kohlschuetter[158] *et al.* in 1966, then McCallum[159] investigated the use of silica gel as a molecular sieve for polyvinyl chloride, polychloroprenes, and polyethylene. Interest in silica gels has increased recently. Halpaap and Klatyk[160] have prepared gels having pore diameters in the range 1–100 nm, and Wacks[161] has described the production and use of molecular sieves rich in silicic acid. Hydrothermal treatment with steam at 800° for 24 hr enlarges pores of 5 nm in silica gels to 500 nm.

*Polymethacrylate gels*

Cross-linked poly(methylmethacrylate) gels useful for low molecular weight (<2500) materials were described[162] in 1964, and co-polymerization of methylmethacrylate and ethylene glycol dimethacrylate in the presence of inert substances, which are subsequently removed, gives gels which show a limited ability for molecular sieving.[163,164]

*Polystyrene gels*

The first attempt, in 1960, to use polystyrene gels cross-linked with divinylbenzene is credited to Vaughan,[70] whose attempts were not very successful as a result of adsorption and partition effects. Although Moore[26] is now commonly credited[11,41] with the successful development of polystyrene gels suitable for non-aqueous molecular-sieve chromatography in 1964, Tipton, Paulis and Pearson[165] reported in the same year a successful separation by molecular size of lipids on polystyrene. Rigid polystyrene gels for non-aqueous solvent systems are commercially available, in 11 different porosities, as "Styragel ®" and also as "Bio-Beads S" (See Appendix II). Partially sulphonated polystyrene beads for molecular-sieve chromatography of aqueous solutions are available commercially as "Aquapak A440" and as "Bio-Beads SM" (two pore sizes).

A great deal of work has been done in the past 5 years with polystyrene gels; it has mostly been published under the title "gel permeation chromatography" and has largely concerned the fractionation, estimation of molecular weights, and the determination of the molecular weight distribution of hydrophobic polymers. Recent applications have included the study of lipid–protein complexes[166] and the separation[167] of high-boiling petroleum fractions without the thermal hazards of distillation; these separations were achieved on a molecular size basis, although some adsorption effects were also observed.

In 1968, Greber and Haussmann[168] described the preparation of polystyrene or poly(α-methylstyrene) cross-linked with substituted chlorosilanes, and Makarova and Egorov[169] have prepared co-polymers of styrene and *m*- or *p*-divinylbenzene. Chloromethylated and (2-aminoethyl)aminomethylated derivatives of 1% cross-linked polystyrene beads are available commercially.

*Other synthetic gels*

The use of cyclodextrin gels has been mentioned above. Coupek and Heitz[170] examined the properties of polyvinylalcohol gels. Heitz and Platt[171] have described the preparation of cross-linked co-polymers of vinyl acetate with divinyladipate and of butanediol–1,4-divinyl ether, which give sieving of molecules up to molecular weight 4000. When inert diluents are included in the co-polymerization, the pores are large enough for molecules of molecular weight up to $1 \times 10^6$. The preparation of resins and gels within silica gel pores has also been studied;[169] the inorganic lattice can be removed subsequently by treatment with sodium hydroxide or hydrofluoric acid. Polymers with particular porosities are obtained by varying the properties (specific surface area, pore volume and dimensions) of the silica gel. *N*,*N*-diallyltartardiamide has been used[172] for cross-linking gels that can be dissolved in 2% periodic acid.

*Cellulose gels*

In 1967 the molecular-sieve properties of both unmodified and cross-linked decrystallized cotton cellulose were reported by Martin and Rowland[173] to be similar to the dextran and polyacrylamide gels of smallest pore-size. Determann *et al.* described new gels from cellulose,[174] and also the preparation[175] of rigid, chemically stable cellulose gel beads suitable as sieves for the molecular weight range $10^4$–$10^7$. The preparation involves the regeneration with benzoic acid in benzene of 2, 6 and 10% solutions of cellulose containing Schweitzer reagent. Martin *et al.* have recently reported an MSC technique for the characterization of chemically modified cellulose,[176] and the molecular-sieve properties of decrystallized cotton cross-linked with formaldehyde.[177] It appears rather strange that Rinaudo and Merle[178] should claim very recently to be able to "disclose the first results obtained by GPC in the cellulose field" (for GPC read MSC).

## COLUMNS

There is a great deal of expertise involved, and there are many factors to consider, when setting up a column for molecular-sieve chromatography; a good account of these aspects of the technique has been given by Fischer.[41]

If careful attention to important details is given, molecular-sieve chromatography can be carried out successfully in a simple straight glass tube constricted suitably at one end. Columns 0·3–0·5 m long × 15–25 mm diameter have been used in many applications. Columns longer than this may give difficulty through compaction if the softer gels are involved; columns of smaller bore may give "wall effects." The constriction should be such as to allow a good bed support of glass wool and sand to be formed and should also allow direct connection of 1-mm diameter plastic tubing, so that minimum dead space, that would give mixing of separated zones, occurs beneath the molecular-sieve bed. Smith and Feldman[179] have discussed the resolution obtainable with MSC columns.

The use of columns of square cross-section has been described,[180] and there have been several reports[181] of the use of "microcolumns" (1–3 mm diameter) for work with microgram amounts. Wasteson[182] has used polythene tubes (1·7–3 × 60–100 mm) and glass capillaries (0·8–1·2 × 60–100 mm) for analytical studies on chondroitin sulphate at the microgram level: Sephadex G-200 dextran was used with 1 *M* sodium chloride as eluent. The total volume of the columns was 0·5–7 ml and vibration was applied when packing the columns; 100 $\mu$g samples in 50–100 $\mu$l of eluent were used. The ultimate in small-scale operation has probably already been attained[183] through the use of single Sephadex ® particles.

Scaling-up for preparative work has also been investigated,[184,185] and applications range from the purification of 5-g quantities of alkaline phosphatases[186] to the desalting of 1500 l of solutions per hr at almost negligible cost.[184]

Specially designed columns for all scales of working are now available commerically from sources that have been detailed by Fischer.[41]

## DETECTORS/MONITORS

Continuous monitoring of some differential function of the effluent from any chromatographic column is preferable to the collection and subsequent examination of small, arbitrary fractions.[187] There is quite an array of possible detectors for molecular-sieve chromatography, one of which may be preferable to the others for some particular analysis.

Differential refractometers with micro flow-through cells are widely used[188,189] although they are relatively insensitive (sensitivity ~10 μg/ml) and are useless for studies involving solvent temperature changes or gradient elution. The other common method is ultraviolet absorption spectrophotometry, sensitive to ~1 ng, but of course not universally applicable; nevertheless this principle is still widely used.[9,190] An important extension of that technique was the direct ultraviolet scanning of gel columns, proposed by Brumbaugh and Ackers.[191] This gives several advantages, *e.g.*, (*a*) a record of the actual development of a reaction boundary is obtained (as in the ultracentrifuge); (*b*) scanning at different wavelengths gives concentration profiles of individual components within a reaction boundary or zone; (*c*) the distortions due to outflow adapters and flow-cells are eliminated, and adsorptions of substrates at low ionic strength can be detected. The technique appears to be particularly suitable for studies of interacting systems of macromolecules.

Infrared spectroscopy[192] has been used recently,[83] and the continuous measurement of conductivity can also be used; suitable apparatus is available commercially. Jackson[193] proposed that differences in dielectric constant could be measured, and a thermal detector to measure the temperature effects that arise when solutes interact with the column packing was described.[194]

Radioactive labelling[195] and polarography[196] can also be used, and there has been a revival [197] of true chromatography, in which polysaccharides are treated with reactive dyestuffs prior to chromatographic separation. Shipman and Cole[198] monitored the separations achieved on a constant-flow column by following the changes in the effluent drop volume. Runyon *et al.* have described[199] the use of multiple detectors for molecular weight determinations and for composition analysis of co-polymers separated on polystyrene columns.

Increased sensitivity was achieved by the adaptation[200] to MSC of the flame-ionization and argon-ionization techniques commonly used in GLC; the eluent is collected and conveyed to the detector on a moving hot wire. This technique has been widely used[181] since it is applicable to all classes of compounds in organic solvents and is independent of the method of chromatographic development. Scott and Lawrence[201] have recently devised a means of increasing the sensitivity to 1·1 μg/ml. Conductivity[202,203] and differential vapour-pressure[204] detectors have also been employed.

## SPECIAL EXPERIMENTAL TECHNIQUES

Hanson[205] has recently described a high-speed desalting technique for kinetic studies such as hydrogen exchange in DNA. Small columns (10·5 × 40–55 mm) are used at pressures up to 420 kn/m$^2$; 25-μl samples are applied, and the process takes 20–100 sec for completion. "Wall effects" are important, and the molecular-sieve used is discarded after each run.

The basket centrifuge technique[206] is useful for preparative desalting and for the removal of low molecular weight impurities from relatively concentrated solutions of polymeric materials, and what appears to be an extension of the technique has been described recently.[207] A slurry of the molecular-sieve gel is spun in a centrifuge-tube fitted with a riddle plate, through which can pass the excess of solvent. Then the solution to be treated is placed on top of the gel, which is again centrifuged. The procedure gave γ-globulin G that was almost immunoelectrophoretically pure. It is claimed that the basis of separation is not the usual one, but depends on the solubility of the protein in a medium poor in salt.

"Interrupted-flow" chromatography[208] has been described, in which the solvent flow can be interrupted, for delays up to 62 hr, with no difference in the effective resolution as indicated by the chromatograms for a solute of molecular weight $1{\cdot}6 \times 10^5$. Fox *et al.*[209] have given details of the construction, operation, and some applications of an apparatus for continuous chromatography with molecular-sieve gels; continuous chromatographic analysis on an automated basis has also been described by Barker *et al.*[89] The recycling technique of Porath and Bennich,[210] wherein the eluent from a column can be pumped back into the inlet has been used on the gram scale[211] for the fractionation and purification of histones.[212] A modified technique that eliminates the danger of "overtaking" has been described.[186] Upward-flow techniques offer the possibility of higher flow-rates than is normally possible with long beds of soft gels. Short columns connected in series provide another alternative.

The use of flow-rates of up to 12·5 ml/min, giving reductions in the process time from 4 hr to 30 min, has been shown[213] to be readily attainable through optimization of the operating parameters, without significant sacrifices in terms of resolution or peak width; the peak-spreading with increasing flow-rate was found to be much less than predicted by the van Deemter equation.

Hellsing[214] described an interesting method, based on steric exclusion effects, for extending the fractionation range of a molecular-sieve to higher molecular weights by the addition of a suitable neutral polymer to the chromatographic eluent. A human albumin was eluted more slowly from a cross-linked dextran column when the eluent contained dissolved polyethylene glycol, polyvinyl alcohol, or Ficoll ®, a high polymer of sucrose. Burke[215] has reported that the reversible adsorption of steroids on dextran gels is counteracted by the addition of serum or plasma, although the adsorption of steroids on polyacrylamide gel is not reduced. A similar effect was found in a study of proteins; high recoveries were not obtained unless the dextran gel column was first treated with serum to block the active sites.[216]

Heitz and Coupek[217] gave details for the compressive packing of columns, and Edwards and Helft[218] have reported that compressed beds give increased resolution, elution zones being sharpened and the void volume decreased. The technique involves applying compression (suitable jacketed columns are available commercially), increasing the flow-rate, then increasing the compression again, *etc.*

The use of less common solvents frequently extends the applicability of MSC. Aqueous hexafluoroacetone[219] was useful for the study of large peptides derived from chymotrypsinogen A, although the Sephadex ® gel beads were more fragile and the flowrate was decreased. The use of illite as a sieve for use with "Cadoxen" solutions[93] has already been mentioned, and this powerful solvent has been used in a study of regenerated celluloses on agarose gel.[220]

## COMBINED TECHNIQUES

The combination of two distinct techniques to give more powerful methods of analysis is now well-known, *e.g.*, GLC/Infrared and GLC/Mass Spectroscopy. Radioimmuno-assay has been combined[216] with MSC to detect antibody to the basic proteins of myelin, and immuno-MSC was used in the study of cerebral fluids[221] following a technique published by Hanson *et al.*[222] in 1966. Brain proteins have been separated[223] by a combination of MSC and electrophoresis. The distribution of protein-bound copper in human serum has been determined[224] by a combination of

MSC and neutron-activation analysis. The branching distribution in polymers has been studied[225] by concurrent MSC and sedimentation velocity experiments.

## THIN-LAYER CHROMATOGRAPHY WITH MOLECULAR SIEVES

The advantages offered by thin-layer chromatography—speed, sensitivity and simplicity—are well-known, and the use of molecular-sieves to form the thin-layer coatings was first applied in 1962.[226,227] The combination is, in general, a happy merger—the general principles and techniques of thin-layer chromatography still apply, and virtually all of what has been summarized above from the viewpoint of work with molecular-sieves in columns also applies. The technique is now widely used, and not only for proteins, as was undoubtedly the case until about 1967. Several reviews have dealt adequately with the relevant literature[17,41,44,228] and these give sufficient experimental detail to allow the novice to take up the technique.

It is customary to use the smallest particle-size grades available, and the dextran,[229–231] polyacrylamide,[229] agarose,[232] and Sephadex ® LH-20 gels[233] have all given good results for particular systems. In addition to work with proteins,[229,231] the technique has been applied to dyestuffs,[233] tetrazolium salts,[230] humic acids;[234] and antibiotics.[235] In the latter investigation, the fact that the chromatoplates were eluted with aqueous buffer and not with organic solvents was exploited, since a specific and sensitive bioautography technique[236] could be applied directly to the identification of the 17 antibiotics involved. After development, the chromatoplate was taken from the elution chamber and pressed on to a seeded agar plate covered with a sheet of lens tissue paper. After "printing" for 30 min, the chromatoplate was taken off, the lens tissue removed, and the agar plate incubated at the optimal growth temperature of the test organism. As expected,[237] the resolution on dextran gel depended on adsorption rather than on graded accessibility—the antibiotics that were separated did not differ appreciably in molecular weight.

Maier[238] has extended the application of thin-layer molecular-sieve chromatography by eliminating the need for destruction of the sample at the identification stage. Lissamine Rhodamine B200 is one of several useful fluorescence-quenching agents; on exposure of the treated plate to ultraviolet light the separated zones are seen as dark areas against an orange background.

## APPLICATIONS OF MSC TO LOW MOLECULAR WEIGHT COMPOUNDS

The following short selection of recent applications of MSC serves to show that MSC has applications to molecules of all molecular weights.

One of the simplest fractionations achievable by MSC is the separation of small molecules from polymers (*i.e.*, desalting) *e.g.*, the recovery[239] of apoferritin from 2% ammonium sulphate solution. There are very many such examples.

Cross-linked dextrans have been used for the separation of paraffinic classes in petroleum distillates,[240] and for the removal[241] of penicilloylated protein and high molecular weight self-aggregates of purified benzylpenicillin, both of which can cause humans to have allergic reactions to penicillin. Polyacrylamide gel gives[242] rapid separation of mono- and oligosaccharides (up to 13 glucose units); quantitative calibration over the range 10–100 $\mu$g can be effected. The plot of elution volume *vs.* log (mol. wt.) is linear for mol. wt. $\geqslant$ 600. For smaller molecules it appears that the mechanism of separation differs from pure molecular-sieving. The technique has also been applied to the analysis and characterization of glucose syrups.[243]

Triglycerides can be separated from free fatty acids[244] and from diol lipids.[245] Dextran gels have been used for the separation of salicyclic and acetylsalicyclic acids,[246] of benzoic acid from volatile fatty acids,[247] of maleic from fumaric acid,[196] of ethylene glycol from di-, tri-, and tetraethylene glycol,[38] and for the fractionation and identification of glucosides.[248]

Oestrogens[249] can be investigated, and cyclic monomers and oligomers in Nylon 66 have been studied.[250] The dissociation constants of organic acids and bases such as phenylacetic acid and *N*,*N*-dimethylaniline can be determined.[127] Pontis[251] has achieved separation of the first ten members of a series of fructose oligomers.

## APPLICATIONS OF MSC TO INORGANIC MATERIALS

The following few references indicate that the use of MSC is not confined to organic molecules.

Ortner and Spitzy[252] reported that 1–1000 $\mu$g amounts of all the alkali metal chlorides could be separated successfully on dextran that had been swollen in methanol–water mixtures. Egan[253] studied the behaviour on gel columns of the chlorides and nitrates of alkali and alkaline earth metals, the nitrates of Mn(II), Tl(I) and Ag, and $F^-$, $Cl^-$, $NO_3^-$ and $SO_4^{2-}$ as sodium salts. Egan discussed the electrolyte behaviour on gels in terms of hydrated ionic size, adsorption, and chemical interaction. The applications of gel columns to the qualitative and trace analysis of anions and cations were carefully investigated.[254,255] Streuli and Rodgers[256] gave references to earlier studies of inorganic systems, and studied the existence of the species $MoO_4^{2-}$, $Mo_7O_{24}^{6-}$, and $Mo_8O_{26}^{4-}$ at fixed pH values in acidified molybdate solutions.

Dextran gels can be used for the separation of chromium(III) hexacyanoferrate(II) complexes[257] and also for the alkaline earth metal ions.[258] Inorganic phosphate polymers[259] and polydimethylsiloxanes[260] have been studied, and the technique has been useful for the purification of commercial Alizarin Red S required as a reagent for the determination of aluminium in silicates.[261] Oligophosphates and an iron(III) monomer/polymer system have been used[262] to evaluate the performance of various molecular-sieves; the results indicated that the various molecular-sieves available should always be tested in order to find that giving the optimum performance for the system involved.

MSC has been useful for the study of calcium and magnesium salts in normal and stone-forming urines.[263] The maximum recovery of anions and cations was obtained with ammonium acetate and formate buffers at pH 4·0–6·0. The anions were clearly separated and eluted before the cations, with magnesium eluted before calcium. The technique has been applied with less success to the study of calcium and magnesium[264] not bound to serum.

There are both advantages and limitations in using MSC to study metal ion binding; the effect of 2′- and 3′-cytidylic acid on the binding of copper(II) ions by RNase has been studied,[265] and the binding of mercury in rats has been investigated[266] by means of $^{203}HgCl_2$. Studies on dextran gels enabled the optimum conditions for the labelling of human fibrinogen with $^{131}I$ to be found, and also gave a way of purifying the product from iodine not bound to the protein.[267]

## APPLICATIONS OF MSC TO ENZYMATIC ANALYSIS

Enzymatic methods of analysis offer the advantages of great sensitivity, specificity, and speed, and their development is attracting much attention at the present time. The following references show that MSC is facilitating these advances.

The phosphors present in firefly tail extracts are the basis of sensitive analytical procedures: luciferin, dehydroluciferin and ATP can be separated on low-porosity dextran gels, and luciferase can be separated from other kinase-type enzymes on high-porosity gels. As a result, enzyme preparations with greatly increased specificity have been obtained.[268] MSC has also been used in the purification of ficin[269] and other proteolytic enzymes.[270] The cellulase involved in the solubilization of cotton has been fractionated into a CM-cellulase, a cellobiase, and an inactive substance. It was found, however, that only when these three components were recombined was the ability to convert cellulose completely into water-soluble products regained.[271] NAD-glycohydrolase from streptococci has also been fractionated into three fractions.[272] Dextran gels can be used[273] in a simpler, rapid, reliable method for the separation from urine of lactate dehydrogenase, alkaline and acid phosphatase, leucine aminopeptidase, arylsulphatase, and $\beta$-glucuronidase.

Henn and Ackers[274] have contributed a series of researches on the interaction of protein systems, and, as examples of a field of study in which MSC offers great advantages, papers reporting molecular weight estimations of flexible disordered proteins[275] and polypeptide chains[276] continue to be published frequently. The protein-binding of small molecules[64] and the estimation of molecular size and molecular weights of biological compounds[44] have been reviewed.

*Affinity chromatography and the insolubilization of enzymes*

Following a preliminary publication[277] in 1964, Silman and Katchalski[278] described the coupling of enzymes to polystyrene gels in 1966. Porath, Axén and Ernback[279] published a method in 1967 for the formation, by reaction with cyanogen bromide, of reactive polysaccharide intermediates which facilitated the coupling of enzymes to dextran; this was extended,[280] in 1969, by the coupling of acetylcholinesterase and butyrylcholinesterase to agarose gels. The coupling of yeast hexokinase to dextran gels was also reported.[281] The technique can be extended[282] to the purification of synthetic peptides by coupling the necessary conjugate protein. Coupled enzymes are available commercially.

In 1968, Cuatrecasas *et al.*[283] used Porath's cyanogen bromide technique[279] to couple biocytin (and chymotrypsin) to agarose gels; the resulting biocytin–sepharose column gave a single-step purification of avidin from egg white. Cuatrecasas suggested that separations on such columns should be termed "Affinity Chromatography," and this term has been used by Chua and Bushuk,[284] who purified wheat proteases on an agarose column to which haemoglobin was found covalently. Since then, rapid advances have been made. Chymotrypsin can be attached chemically to water-insoluble polymers by means of 2-amino-4,6-dichloro-*S*-triazine;[285] papain has been "insolubilized" with glutaraldehyde;[286] and Guilbault and Das[287] have "immobilized" urease and cholinesterase on starch and polyacrylamide gels. Unfortunately, the proliferation of terms describing essentially the same basic principle appears to have begun once again. Recently, Omenn *et al.* have summarized[288] papers describing the attachment of antibodies, hormones, enzyme substrates and polypeptide fragments to molecular-sieves as well as to other chromatographic supports.

## APPLICATIONS OF MSC TO THE DETERMINATION OF MOLECULAR WEIGHT AND MOLECULAR WEIGHT DISTRIBUTION: CALIBRATION

These are perhaps the best known applications of MSC. The amount of published work involved is already voluminous, particularly for studies of proteins and synthetic polymers, and this aspect of the subject has been given prominence in several of the reviews cited above. Therefore only a few very recent references are given here to summarize the present situation.

MSC has been described as "the poor man's centrifuge" and since the main mechanism of the process involves chromatographic separation on the basis of molecular dimensions, it is one of the simplest of matters to establish whether a polymer system is heterogeneous or homogeneous and also whether the molecular-weight distribution is broad, narrow, skew, *etc.*

If the molecular-sieve column can be calibrated in terms of the elution volumes of adequately characterized standards of known molecular weight, then *estimations* [$\pm(5\text{–}10)\%$] of molecular weights can be made rapidly and conveniently with very small amounts of material.

A main difficulty is that although there is a selection of materials covering a fairly wide range of known molecular weight available commercially, these are usually proteins, dextrans, or polystyrenes (Appendix III). Although there is some evidence that dextrans act as reasonable standards for other types of polysaccharide, dextrans are certainly not valid as standards for proteins and *vice-versa.*

Over the past 10 years, at least 15 different relationships between the molecular weights of polymers and various expressions for their elution volume parameters have been "discovered." Several of these relationships are, however, mathematically equivalent, or differ only in the evaluation of some constant.

In 1965, Meyerhoff[289] introduced an expression relating the elution volume to log $(M^{1/2}\eta^{1/3})$ where $M$ is the molecular weight and $\eta$ the intrinsic viscosity. This form of plot gives a series of parallel straight lines for different series of polymers if the same column, solvent and temperature are used; it therefore gives a useful qualitative test for polymer types and its validity was later confirmed experimentally by Grubisic, Rempp and Benoit.[290]

Three important papers then followed in close succession, and the following order observes their priority in terms of the submission dates. Rohn[291] plotted the intrinsic viscosities of polyvinyl chloride fractions against their elution volumes and deduced that the column permeation of chain macromolecules was governed "by some sort of hydrodynamic size"; he took the intrinsic viscosity ($\eta$) to be a measure of the hydrodynamic volume of a polymer in solution. Wild and Guliana[292] studied the effect of chain-branching in polyethylene, and found that a single relationship for both linear and branched polyethylenes was obtained by plotting $\eta M$ against the elution volumes. Wild and Guliana reached the same conclusion as Rohn,[291] *i.e.*, that the separation on gels was dependent on the hydrodynamic volume of the polymer molecules, and they suggested that the effect would hold for other polymer–solvent systems. Grubisic, Rempp and Benoit[290] then proposed that a "universal calibration," in which the elution data for all classes of polymers would lie on one line, was obtained if the elution volume was plotted against log $(\eta M)$, a function giving, on the basis of Einstein's viscosity law, a direct measure of the hydrodynamic volume of

dissolved particles. They showed the relationship to hold for linear, comb-, ladder-, and star-shaped polystyrenes, and for polymethacrylates, polyphenylsiloxanes, polyvinyl chloride, and graft co-polymers.

Refinements,[293], corrections,[294] and a case where the universal calibration was inadequate[295] have been reported. The universal calibration only holds[293] when the molecular weight of the solute is sufficiently high for random-coil statistics to be valid. Other forms of calibration have been based on carbon number,[296] and on the unperturbed dimensions of a polymer.[297]

Fractionated polymers of narrow molecular weight distribution[298] are not always available as calibration standards, and several papers[299–303] have discussed the basis for effecting calibration with unfractionated polymers of broad molecular weight range.

## THEORETICAL ASPECTS OF MSC

The earlier theoretical treatments were reviewed by Anderson and Stoddart.[48,49] Several of the reviews quoted above have given prominence to discussions of the many theoretical treatments that have been published, and only a brief summary and an account of the most recent progress is justified here.

In the earliest work, the accepted but over-simplified concept was that molecules separated on the basis of their ability to penetrate the pores of the molecular-sieves, with the largest molecules emerging from the columns first since they were unable to enter the pores and so had to take the shortest possible path. It was shown that with certain solvents this was not invariably the case;[304] several systems where effects other than molecular-sieving are involved have been mentioned above.[44,155,156] Skalka[305] has shown that, in eluents of low ionic strength, the molecules of heparin are altered to an extent such that they cannot penetrate the otherwise accessible pores of lightly cross-linked dextran gels. The "aromatic effect"[123] and the planarity of molecules[306] also influence the mechanism; phenolic and indolylic compounds show greater adsorption than biphenyl or naphthyl compounds. Substitutions that extend planarity may result either in ion-exclusion or in adsorption *e.g.*, carboxy and nitro groups decrease adsorption, hydroxy and methoxy groups increase adsorption.[306] Hendrickson[307] has found that phenols elute at a position that would refer to a compound 4·67 carbon atoms larger than the corresponding aromatic hydrocarbon, and he also reiterated that different solvents may give different elution orders.

Although Giddings,[308] Smith and Kollmansberger,[309] and Giddings and Malik[310] had made significant contributions, Yau and Malone[311] stated in 1967 that the mechanism was not completely understood at that time, and pointed out that the finite diffusion rate,[309,310] *i.e.*, a non-equilibrium mass-transfer mechanism, must be taken into account in addition to the assumption of instant equilibrium between the mobile and stationary phases that was implicit in the earlier over-simplified theories. The importance of the framework-limited partition coefficient was pointed out[312] and, in experiments with columns having ~11000 plates/m, Hendrickson[307] showed that although diffusional spreading was not detected for small molecules, it was extensive for polystyrene of mol. wt. $1 \times 10^4$. Carmichael[313] then evaluated the contributions to the elution curve of a polydisperse sample, from the column itself and from sources exterior to the column, thus extending the results of Vink.[314] Very recently, Polson and Katz[36] have published a quantitative theory and Fuge and Hummel [315] have discussed the principles and described a model for the molecular sieving process.

## CONCLUSIONS

Molecular-sieve chromatography is an important and rapidly developing branch of chromatography with great potential, that has yet to be utilized fully in branches of analytical chemistry. Hendrickson[307] has described the technique as a "liquid phase size spectrometer," but its advantages are not confined to the investigation of size and shape in polymers; in the purification of low molecular weight azo and azomethine dyes, MSC has shown[316] the presence of up to 6 components in recrystallized "analytical samples." A recent paper has concluded[317] that "a liquid chromatograph comprising a peristaltic pump, a small (20–30 × 1 cm) xerogel column, a simple light-scattering or refractive index detector and a fraction collector may well in future find as many uses as a gas chromatograph in the organic chemistry laboratory." It is hoped that this Review has shown the justification for our belief that this represents rather too limited a view.

**Zusammenfassung**—Ziel dieser Übersicht ist es, den analytischen Chemikern eine allgemeine Einführung in die Chromatographie mit Molekularsieben zu geben. Es handelt sich um eine Form der Flüssigkeitschromatographie, bei der Molekularsiebe die primäre Grundlage für die Trennung darstellen, obwohl auch andere Effekte häufig eine Rolle spielen. Das Verfahren kann für anorganische und organische, monomere und polymere Moleküle in wäßrigen oder nichtwäßrigen Systemen werwendet werden. Der Bereich wird abgegrenzt, in dem Molekularsiebe in Xerogel- und Aerogelform zur Zeit zu haben sind; es wird mehr Wert auf die experimentellen Arbeitsbedingungen bei ihrem Gebrauch gelegt als auf mechanistische und theoretische Erwägungen. Die zitierte Literatur wurde kritisch ausgewählt, um eine ausgewogene aktuelle Übersicht zu geben, die allgemeinen analytischen Möglichkeiten aufzuzeigen und einen Ausblick auf die zukünftige Entwicklung des Verfahrens zu geben. Ein Anhang führt die kommerziellen Bezugsquellen für Molekularsiebe und Eichstandards auf.

**Résumé**—L'objet de cette revue est de donner aux chimistes analystes une introduction générale à la chromatographie sur tamis moléculaire, une forme de chromatographie liquide dans laquelle la dimension moléculaire constitue la base primordiale pour la séparation, quoique d'autres influences soient aussi fréquemment mises en jeu. On peut utiliser la technique pour les molécules minérales et organiques tant monomères que polymères, dans des systèmes aqueux ou non aqueux. On décrit la gamme de tamis moléculaires xerogel et aérogel disponibles à présent, et l'on fait ressortir les techniques expérimentales mises en jeu dans leur emploi plutôt que les considérations de mécanisme et de théorie. Les références citées ont été sélectionnées de manière critique pour former une revue équilibrée à jour, et aussi pour montrer les potentiel et domaine analytiques généraux pour le développement futur de la technique. Un appendice donne la liste des sources commerciales de tamis moléculaires et les standards de calibrage.

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## APPENDIX I*

*Commercial Sources of Aerogels for MSC*

| Trade name | Chemical type | Number of different pore sizes available | Available from |
|---|---|---|---|
| Porasil ® | Porous Silica | 6 | A |
| CPG ® | Porous Glass | 8 | B, C, D |
| Bio-Glas ® | Porous Glass | 5 | E |

A. Waters Associates Ltd., Framingham, Massachusetts 01701, U.S.A. or Meadow Mills, Water Street, Portwood, Cheshire, U.K.

B. Chromatography Products, Corning Glass Works, Corning, N.Y. 14830, U.S.A.

C. As for A.

D. B.D.H. Ltd., Poole, U.K.

E. Bio-Rad Laboratories, 32nd and Griffin Avenue, Richmond, California 94804, U.S.A.

* The authors of this review have no commercial interest in any of the companies producing materials for molecular-sieve chromatography. The Appendices list all the products of which they are aware; there may exist others, and, if so, their omission has no other significance and there is no implication regarding their suitability for molecular-sieve chromatography. The order in which the products are listed above has no significance; no product is recommended in preference to any other.

## APPENDIX II

*Commercial Sources of Xerogels for MSC*

| Trade name | Chemical type | Number of different pore sizes available | Available from |
|---|---|---|---|
| Sephadex G ® | Dextran | 8 | A |
| Sephadex LH20 ® | Substituted dextran | 1 | A |
| Sepharose ® | Agarose | 3 | A |
| Bio-Gel A ® | Agarose | 6 | B |
| Sagavac ® | Agarose | 5 | C |
| Gelarose ® | Agarose | 5 | D |
| Sea-Sep ® | Agarose | | E |
| Bio-Gel P ® | Polyacrylamide | 10 | B |
| Bio-Beads S ® | Polystyrene | 5 | B |
| Bio-Beads SM ® | Substituted Polystyrene | 2 | B |
| Styragel ® | Polystyrene | 11 | F |
| Aquapak ® | Substituted Polystyrene | 1 | F |

A. Pharmacia Fine Chemicals AB, Uppsala, Sweden (also 75 Uxbridge Road, London W.5, U.K.)
B. As for Appendix I, E.
C. Seravac Laboratories Ltd., Moneyrow Green, Holyport, Maidenhead, U.K.
D. Litex, P.O. Box 7, DK-2600, Glostrup, Denmark.
E. Marine Colloids Inc., P.O. Box 70, Springfield, N.J. 07081, U.S.A.
F. As for Appendix I, A.

## APPENDIX III

*Commercial Sources of Molecular-Weight Calibration Standards for MSC*

| Type | Number available | Mol. wt. range covered | Available from |
|---|---|---|---|
| Dextrans | 10 | $10^4$–$2 \times 10^6$ | A |
| Proteins | 18 | 1450–$4{\cdot}8 \times 10^5$ | B, C, D, E, F |
| Polystyrenes | 12 | $9{\cdot}7 \times 10^4$–$2{\cdot}1 \times 10^6$ | D, G, H, J, K, L |
| Polypropylene glycol | 4 | 790–3900 | J |

A. As for Appendix II, A.
B. C. F. Boehringer & Son, 68 Mannheim-Waldhof, Germany.
C. Calbiochem, Box 54282, Los Angeles, California 90054, U.S.A. (also 10, Wyndham Place, London W.1, U.K.).
D. Mann Research Laboratories, Inc., 136 Liberty Street, New York, N.Y. 10006, U.S.A.
E. Serva Entwicklungslabor, P.O. Box 1505, 69 Heidelberg, Germany.
F. Worthington Biochemical Corporation, Freehold, N.J. 07728, U.S.A.
G. Arro Laboratories, Inc., Joliet, Illinois 60431, U.S.A.
H. Pressure Chemicals Co., Pittsburgh, Penn. 15201, U.S.A.
J. As for Appendix I, A.
K. Dow Chemical Co., Midland, Michigan, U.S.A.
L. National Bureau of Standards, Washington, D.C., U.S.A.

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# CONTRIBUTIONS TO THE BASIC PROBLEMS OF COMPLEXOMETRY—XXIV*

## DETERMINATION OF ALUMINIUM IN THE PRESENCE OF VERY LARGE AMOUNTS OF MANGANESE

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**Summary**—A simple complexometric determination of aluminium in the presence of a large amount of manganese has been developed. For such determinations only triethylenetetraminehexa-acetic acid (TTHA) can be used. In slightly acidic medium TTHA forms with aluminium a binuclear complex $Al_2L$. The complex is formed almost immediately at room temperature. The excess of TTHA is back-titrated with copper sulphate at pH about 5·3, with Glycinecresol Red as indicator. Reliable results were obtained for Mn:Al ratios up to at least 20. The sum of Al + Fe can be determined by the same method. Very large amounts of calcium and magnesium do not interfere.

COMPLEXOMETRIC determination of aluminium in the presence of large amounts of manganese cannot be performed without previous separation of either the manganese or the aluminium, which is tedious and very often reduces the accuracy. This is because the difference between the stability constants of the Al–EDTA and Mn–EDTA complexes is not large enough for their separate determination (log $K_{AlL} = 16{\cdot}1$, log $K_{MnL} = 14$). For the same reason we cannot use DCTA or DTPA (diethylenetriaminepenta-acetic acid) as titrant.

Recently we have described[1–4] the very interesting properties of triethylenetetraminehexa-acetic acid (TTHA) as a volumetric reagent. TTHA tends to form not only simple 1:1 complexes but also binuclear complexes. Under simple conditions it is possible to titrate some bivalent metals, and aluminium, iron and gallium, as these complexes, $M_2L$. On the other hand such metals as thorium, zirconium, indium, bismuth, manganese and rare earths are determinable as the simple 1:1 complexes. This offers a great number of new possibilities in complexometry, for solving problems which cannot be dealt with by EDTA titrations. In this respect TTHA is unique as a volumetric reagent.

During further investigation of TTHA titrations we have found that the $Al_2$TTHA complex is formed almost immediately at room temperature. From previous experience we expected that the manganese–TTHA complexes (MnL or $Mn_2L$) should be considerably weaker than the aluminium complexes. This seemed very promising for determination of aluminium in the presence of manganese. We have chosen an indirect determination of aluminium, based on back-titration of excess of TTHA with copper sulphate solution, with Glycinecresol Red as indicator. This indicator is well known as a very sensitive indicator for copper and does not react with lead, zinc, cadmium, manganese *etc.*[5] The optimal pH for the titration is 5·1–5·5. The work was

* Part XXIII, *Talanta* 1967, **14**, 313.

done although the stability constants of the TTHA complexes of aluminium, manganese and copper were not known at that time (but see *Discussion*).

## EXPERIMENTAL

*Reagents*

*TTHA solution* 0·05*M*. Prepared by dissolution of 24·723 g of the free acid (Dojin Pharmaceutical Laboratories, Kumamoto, Japan) in 130 ml of warm 1*M* sodium hydroxide, and dilution to 1 litre with redistilled water, and standardized by titration with 0·05*M* lead nitrate, with Xylenol Orange as indicator.

*Acetate buffer, pH* 5·3–5·5. Prepared by neutralization of 1*M* acetic acid with 1*M* sodium hydroxide to the desired pH, with potentiometric control.

*Indicators*. A 0·5% aqueous solution of Xylenol Orange and a 1% aqueous solution of Glycinecresol Red (both Lachema, Brno).

*Aluminium, copper, iron, manganese, calcium and magnesium solutions*, 0·05*M*. Prepared by dissolving the appropriate amounts of reagent-grade chemicals (chloride, nitrate or sulphate) in redistilled water, and standardized complexometrically.

*Recommended procedure*

To the slightly acid solution (pH 3–4) containing aluminium and manganese (up to an Mn:Al ratio of 20) add an excess of 0·05*M* TTHA—enough to complex all aluminium (1 ml of TTHA = 2·698 mg of Al)—and adjust the pH of the solution to 5·3–5·5 with acetate buffer and dilute to 150–200 ml with water. When large amounts of manganese are present let stand for 15–20 min to ensure complete formation of the $Al_2$TTHA complex. Then add a few drops of Glycinecresol Red and titrate with 0·05*M* copper till the colour changes from yellow-green to violet.

*Remarks*. Not more than 10–15 ml of 0·05*M* TTHA in excess can be used, because in the back-titration with copper, the blue Cu–TTHA complex is formed, which in large concentrations makes the colour change less distinct. The need to stand the solution for some time before the back-titration if a lot of manganese is present arises because very large concentrations of manganese hinder the $Al_2$TTHA complex formation and the results for aluminium would otherwise be low. Boiling the solution, as is usually done in aluminium–EDTA titrations, has an unfavourable influence on $Al_2$TTHA formation.

By the method described, aluminium can be reliably determined even at low concentration, as illustrated in Table I. Besides manganese, large amounts of calcium and magnesium are tolerable.

TABLE I.—DETERMINATION OF ALUMINIUM WITH TTHA IN THE PRESENCE OF MANGANESE AND CALCIUM

| Added, *mg* | | | 0·05*M* TTHA taken, | 0·05*M* $Cu^{2+}$ used, | Al found, | Error, |
|---|---|---|---|---|---|---|
| Al | Ca | Mn | *ml* | *ml* | *mg* | *mg* |
| 1·43 | | 41 | 2·97 | 4·99 | 1·28 | −0·15 |
| 1·43 | 100 | 14 | 2·97 | 4·96 | 1·32 | −0·11 |
| 4·28 | 50 | — | 4·95 | 6·73 | 4·27 | −0·01 |
| 4·28 | 6 | 69 | 4·95 | 6·73 | 4·27 | −0·01 |
| 7·13 | 20 | 27 | 9·89 | 14·58 | 7·02 | −0·11 |
| 7·13 | 40 | 137 | 9·89 | 14·63 | 6·95 | −0·18 |
| 14·25 | 200 | 274 | 9·89 | 9·22* | 14·25 | 0 |
| 35·63 | 200 | 137 | 19·79 | 13·21 | 35·57 | −0·06 |

* Standing time 30 min.

The same method can be used for the determination of the sum Al + Fe up to an amount of 25 mg of Fe/200 ml. At high concentrations the yellow $Fe_2$TTHA complex makes the colour change at the end-point less sharp. Manganese, calcium and magnesium do not interfere in the determination.

Near the end of our experimental work two papers by Harju and Ringbom appeared,[6,7] which extended Ringbom's general theory of complexation to TTHA titrations.[8] Both papers gave valuable information about the stability constants of the ML and $M_2L$ complexes of 19 common elements with TTHA, as well as their conditional constants, and estimates of the optimal conditions for complexometric determination of various metals, which were in good agreement with our previous findings. We were interested in Professor Ringbom's opinion and asked him for comments. We are most grateful for the following discussion by Professor Ringbom.

## DISCUSSION

(*A. Ringbom, Åbo Academy, Åbo, Finland*)

The method described is based on back-titration of excessive TTHA with copper solution. In the back-titration the displacement reaction (charges omitted for simplicity)

$$Al_2L + 2Cu = Cu_2L + 2Al \qquad (1)$$

could take place, resulting in a negative error which would greatly depend on the transition point of the indicator used. If a $10^{-3}M$ aluminium solution containing no manganese is titrated at pH 5·4, pAl at the equivalence point is 6·0.[7] The corresponding pCu value can be estimated from the overall stability constants:

$$\frac{[Cu_2L][Al]^2}{[Al_2L][Cu]^2} = \frac{\beta_{Cu_2L}}{\beta_{Al_2L}} = \frac{10^{32.6}}{10^{28.6}} = 10^{4.0} \qquad (2)$$

If $[Cu_2L]$ is of the same order of magnitude as $[Al_2L]$ (*i.e.*, if 100% excess of TTHA is used), the equivalence point corresponds to pCu $\sim$ 8·0. In other words, if $pCu_{trans}$ exceeds 8, no aluminium will be displaced by copper. If $pCu_{trans} = 8$, the titration error is zero, but if $pCu_{trans}$ is smaller than 8, a displacement will take place and a negative error arises. According to recent investigations by Harju[9] the transition point $pCu_{trans}$ of Glycinecresol Red is between 6·8 and 7·0. This means that a negative error will arise if back-titration is performed to the transition point or to a complete colour change. The first shade of the mixed colour should be taken as the end-point.

The negative error caused by unreacted aluminium can be expressed by [Al′], *i.e.*, the sum of the concentrations of free and hydrolysed aluminium ions. $\alpha_{Al(OH)}$ at pH 5·4 can be estimated to be $10^{0.7}$ [8], hence $[Al'] = 10^{0.7}[Al]$. Considering that $[Al_2L] \sim \frac{1}{2}C_{Al}$ and $[Cu_2L] = \frac{1}{2}C_{Cu}$ ($C$ denotes total concentration) one obtains from equation (2)

$$[Al'] = 10^{2.7}\left(\frac{C_{Al}}{C_{Cu}}\right)^{1/2}[Cu] \qquad (3)$$

If manganese is present, a possible formation of $Mn_2L$ may cause a positive error, *i.e.*, the equivalence point of the back-titration will be shifted towards lower pCu values. If much manganese is present, the titration should therefore be performed to a colour change more complete than when no manganese (or only a very small amount) is present.

$[Mn_2L]$ at the end-point can be calculated from the expression

$$\frac{[Mn_2L]}{[Cu_2L]}\frac{[Cu]^2}{[Mn]^2} = \frac{\beta_{Mn_2L}}{\beta_{Cu_2L}} = \frac{10^{21.2}}{10^{32.6}} = 10^{-11.43} \qquad (4)$$

or, considering that $[Cu_2L] \sim \frac{1}{2}C_{Cu}$ and $[Mn] \sim C_{Mn}$

$$[Mn_2L] = \tfrac{1}{2}C_{Cu}\left(\frac{C_{Mn}}{[Cu]}\right)^2 10^{-11.4} \qquad (5)$$

A strictly correct expression for the theoretical titration error contains in addition to $[Mn_2L]$ and [Al′] the concentrations of a number of other species ([L′], [AlL′], [MnL′], [CuL′], [Cu′],) but according to considerations based on the equilibrium

equations all these species can be disregarded at pH 5·4 if pCu is not far from 7. Consequently, if a titration is performed to $[Cu]_{endp}$:

$$\text{Titration error in } \% \sim \left\{\frac{2[Mn_2L] - [Al']}{C_{Al}}\right\} 100$$

$$= \frac{C_{Cu}}{C_{Al}} \left(\frac{C_{Mn}}{[Cu]_{endp}}\right)^2 10^{-9.4} - \frac{[Cu]_{endp}}{(C_{Al}\, C_{Cu})^{1/2}} 10^{4.7} \quad (6)$$

For instance, if $C_{Al} = C_{Cu} = 10^{-3}M$, $C_{Mn} = 10^{-2}M$ and $pCu_{endp} = 7{\cdot}0$, the error will be $10^{0.6} - 10^{0.7} = 1\%$.

Of course, such calculations of titration errors cannot give precise answers. The values of the constants are of limited accuracy, there exists some uncertainty about the hydrolysis of aluminium, and the first term of equation (6) is very sensitive to even small changes of $pCu_{endp}$ and $C_{Mn}$. Also kinetic factors may affect the results. Therefore, it is remarkable that, for the titrations in Table I, calculations based on equation (6) will in most cases give titration errors of the right order of magnitude.

**Zusammenfassung**—Ein einfaches komplexometrisches Verfahren zur Bestimmung von Aluminium in Gegenwart einer großen Menge Mangan wurde entwickelt. Für solche Bestimmungen kann man nur Triäthylentetraminhexaessigsäure (TTHA) verwenden. In schwach saurem Medium bildet TTHA mit Aluminium einen zweikernigen Komplex $Al_2L$. Der Komplex bildet sich bei Zimmertemperatur fast augenblicklich. Der Überschuß von TTHA wird ungefähr bei pH 5,3 mit Kupfersulfat zurücktitriert, Glycinkresolrot dient dabei als Indikator. Für Mn:Al-Verhältnisse bis mindessens 20 wurden zuverlässige Ergebnisse erhalten. Die Summe von Al + Fe kann nach dem selben Verfahren bestimmt werden. Sehr große Mengen Calcium und Magnesium stören nicht.

**Résumé**—On a élaboré un dosage complexométrique simple de l'aluminium en la présence d'une grande quantité de manganèse. Pour de telles déterminations, on ne peut utiliser que l'acide triéthylènetétraminohexacétique (TTHA). En milieu légèrement acide, le TTHA forme avec l'aluminium un complexe binucléaire $Al_2L$. Le complexe se forme presqu'immédiatement à température ordinaire. L'excès de TTHA est titré en retour au moyen de sulfate de cuivre à un pH d'environ 5,3, avec le Rouge de Glycinecrésol pour indicateur. On a obtenu des résultats exacts pour des rapports Mn:Al allant jusqu'à 20 au moins. On peut déterminer la somme Al + Fe par la même méthode. De très grandes quantités de calcium et de magnésium ne gênent pas.

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# CATION-EXCHANGE BEHAVIOUR OF SEVERAL ELEMENTS IN FORMIC ACID SOLUTION

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**Summary**—The cation-exchange characteristics of 20 elements towards the strongly acidic cation-exchange resin Dowex 50 × 8 in media containing varying concentrations of formic acid and mixtures of formic acid with aqueous dioxan were investigated. Possible separations are indicated and discussed. Bismuth may be quantitatively separated from copper.

IN THE PRECEDING PAPER,[1] ion-exchange distribution coefficients were reported for twelve different metal ions, which indicated that formic acid may be a promising medium for chromatographic separations on cation-exchange resins. Zirconium was separated quantitatively from thorium and from gallium with 0·05*M* formic acid. Only a few studies on the sorption of metal formate complexes on cation-exchange resins have been reported.[2–4] However, no attempt has been made to investigate systematically the cation-exchange behaviour of the elements in aqueous formic acid solutions and mixed aqueous–organic solvent media containing formic acid. To obtain a more complete picture of the sorption characteristics and the separation possibilities of elements in media containing formic acid, distribution coefficients were determined for 20 elements.

## EXPERIMENTAL

### *Reagents*

*Ion-exchange resin.* Dowex 50-W × 8 (100–200 mesh) in the $Na^+$ form was converted into the $H^+$ form by washing it with 3*M* hydrochloric acid, then with water and finally drying in air.

*Standard metal ion solutions*, 0·05*M*. Prepared in demineralized water from the chlorides, nitrates or sulphates of the metals. When necessary, enough mineral acid was added to prevent hydrolysis. $Pr_2O_3$, $Nd_2O_3$ and $Sm_2O_3$ were first fused with sodium carbonate, then dissolved in the minimum amount of hydrochloric acid and diluted with demineralized water.

### *Apparatus*

For separations 12-mm bore glass columns were used.

### *Determination of metal ions*

All the metal ions were determined titrimetrically with standard 0·002*M* EDTA.

### *Determination of distribution coefficients*

The batch equilibrium method was used to determine the coefficients according to the relationship

$$K_d = \frac{\mu g \text{ of element/g of resin}}{\mu g \text{ of element/ml of solution}}.$$

Each equilibrium experiment was performed in a 100-ml conical flask containing 25 ml of a mixture of 0·5 ml of test solution and 24·5 ml of formic acid solution. To this mixture 0·5 g of air-dried resin in the $H^+$ form was added and the mixture was agitated for 3 hr on a water-bath maintained at 30 ± 1°. The mixture was then filtered and the metal in the filtrate was determined.

### *Separation of bismuth from copper*

A slurry of 1 g of air-dried resin in water was poured into a column. The column was saturated by the passage of 50 ml of 23*M* formic acid, and then the mixture of bismuth and copper solutions was

added (in 23*M* formic acid). The bismuth was eluted by passing 23*M* formic acid through the column, at a flow-rate of 3·0 ml/min. The copper was sorbed on the resin and the bismuth passed into the effluent. The copper was eluted with 2·5*M* hydrochloric acid at a flow-rate of 1·5 ml/min.

## RESULTS AND DISCUSSION

The distribution coefficients of 20 metal ions in formic acid of varied concentration (3·0–23·0*M*) are given in Table I, and in 3*M* formic acid–aqueous dioxan mixtures (1 : 1) in Table II. The quantitative separation of bismuth from copper is summarized in Table III.

### *Sorption behaviour in formic acid solutions*

*Zinc(II), manganese(II), nickel(II) and cobalt(II).* Zinc and manganese have almost the same behaviour over the entire range of formic acid concentration, and therefore cannot be separated at any acid concentration. Nickel and cobalt show some interesting results. The sorption of nickel decreases with acid concentration until a minimum is reached at an acid concentration of 7*M*. The sorption then begins to increase and nickel is completely sorbed from 11–23*M* formic acid. Cobalt on the other hand is completely sorbed from formic acid solutions of all concentrations. Therefore the following separations appear to be possible: zinc–nickel and manganese–nickel in 11*M* formic acid, nickel–cobalt in 7*M* formic acid, zinc–cobalt and manganese–cobalt in 3*M* formic acid solution.

*Lanthanum(III), yttrium(III), cerium(III), neodymium(III), samarium(III) and praseodymium(III).* Lanthanum and yttrium show almost the same sorption behaviour over the entire range of acid concentrations studied. No separation of these two ions appears to be possible. Cerium(III) is strongly sorbed from low formic acid concentrations. However as the formic acid concentration is increased to 5*M* the sorption decreases and then remains almost constant up to 23*M* acid. As expected, neodymium, samarium and praseodymium behave in a similar fashion to cerium(III). Therefore no separation of rare earths is possible, and lanthanum(III) or yttrium(III) can only be separated from binary mixtures with cerium, samarium, neodymium and praseodymium in 3*M* formic acid solution.

*Zirconium(IV) and thorium(IV).* With increasing concentration of the acid the zirconium sorption gradually increases, while thorium is totally sorbed at all concentrations of formic acid. The separation of zirconium from thorium is therefore possible in 3*M* formic acid.

*Magnesium(II) and barium(II).* These metal ions show identical behaviour and their $K_d$ values remain almost stationary at varying formic acid concentration.

*Gallium(III) and indium(III).* Gallium is strongly sorbed at all concentrations of the acid. No appreciable variation in sorption of indium is observed with increase in the acid concentration. Therefore indium can be separated from gallium in 3*M* formic acid.

*Copper(II), bismuth(III), cadmium(II) and lead(II).* The sorption of copper increases with increase in the acid concentration while that of bismuth decreases and is lowest in 23*M* formic acid. Therefore bismuth is easily separated from copper quantitatively, by eluting it with 23*M* formic acid (see Table III). The distribution coefficient of cadmium remains constant up to a formic acid concentration of 13*M*. From 13–23*M* formic acid a variation in the $K_d$ values is observed which cannot be explained. Lead shows an interesting behaviour; its sorption gradually increases

TABLE I.—DISTRIBUTION COEFFICIENTS IN FORMIC ACID OF VARYING CONCENTRATION

| Metal ion | Formic acid concentration | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 3*M* | 5*M* | 7*M* | 9*M* | 11*M* | 13*M* | 15*M* | 19*M* | 21*M* | 23*M* |
| Cu(II) | 311 | $>10^3$ | $>10^3$ | $>10^3$ | $>10^3$ | $>10^3$ | $>10^3$ | $>10^3$ | $>10^3$ | $>10^4$ |
| Zn(II) | 121 | 226 | 221 | 182 | 210 | 600 | $>10^3$ | $>10^3$ | $>10^3$ | $>10^3$ |
| Mn(II) | 128 | 369 | 425 | 662 | 459 | 274 | $>10^3$ | $>10^3$ | $>10^3$ | $>10^3$ |
| Cd(II) | 218 | 250 | 228 | 262 | 250 | 700 | 450 | 527 | 450 | 700 |
| Mg(II) | 231 | 315 | 325 | 325 | 347 | 272 | 305 | 325 | 287 | 257 |
| Ba(II) | 146 | 146 | 133 | 95 | 95 | 87 | 255 | 179 | 95 | 52 |
| Pb(II) | 58 | 77 | 125 | 125 | 182 | 600 | 600 | 600 | 762 | $>10^3$ |
| Ni(II) | $>10^3$ | 861 | 162 | 269 | T.A. | T.A. | T.A. | T.A. | T.A. | T.A. |
| Co(II) | T.A. | T.A. | T.A. | T.A. | T.A. | T.A. | T.A. | T.A. | T.A. | T.A. |
| Bi(III) | T.A. | T.A. | $>10^3$ | $>10^3$ | 804 | 316 | 173 | 96 | 56 | 52 |
| La(III) | 760 | 616 | 350 | 172 | 160 | 217 | 200 | 200 | 200 | 200 |
| In(III) | 616 | 655 | 655 | 750 | 807 | $>10^3$ | $>10^3$ | 873 | 849 | 950 |
| Ce(III) | T.A. | 366 | 366 | 470 | 470 | 470 | 396 | 340 | 575 | 518 |
| Pr(III) | T.A. | 684 | 733 | 789 | 853 | $>10^3$ | $>10^3$ | $>10^3$ | $>10^3$ | 929 |
| Y(III) | 490 | 495 | 550 | 495 | 550 | 616 | 616 | 700 | 966 | 966 |
| Nd(III) | T.A. | 929 | 789 | $>10^3$ | $>10^3$ | $>10^3$ | $>10^3$ | 929 | 929 | 890 |
| Sm(III) | T.A. | $>10^3$ | 733 | $>10^3$ | 789 | $>10^3$ | 509 | 853 | 853 | 853 |
| Th(IV) | T.A. | T.A. | T.A. | T.A. | T.A. | T.A. | T.A. | T.A. | T.A. | T.A. |
| Zr(IV) | 106 | 106 | 297 | 366 | 911 | 911 | $>10^3$ | $>10^3$ | $>10^3$ | $>10^3$ |
| Ga(III) | T.A. | T.A. | T.A. | T.A. | T.A. | T.A. | T.A. | T.A. | T.A. | T.A. |

T.A. = total adsorption.

TABLE II.—DISTRIBUTION COEFFICIENTS IN AQUEOUS DIOXAN–3*M* FORMIC ACID (1:1) MIXTURES

| Metal ion | Dioxan concentration (% V/V) | | | | |
|---|---|---|---|---|---|
| | 10 | 30 | 50 | 70 | 90 |
| Cu(II) | $>10^3$ | $>10^3$ | $>10^3$ | T.A. | T.A. |
| Zn(II) | $>10^3$ | $>10^3$ | $>10^3$ | $>10^3$ | $>10^3$ |
| Mn(II) | 108 | 543 | 967 | 967 | $>10^3$ |
| Cd(II) | $>10^3$ | 632 | $>10^3$ | $>10^3$ | 700 |
| Mg(II) | $>10^3$ | $>10^3$ | $>10^3$ | 564 | 256 |
| Ba(II) | 133 | 225 | 293 | 200 | 225 |
| Pb(II) | 227 | 878 | T.A. | T.A. | T.A. |
| Ni(II) | T.A. | T.A. | T.A. | T.A. | T.A. |
| Co(II) | T.A. | T.A. | T.A. | T.A. | T.A. |
| Bi(III) | $>10^3$ | $>10^3$ | $>10^4$ | $>10^4$ | $>10^3$ |
| Y(III) | 700 | 250 | 250 | 495 | $>10^3$ |
| La(III) | 15 | 750 | 750 | 750 | T.A. |
| In(III) | 430 | 655 | 581 | 655 | $>10^3$ |
| Ce(III) | 430 | 430 | 470 | 470 | 470 |
| Pr(III) | 684 | 684 | 729 | 854 | 854 |
| Nd(III) | 853 | 789 | 853 | 853 | 853 |
| Sm(III) | 602 | $>10^3$ | 929 | 929 | 853 |
| Th(IV) | T.A. | T.A. | T.A. | T.A. | T.A. |
| Zr(IV) | 22 | 13 | 0·00 | 0·00 | 1·6 |
| Ga(III) | T.A. | T.A. | T.A. | T.A. | T.A. |

T.A. = total adsorption.

TABLE III.—QUANTITATIVE SEPARATION OF BISMUTH AND COPPER

| Volume of effluent | | Bismuth | | Copper | |
|---|---|---|---|---|---|
| Eluent A 23*M* HCOOH, *ml* | Eluent B 2·5*M* HCl, *ml* | Taken, *mg* | Found, *mg* | Taken, *mg* | Found, *mg* |
| 80 | 50 | 0·83 | 0·83 | 1·65 | 1·65 |
| 100 | 50 | 1·67 | 1·67 | 1·65 | 1·65 |
| 100 | 50 | 2·50 | 2·47 | 2·64 | 2·68 |
| 100 | 70 | 3·34 | 3·32 | 2·64 | 2·68 |
| 100 | 70 | 4·18 | 4·12 | 3·30 | 3·24 |

with an increase in formic acid concentration up to 11*M* then it remains constant from 13*M* to 19*M* formic acid. With further increase in the concentration of acid a sharp increase in $K_d$ is observed.

*Sorption behaviour in mixed formic acid–aqueous dioxan media*

*Cerium(III), praseodymium(III), neodymium(III) and samarium(III).* All these metal ions show similar behaviour and are completely sorbed from 3*M* formic acid. However, as aqueous dioxan is added the sorption falls and remains constant.

*Indium(III), cadmium(II), yttrium(III), magnesium(II), manganese(II) and barium(II).* The sorption of indium and manganese increases gradually with increase in the concentration of dioxan, but the reverse order is observed in the case of cadmium and magnesium. No pronounced variation is observed for barium and yttrium.

*Lanthanum(III), lead(II) and copper(II).* An increase in the sorption of these metal ions is observed at first and with increase in dioxan concentration the $K_d$ value also increases and then remains constant.

*Thorium(IV), gallium(III), nickel(II) and cobalt(II).* These are all strongly sorbed from all concentrations of dioxan in admixture with 3*M* formic acid.

The following separations can be easily achieved in the mixed (1:1 v/v) dioxan–formic acid solutions specified: lead–copper, 10% dioxan–3*M* formic acid; barium–lanthanum, 3*M* formic acid–90% aqueous dioxan; cadmium–copper, 70% aqueous dioxan–3*M* formic acid.

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**Résumé**—On a étudié les caractéristiques d'échange de cations de 20 éléments vis-à-vis de la résine échangeuse de cations fortement acide Dowex 50 × 8 dans des milieux contenant des concentrations variables d'acide formique et des mélanges d'acide formique avec le dioxane aqueux. On indique des séparations possibles et en discute. On peut séparer quantitativement le bismuth du cuivre.

**Zusammenfassung**—Die Kationenaustausch-Eigenschaften von 20 Elementen gegenüber dem stark sauren Kationenaustauschharz Dowex 50 × 8 wurden in Medien untersucht, die verschiedene Konzentrationen von Ameisensäure und Gemische von Ameisensäure mit wäßrigem Dioxan enthielten. Trennungsmöglichkeiten werden angegeben und diskutiert. Wismut kann quantitativ von Kupfer getrennt werden.

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# EXTRACTION WITH LONG-CHAIN AMINES—IV
## SEPARATION AND COLORIMETRIC DETERMINATION OF GOLD

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**Summary**—A highly selective method, almost free from interferences, for extraction of gold(III) from sulphuric acid into a chloroform solution of trioctylamine (TOA) is described. The yellow extract is then measured at 325–330 nm. Gold can be determined in the presence of platinum. The method has been applied to the determination of gold in waste dumps. A more sensitive method is based on reaction of the gold with diphenylcarbazide after the separation of the gold from the TOA-chloroform extract. The violet colour is stable and is measured at 560 nm.

RECENTLY Groenewald[1,2] described a very sensitive method for the determination of gold by atomic-absorption spectrophotometry after its previous extraction into a di-isobutyl ketone solution of trioctylamine (TOA) or trioctylmethylammonium chloride (TOMA). The method is suitable for a very low concentration of gold up to $5 \times 10^{-8}M$ (0·01 mg/l.). Gold(III) chloride is extracted from hydrochloric acid–sodium chloride solutions (pH 0–4), and gold(I) and gold(III) cyanide from $10^{-2}M$ cyanide at pH 4–10. Low concentrations of calcium, magnesium ($2 \times 10^{-3}M$) and copper, iron, zinc, cobalt, manganese ($2 \times 10^{-3}$–$5 \times 10$–$^{5}M$) as complex cyanides do not interfere. The method has been applied to the determination of gold in ores. Strelow *et al.*[3] have proposed isobutyl methyl ketone for the extraction of gold(III) chloride from $3M$ hydrochloric acid and also determined gold by atomic absorption. Metals which are partly co-extracted do not interfere in the final determination. Gold(I) cyanide normally present in cyanide waste solutions must be oxidized with solid potassium permanganate and concentrated hydrochloric acid. Ishimori *et al.*[4] have studied the distribution of gold(III) between hydrochloric acid and Primene JM-T in Xylene solution and Maeck *et al.*[5] have examined the extraction of gold(III) as a quaternary ammonium salt ion-pair from various acid media into isobutyl methyl ketone.

During our systematic investigation of extraction properties of long-chain amines, we also have found that gold(III) chloride is quantitatively extracted with a chloroform solution of TOA or TOMA from relatively acidic solutions of it in hydrochloric or sulphuric acid. Extraction from sulphuric acid is selective, and high concentrations of iron, copper, cobalt, nickel *etc* do not interfere. The concentration of gold is directly measured in the chloroform extract at 330 nm. Gold can easily be removed from the extract, dissolved again in *aqua regia*, and after addition of diphenylcarbazide extracted

Part III, *Talanta*, 1971, **18**, 349.

once more into TOMA and measured at 560 nm; this method is more sensitive. The molar absorptivities are $4{\cdot}2 \times 10^3$ l.mole.$^{-1}$ mm$^{-1}$ for the diphenylcarbazide reaction and 226 l.mole$^{-1}$. mm$^{-1}$ for the Au-TOMA-chloride.

## EXPERIMENTAL

*Reagents*

*Gold(III) chloride solution*, 0·05*M*. Standardized gravimetrically and diluted 10- and 100-fold with water to give solutions containing 570 and 57 $\mu$g of gold per ml.

*Trioctylamine (TOA) and trioctylmethylammonium chloride (TOMA) solutions*, 5% *w/v in chloroform*. Both compounds were products of General Mills Inc., Kankakee, Illinois, marketed as Alamine S-336 and Aliquat S-336 respectively.

*Diphenylcarbazide solution*, 1% *w/v*. Dissolve 0·5 g of reagent-grade chemical in 5 ml of conc. acetic acid (with warming) and dilute to 50 ml with water. Filter after 30 min. Prepare fresh daily.

*Absorption spectra*

To 0·5 ml of $5 \times 10^{-3}$ *M* gold(III) chloride in a 150-ml separatory funnel, various amounts of 5*M* sulphuric acid or 11*M* hydrochloric acid were added and the mixtures diluted to 35 ml with water.

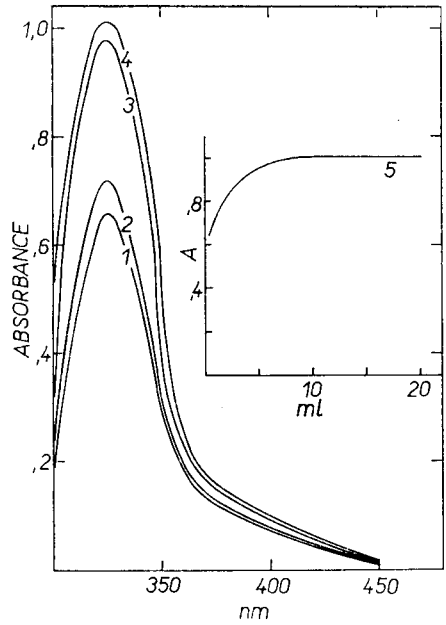


FIG. 1.—Influence of acidity on the extraction of gold with TOA.
0·5 ml of $5 \times 10^{-3}$ *M* $AuCl_3$ and *x* ml of 5*M* $H_2SO_4$, diluted to 35 ml with water. Extracted into 5 ml of 5% TOA in $CHCl_3$ and measured in a 10-mm silica cell. *x*: *1*–0·5, *2*–1, *3*–5, *4*–10, 20, 25 *Inset*: *5*—Influence of $H_2SO_4$ on maximum absorbance.

The solutions were extracted with 5 ml of 5% TOA or TOMA solution by shaking for 2 min. After phase separation the chloroform extract was passed through a dry filter paper into a 10-mm cell and the spectrum recorded between 300 and 450 nm. Figure 1 indicates that extracts from either acid have the same very sharp maximum at 325–330 nm and that the absorbance is constant when the solutions are at least 1·2*M* in sulphuric acid or 3*M* in hydrochloric acid. The colour is stable for at least 40 min.

Because many other metals are also extracted from hydrochloric acid solutions, further experiments were made only with sulphuric acid and with a chloroform solution of TOA (because of shortage of TOMA). Only a single extraction is needed for quantitative extraction of gold. The water phase showed no polarographic wave for gold after the extraction.

Under optimal conditions the Lambert-Beer law is obeyed up to 0·22 mg of Au/5 ml of extractant for sulphuric acid media and up to 0·5 mg/5 ml for hydrochloric acid solutions. If measurement is made against water as reference, the calibration curve is linear but does not pass through the origin of the graph.

*Influence of other ions*

Because of the chemical behaviour and limited possibilities for dissolution of metallic gold, only the presence of nitrate and citrate was investigated. It was found that up to 3% w/v potassium nitrate and 5% w/v citric acid had no effect on extraction and absorbance.

The same procedure was used for the determination of gold in the presence of iron, nickel, cobalt, copper and silver. Table I summarizes some of our results. Chromium(VI), molybdenum(VI), uranium(VI), platinum(IV) and palladium(II) interfere. Other platinum metals were not available but most probably also interfere. Large amounts of silver must be removed as silver chloride (otherwise AgCl is dispersed in the chloroform phase and makes filtration impracticably slow). The interference of chromium(VI), molybdenum(VI) and certain other elements could be reduced by a preliminary extraction of the gold(III) with isobutyl methyl ketone from 0·5*M* hydrochloric acid.[3]

*Determination of gold in the presence of platinum*

Platinum(IV) is quantitatively extracted under our experimental conditions but can easily be stripped into 0·1*M* sodium hydroxide. In this case, the chloroform phase containing the gold becomes cloudy after a while, but this can be avoided by transferring the chloroform to another separatory funnel, and shaking it with 10 ml of 5*M* sulphuric acid, 1 ml of conc. hydrochloric acid and 15 ml of water, then filtering after 5 min and measuring the absorbance of the chloroform phase. A result from such a determination is also included in Table I.

Palladium is only partly stripped from the chloroform phase under these conditions and therefore still interferes.

TABLE I.—DETERMINATION OF GOLD IN THE PRESENCE OF FOREIGN METALS

| Au taken, *μg* | Metals added, *mg* | Au found, *μg* | Error, *μg* | Error, % |
|---|---|---|---|---|
| 171 | 6 Fe + 6 Co + 6 Ni | 169 | 2 | 1 |
| 228 | 32 Cu + 50 Fe + 15 Co + 15 Ni | 227 | 1 | 0·5 |
| 171 | 25 Ag | 166 | 5 | 3 |
| 114 | 19·5 Pt | 103 | 11 | 10 |

*Determination of gold with diphenylcarbazide*

It has been found that diphenylcarbazide gives an intense violet colour with gold(III) chloride, which can also be extracted into a chloroform solution of TOA. The absorption maximum is at 560 nm and the absorbance becomes constant (as a function of the acidity) if extraction is made from 4–5*M* sulphuric acid medium (see Fig. 2). This has the advantage that a simpler spectrophotometer, suitable for visible light and with glass optics, can be used and that the reaction is at least ten times as sensitive as the TOA reaction. Diphenylcarbazide reacts with some other metals and therefore cannot be used directly. All attempts to initiate the colour reaction of diphenylcarbazide in the TOA extract of gold failed. Good results were obtained by isolation of gold from its TOA extract, followed by the diphenylcarbazide reaction, as described below.

*Procedure.* Extract the gold(III) chloride solution with 5 ml of 5% TOA–chloroform solution as before. Transfer the chloroform layer into a silica dish. Extract the remaining aqueous phase with 5 ml of pure chloroform and add this to the first extract. Evaporate the chloroform (on a heated sand-bath), then add 0·3–0·5 g of hydroxylamine hydrochloride, and ammonia until free ammonia can be smelt. Evaporate to dryness and heat over a burner to remove organic matter. Cool, then add 0·3–1·0 ml of *aqua regia* to the residue and evaporate again. Dissolve this residue in water and transfer to a 150-ml separatory funnel. Add 10–20 ml of 5*M* sulphuric acid and 1 ml of 0·5% diphenylcarbazide solution. Warm the contents of the funnel to 40–45° (hot tap-water), then after cooling add 5 ml of TOA solution and shake the mixture for 2 min. Filter the chloroform layer and measure its absorbance at 560 nm. After 15 min the absorbance becomes constant and remains so for at least 6 hr. Platinum gives the same colour and must be separated as described above. A few results for gold alone and in the presence of platinum are given in Table II.

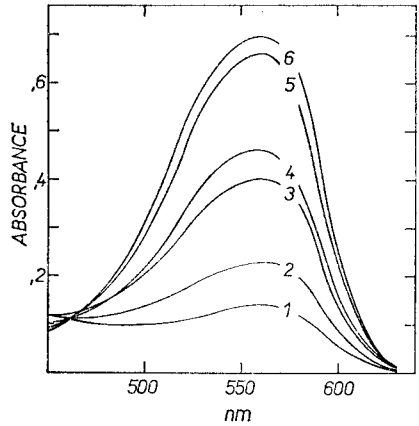


FIG. 2.—Influence of acidity on diphenylcarbazide reaction with gold.
1·8 ml of 5 × $10^{-4}M$ $AuCl_3$ and $x$ ml of $1M$ or $5M$ $H_2SO_4$, diluted to 30 ml with water and 1 ml of 0·5% diphenylcarbazide solution added. Extracted with 5 ml of 5% TOA in $CHCl_3$ and measured in a 10-mm glass cell after 30 min.
$1M$ $H_2SO_4$ added: *1*–5 ml, *2*–10 ml, *3*–20 ml, *4*–25 ml
$5M$ $H_2SO_4$ added: *5*–10 ml, *6*–20, 25 ml.

*Practical applications*

The proposed method is suitable for the determination of gold in ores and silicates.

*Procedure.* Take a 10-g sample in a Teflon beaker and mix it with 50 ml of hydrofluoric acid and 2 ml of conc. sulphuric acid. Evaporate and then add a further 2 ml of conc. sulphuric acid and evaporate to white fumes. To the residue add 5 ml of *aqua regia* and evaporate to dryness. Add 50 ml of 2·5$M$ sulphuric acid and warm on a sand-bath to dissolve the residue. Transfer the solution to a 150-ml separatory funnel and extract with 5 ml of 5% chloroform solution of TOA. Measure in a 10-mm silica cell at 330 nm.

Only one analysed sample was available—a sample from a dump for waste after extraction of gold by the cyanide process, and having a gold content of 2 g/ton. Our analysis gave an average of 2·13 g/ton.

TABLE II.—DETERMINATION OF GOLD WITH DIPHENYLCARBAZIDE IN THE PRESENCE OF PLATINUM

| Au taken, *μg* | Pt taken, *mg* | Au found, *μg* |
|---|---|---|
| 28·5 | 39·1 | 25·0 |
| 57·0 | 39·1 | 58·7 |
| 17·1 | — | 13·6 |
| 57·0 | — | 53·5 |
| 85·5 | — | 83·8 |
| 96·9 | — | 98·6 |

**Zusammenfassung**—Eine hochselektive, von Störungen fast freie Methode zur Extraktion von Gold(III) aus Schwefelsäure in eine Chloroformlösung von Trioktylamin (TOA) wird beschrieben. Der gelbe Extrakt wird dann bei 325–330 nm gemessen. Gold kann in Gegenwart von Platin bestimmt werden. Das Verfahren wurde auf die Bestimmung von Gold in Halden angewandt. Ein empfindlicheres Verfahren beruht auf der Reaktion von Gold mit Diphenylcarbazid nach der Abtrennung von Gold aus dem TOA-Chloroform-Extrakt. Die violette Farbe ist stabil und wird bei 560 nm gemessen.

**Résumé**—On décrit une méthode hautement sélective, presqu'exempte d'interférences, pour l'extraction de l'or(III) d'acide sulfurique dans une solution chloroformique de trioctylamine (TOA). L'extrait jaune est alors mesuré à 325–330 nm. On peut doser l'or en la présence de platine. On a appliqué la méthode au dosage de l'or dans des balayures. Une méthode plus sensible est basée sur la réaction de l'or avec le diphénylcarbazide après la séparation de l'or de l'extrait TOA-chloroforme. La coloration violette est stable et est mesurée à 560 nm.

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# SPECTROPHOTOMETRIC DETERMINATION OF RUTHENIUM WITH 3,4-DIAMINOBENZOIC ACID

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**Summary**—Ruthenium(III) chloride and 3,4-diaminobenzoic acid, in aqueous solution at pH 4·0–4·5, react slowly at room temperature, but more rapidly when the mixture is heated, giving solutions that go through colour transitions from amber to purple-red; maximum absorbance of fully developed solutions occurs at 550 nm. The effects of heating temperature and time, pH, reagent concentration, and other variables have been studied. The system conforms to Beer's law; optimum concentration range, for measurement in 10-mm cells, is about 0·5–2 ppm of ruthenium. Interference from foreign ions, especially other platinum elements, is avoided by a distillation separation of ruthenium. The spectrophotometric mole-ratio and continuous-variation methods indicated the presence of complexes of 1:2 and 1:3 ruthenium-to-reagent stoichiometry. Elemental analysis of solid products isolated from solution confirmed the 1:2 reaction ratio. Several other *o*-diamines gave similar coloured solutions and reaction stoichiometry.

SPECTROPHOTOMETRIC methods published up to 1964 for determination of the noble metals have been evaluated in review articles[1,2] and in an important reference work[3] which lists some 20 spectrophotometric reagents for ruthenium. More recently-described reagents for ruthenium include 1-nitroso-2-naphthol,[4] 2,4,6-tri-(2′-pyridyl)-*s*-triazine,[5] 2,3-diaminopyridine,[6] nitroso-R-salt,[7] oximidobenzotetronic acid[8] and thenoyltrifluoroacetone.[9] In general, chromogenic reagents for ruthenium suffer from interference from other platinum elements, especially from osmium, so that a separation of ruthenium is required. The improved economics of the platinum elements and their use in a variety of applications is an incentive for a continued search for methods possessing high sensitivity and selectivity.

The following diamines (abbreviated designations shown in parentheses) gave colour reactions with ruthenium(III,IV): 3,4-diaminobenzoic acid (DABA), *o*-phenylenediamine (OPDA), 3,4-diaminotoluene (DAT), 2,3-diaminopyridine (DAPy), 4,5-diaminopyrimidine (DAPm), 3,4-diamino-5-hydroxypyrazole sulphate (DAHPz), and *N*,*N*,*N*′,*N*′-tetramethyl-*o*-phenylenediamine (TMOPDA). Factors such as solubility of reagent and product, rate of reaction, intensity and stability of colour, and reaction with other metal ions varied widely for the different reagents. 3,4-Diaminobenzoic acid was chosen as the most promising reagent for detailed study because of its adequate solubility in water and the intensity and stability of the coloured solutions formed with ruthenium.

## EXPERIMENTAL

### *Apparatus*

Absorbance measurements requiring high precision at fixed wavelength were made with a Beckman Model DU spectrophotometer equipped with a photomultiplier. Spectral scanning in the study of

the effect of variables was made with a Cary Model 14 spectrophotometer and with a Beckman Model DK-1 spectrophotometer equipped with a cell thermostat. Matched silica cells of 10·0 mm optical path were used.

*Reagents*

3,4-*Diaminobenzoic acid.* This was used as received, except for studies of the reaction stoichiometry, for which it was recrystallized. The reagent solution, 0·100 mg per 25 ml, was prepared by dissolving the solid in either hot water or 0·1*M* hydrochloric acid. The aqueous solution should be prepared daily; solutions in acid are stable for several days at room temperature, and show no sign of decomposition in several weeks when stored under refrigeration.

*Ruthenium trichloride.* A stock solution was prepared by dissolving 2·5 g of the salt in distilled water containing sufficient hydrochloric acid to make the final acidity 0·10*N*. The mixture was filtered, allowed to stand for two weeks, then filtered again and diluted to 2 l. The solution was standardized gravimetrically by evaporation of measured portions to dryness in porcelain boats, and reduction by hydrogen at 550° to constant weight. Closely agreeing results showed the stock solution to contain 439·3 ppm of ruthenium. The dark brown solution showed no visible change throughout the investigation period.

Because the "$RuCl_3$" starting material apparently contained both ruthenium(III) and ruthenium(IV), evaluation of the reaction with DABA required preparation of solutions containing the ruthenium in essentially a single oxidation state. Ruthenium(IV) solution was prepared by direct chlorination of a portion of the stock solution, made 1*M* in hydrochloric acid; after boiling to remove dissolved chlorine, the solution was bright yellow. Ruthenium(III) solution was prepared by refluxing a portion of the acidified stock solution with ethanol for several hours; from the colourless solution excess of ethanol was removed by evaporation. Tests with DABA showed that only ruthenium(III) gave the desired reaction. For later use in isolation of reaction products, ruthenium(III) chloride and bromide were prepared by distilling a sodium bismuthate–sulphuric acid slurry of the commercial ruthenium trichloride, the $RuO_4$ being received in hydrochloric or hydrobromic acid; the solid ruthenium halide was obtained by evaporation of the receiving solution.

The pH buffer used in the recommended procedure was 0·91*M* in acetic acid and 0·75*M* in sodium acetate.

All other reagents were ACS reagent grade or equivalent.

*Recommended procedure*

The sample, consisting of ruthenium(III) chloro-complexes in aqueous hydrochloric acid and containing 10–40 $\mu$g of ruthenium, was placed in a 25-ml volumetric flask; 1·00 ml of DABA reagent solution was added, along with acetate buffer (4–6 ml) to adjust the pH to 4·5. The sample solution and a similar blank were heated in a water-bath for 1 hr at 75 $\pm$ 1°. After cooling, the sample and blank were made up to volume with distilled water and allowed to stand 10–15 minutes. Absorbance of the purple-red solution was measured at 550 nm against the reagent blank.

## RESULTS AND DISCUSSION

### *Calibration, range and sensitivity*

Calibration data are given in Table I; each absorbance value is the average of triplicate samples, the maximum deviation being 0·003 absorbance unit. The system conforms to Beer's law over the range studied. The concentration range for least relative error, for measurement in 10-mm cells, is 0·5–1·7 ppm. At 550 nm the molar absorptivity of ruthenium is $4{\cdot}16 \times 10^3$ l.mole$^{-1}$.mm$^{-1}$.

### *Reproducibility and stability*

Twenty-six samples, each at concentration 0·878 ppm ruthenium, developed by the recommended procedure, gave a mean absorbance of 0·360, with a standard deviation of 0·002. Data for this study were collected over a period of several months. For the stability test, samples were developed as usual, and the absorbance was measured at frequent time intervals. The absorbance was unchanged for at least 8 hr, and for most samples no change had occurred in 24 hr.

TABLE I.—CALIBRATION DATA

| Ruthenium, *ppm* | Absorbance | Specific absorptivity, $ppm^{-1}\ mm^{-1}$ |
|---|---|---|
| 0·527 | 0·216 | 0·0410 |
| 0·703 | 0·290 | 0·0412 |
| 0·878 | 0·360 | 0·0410 |
| 1·05 | 0·434 | 0·0413 |
| 1·23 | 0·503 | 0·0409 |
| 1·41 | 0·581 | 0·0412 |
| 1·58 | 0·654 | 0·0414 |
| 1·76 | 0·727 | 0·0413 |
| | Average | 0·0412 |
| | Standard deviation | 0·0002 |

*Effect of pH*

In a series of 1·05-ppm ruthenium samples, pH was adjusted with hydrochloric acid or with sodium hydroxide, and the solutions were allowed to stand at room temperature for 1 hr before absorbance measurement. Below pH 3, colour reaction was slight; above pH 7 the absorbance was essentially constant (0·37) but significantly lower than that obtained by using the recommended procedure (see Fig. 1, curve 1). At pH above 7, colour could be developed rapidly by heating at 75°, but reproducibility and colour stability were unsatisfactory. Curve 2 of Fig. 1 shows the results for 1·05-ppm ruthenium samples with pH adjustment by acetic acid–sodium acetate buffer (total acetate, 1·66*M*) and colour development at 75° for 1 hr. Because ruthenium is known to form complex ions with acetate,[12,13] tests were made to

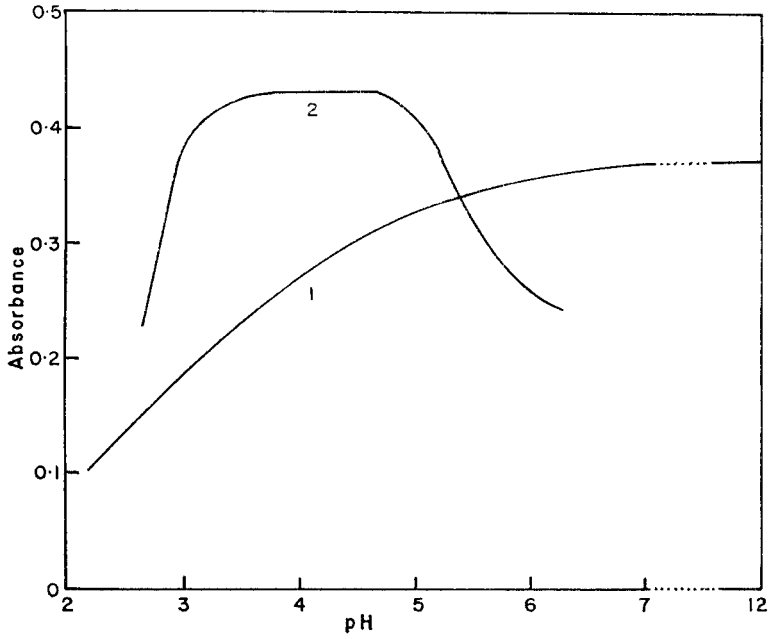


FIG. 1.—Effect of pH on absorbance.
1. Solution developed at room temperature.
2. Solution developed at 75°, acetate buffer.

determine any possible concentration effect of acetate ion, by using solutions containing varying quantities of pH 4·5 buffer, and also solutions in which the pH was adjusted to 4·5 by adding sodium hydroxide to the acidic ruthenium solution. Within experimental error the developed solutions had identical absorbances.

*Effect of temperature and time*

Samples containing 1·05 ppm ruthenium were treated by the recommended procedure, except that temperature and heating time were varied. Absorbance at 550 nm was measured at the end of the heating period, and again after 12 hr. Colour development at room temperature was quite slow (4–5 hr). At 60–65° at least 1 hr was required to attain maximum absorbance. At 70–75° maximum absorbance was attained in 30 min, and times up to 2 hr could be used without adverse effect; if the solutions were then stored at room temperature, the absorbance remained constant for 12 hr. At temperatures near 100° the absorbance increased rapidly during the first 20–30 min, then slowly decreased on additional heating. After the solutions had been standing for 12 hr the absorbance again increased, but to values less than those attained by samples prepared at 75°.

Colour-developed solutions measured at 25, 37 and 60° showed no significant change in the spectral curve or the wavelength of maximum absorption. A very slight decrease in absorbance at 550 nm with increasing measurement temperature indicated that normal fluctuations in room temperature would not produce significant deviations in absorbance.

*Effect of reagent concentration*

Solutions were developed as in the standard procedure except that the amount of DABA reagent was varied to give a mole ratio of DABA to ruthenium ranging from 0·25 to 256. The absorbance, for a given amount of ruthenium, attained a constant value at a reagent-to-metal mole ratio of about 30. In the standard procedure, 1·00 ml of reagent provides a ratio greater than 150. Solutions with a ratio less than 2 were blue-purple, with maximum absorption at 565 nm, whereas solutions containing a higher ratio were purple-red, with absorption peak at 550 nm.

*Effect of ethanol*

Oxidation of DABA reagent (see later discussion) gives rise to a pale yellow product, which is revealed in both reference blank and developed ruthenium samples by a weak absorption centred at 450 nm. Absorption at this wavelength is substantially less when ethanol is present. In the recommended procedure, ruthenium(III) is most conveniently obtained by reduction with ethanol, the excess of which is removed by evaporation. Large amounts of ethanol in the developed solution interfere, presumably by altering the buffer system, causing an increase in the apparent pH of the solution. However, amounts of ethanol up to 10% by volume can be tolerated and indeed are advantageous in decreasing the 450-nm absorption.

*Effect of foreign ions*

Interference tests were conducted over the entire optimum concentration range for ruthenium by developing the samples in the presence of the foreign ion. Metal ions were added as their chlorides [except iron(II), added as ferrous ammonium

sulphate], and anions were added as their alkali metal salts. Salts of periodic-table group VIII and of gold and uranium, were refluxed along with the ruthenium in ethanol solution. Other foreign ions were added directly to the reduced ruthenium sample. Concentration of foreign ion is conveniently expressed as the mole ratio of foreign ion to ruthenium. An ion is said to interfere if a mole ratio less than 1·0 produces an absorbance deviation greater than 0·01 from that of a sample containing ruthenium alone. Results of the interference tests are shown in Table II; the metals are designated by the oxidation state originally added; for those metals treated along with ruthenium in the reduction step with ethanol, a lower oxidation state may be the actual state tested. Previous work in this laboratory[10] has shown that platinum(IV) readily oxidizes the DABA reagent, whereas platinum(II) does not. Platinum(IV) solutions that were refluxed with ethanol did not oxidize the DABA reagent, indicating that platinum(IV) had been reduced. Iron(III) oxidized the DABA reagent; on refluxing with ethanol the iron(III) colour disappeared and the resulting solution failed to oxidize the reagent. Oxidation of the reagent by osmium was not prevented by refluxing with ethanol. Alkali-metal and ammonium cations, and the anions fluoride, chloride, bromide, iodide, nitrate, sulphate and phosphate gave no indication of interference at molar concentration ratios much greater than 100. The high tolerance for common masking agents (*e.g.*, tartrate and citrate) is noteworthy.

TABLE II.—SUMMARY OF INTERFERENCE TESTS

| Ion | Tolerance, mole ratio Ion:Ru | Ion | Tolerance, mole ratio Ion:Ru |
|---|---|---|---|
| Rhodium(III) | 10 | Zinc(II) | 100 |
| Palladium(II) | 1 | Cadmium(II) | 100 |
| Platinum(IV) | 1 | Lead(II) | 10 |
| Iridium(IV) | 10 | Mercury(I) | 10 |
| Osmium(III) | 0·5 | Mercury(II) | 1 |
| Iron(II) | 5 | Uranium(IV) | 10 |
| Iron(III) | 1 | Gold(III) | 5 |
| Cobalt(II) | 5 | Tartrate | 100 |
| Nickel(II) | 5 | Citrate | 60 |
| Copper(I) | 5 | Oxalate | 5 |
| Copper(II) | 1 | EDTA | 2 |

The favourable tolerance for lead, zinc and uranium is of special significance. Lead is commonly used as a collector for the platinum metals in assay procedures. Ruthenium is a fission product of uranium; the uranium fuel for the Experimental Breeder Reactor II contains 2·0% ruthenium and smaller amounts of palladium and rhodium and part of the processing system for that reactor uses molten zinc and zinc–magnesium alloys in the separation of ruthenium from uranium.[4] Of all the metals tested, none interfered as a result of producing a colour similar to that of ruthenium in the recommended procedure. All the platinum elements except osmium can be tolerated at a molar ratio to ruthenium of one or greater. Under the conditions of the recommended procedure, platinum was the only element to form a coloured solution with DABA. In the DABA method for platinum reported by Johnson and Ayres,[10] the reagent and platinum solution, at pH less than 3, was heated for 15 min at near boiling, then cooled to room temperature; the pale amber solution changed to blue-green (absorption peak at 715 nm) upon addition of sodium

hydroxide and the colour intensity increased rapidly in the pH range 6–8, then levelled off at pH 10–12.

In order to compare their behaviour under identical conditions, a solution containing equimolar quantities of ruthenium(III) and platinum(II) was developed, by the recommended procedure, in an absorption cell maintained for 1 hr at 75° in a Beckman DK-1 spectrophotometer. The absorbance was measured at 75° and again after cooling to room temperature. The spectra are shown in Fig. 2. The platinum absorption at 715 nm disappeared on cooling of the solution, but reappeared when the solution was again heated, or when the cooled solution was made alkaline. These observations offer some interesting possibilities for the simultaneous determination of ruthenium and platinum, using DABA reagent under compromise conditions of pH and development procedure.

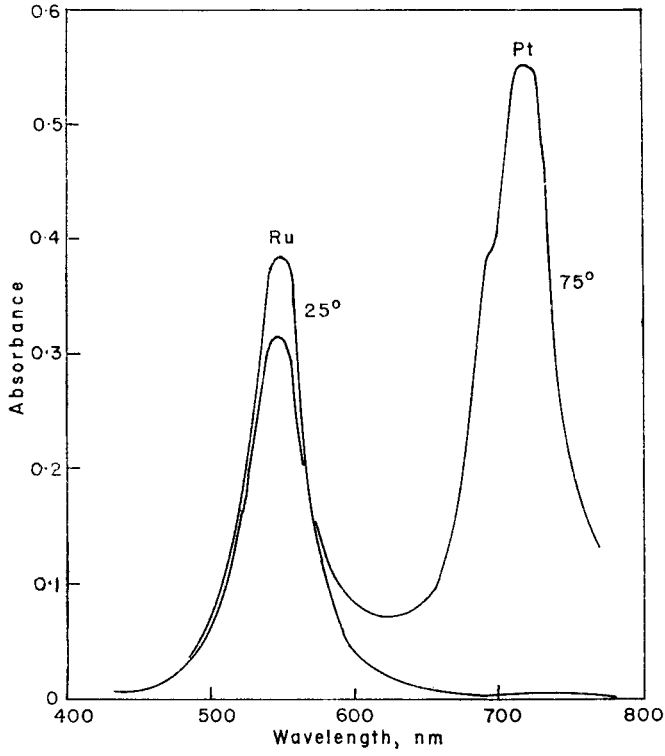


FIG. 2.—Spectral curves of DABA complexes of ruthenium and platinum.

*Separation of ruthenium*

Osmium interferes in the determination to an extent that requires its removal. The problem of interferences in ruthenium determination is simplified by the fact that this element may be separated quantitatively from the other platinum elements by distillation of its tetroxide. Although osmium tetroxide is also volatile, selective oxidation permits their separation. Solutions containing 43·9 $\mu$g of ruthenium and 825 $\mu$g of osmium (a tenfold molar excess) as their chloro-complexes in dilute hydrochloric acid solution were treated with about 2 ml of 30% hydrogen peroxide

by dropwise addition. The solution was boiled gently to volatilize osmium tetroxide, then was evaporated to near dryness with concentrated hydrochloric acid. Addition of 2 ml of 0·1*M* hydrochloric acid and 15–20 ml of 95% ethanol, and slow evaporation to about the original volume served to reduce the ruthenium to the +3 state, which was then determined by the recommended procedure. Results for duplicate samples were as follows: Ru taken, 43·9 μg; Ru found, 43·9 and 44·1 μg.

Distillation of ruthenium tetroxide, for separation from other platinum and base metals, followed essentially the method of Larsen and Ross,[14] except that the receiving solution was 6*M* hydrochloric acid rather than 1*M* sodium hydroxide containing sodium hypochlorite; also, a second bubbler was used as a precaution against possible loss of ruthenium. Five minutes of gentle boiling was sufficient for complete volatilization of the ruthenium tetroxide by the use of sodium bismuthate–sulphuric acid in the still. The receiving solutions and washings were evaporated and then reduced with ethanol as described previously, and the ruthenium was determined with DABA by the recommended procedure. The results are shown in Table III.

TABLE III.—RUTHENIUM SEPARATED BY DISTILLATION

| Ru taken, *mg* | Ru found, *mg* |
|---|---|
| 0·439 | 0·435 |
| 0·439 | 0·439 |
| 43·9 | 43·5 |
| 43·9 | 43·4 |
| 43·9* | 43·4 |

* Sample contained a ten-fold molar excess of iridium, platinum, rhodium and palladium.

*The prior reduction*

The reactive properties of ruthenium in solution are markedly influenced by pH, concentration of associated anions, temperature and time of heating and solution age. The starting material for the present investigation, nominally ruthenium trichloride, is more properly described as ruthenium(III,IV) chloride, with the +4 state possibly predominating; the solution in hydrochloric acid contains a variety of chloro-complexes. When such a solution, or a solution of ruthenium(IV), is reacted with DABA, the characteristic absorption at 550 nm is invariably less than that of a solution containing an equivalent amount of ruthenium(III) and an absorption of moderate intensity at 450 nm appears which is not present in the DABA reagent solution. The absorption at 450 nm was identified as due to an oxidation product of DABA. Reduction of the ruthenium to the +3 state before addition of DABA was therefore indicated, to preclude oxidation of the reagent.

Several reducing agents that were tried, although reducing ruthenium(IV), were unsatisfactory. Excess of hydroxylammonium chloride prevented the desired reaction with DABA. Reduction with zinc or magnesium could not be controlled to prevent reduction to ruthenium(II) or to the metal. Titanium(III) sulphate gave a bright yellow precipitate with DABA. A large excess of iodide cannot be tolerated because of oxidation of iodide by atmospheric oxygen, catalysed by ruthenium compounds,[15] and also because of formation of insoluble $RuI_3$. Reduction by refluxing the

ruthenium(III,IV) with ethanol in hydrochloric acid had the advantage of giving ruthenium(III) without introducing any metallic or other interfering components. The solutions do not contain ruthenium(II), the chloro-complexes of which are bright blue, unreactive toward DABA, easily air-oxidized, and disproportionate to ruthenium(III) and the metal. Furthermore, all known compounds of ruthenium(II) are diamagnetic, whereas those of ruthenium(III) are generally paramagnetic; the isolated ruthenium–DABA product (described later) is paramagnetic, as shown by its electron spin resonance spectrum.

In the reduction step with alcohol, the original brown ruthenium solution first became much darker, then gradually turned bright yellow and within an hour was colourless and clear. Usually the refluxing was continued for 24 hr; heating for 48 hr produced no change in appearance of the solution. The reduced ruthenium solutions showed no visible change on standing for several months. For proper reduction by this method the molar ratio of hydrochloric acid to ruthenium should be at least 230; if insufficient acid was present, small floating flecks of ruthenium metal were observed after 5–10 hr of heating. Occasionally, under conditions of low acidity and prolonged heating, ruthenium plated out as a bright mirror on the wall of the flask. Prolonged heating in a sealed tube at 90–100° produced bright blue solutions characteristic of ruthenium(II).

Upon refluxing with ethanol under the same conditions as used for ruthenium, solutions containing the chloro-complexes of rhodium, iridium and palladium quickly formed finely divided grey or black precipitates, leaving colourless solutions. After standing for several days the dark precipitate of palladium redissolved, giving the familiar yellow colour of palladium(II) chloride. Platinum(IV) solution, after many hours of heating with ethanol, showed a small amount of bright mirror deposit on the flask wall. Gold chloride readily produced a metallic plating on the flask. The osmium solution gave no visible change. In all cases of reduction to the metallic state, this reaction could be prevented or retarded by increasing the acid concentration.

From these observations, it might appear possible to accomplish a separation of ruthenium and osmium from the other platinum elements (and gold) by reducing the latter group to the metallic state with ethanol. However, when another platinum element is present with ruthenium, the deposited metal apparently causes more rapid reduction of ruthenium.

*Absorption spectra of ruthenium solutions*

In Fig. 3, curve 1 is the absorption spectrum of the standard ruthenium(III,IV) solution (43·9 ppm ruthenium) in 0·1*M* hydrochloric acid. Curve 2 is the spectrum of an identical solution after reduction with and evaporation of excess ethanol and dilution to the original volume with 0·1*M* hydrochloric acid. Curve 3 is the spectrum of 22·0 ppm of ruthenium(IV) prepared by chlorination of the standard solution in 1·0*M* hydrochloric acid; this spectrum agrees quite well with the one published by Pantani[16] for a hydrochloric acid solution of ruthenium(IV) chloride. A solution reduced with ethanol (curve 2), upon oxidation with ammonium peroxydisulphate, gives a solution having a spectrum identical with curve 3. In order to determine whether the colourless ruthenium solutions formed by reduction with ethanol might contain an alcoholate complex, the reduced solution was evaporated with concentrated hydrochloric acid to near dryness, then restored to its original volume with 0·1*M*

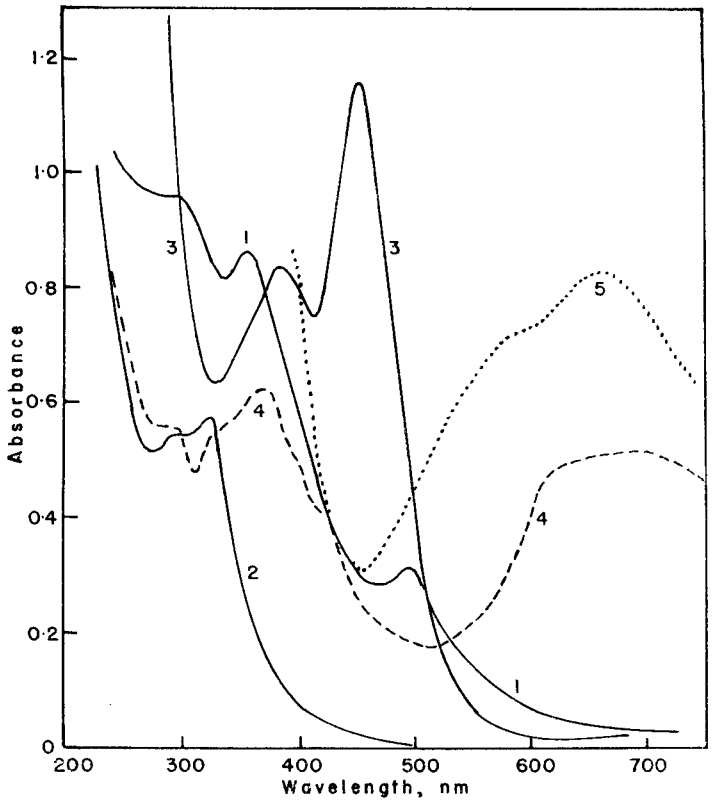


FIG. 3.—Spectral curves of various ruthenium solutions.
1. Ru(III,IV), 43·9 ppm, in 0·1*M* HCl.
2. Solution 1 after reduction with ethanol.
3. Ru(IV), 22·0 ppm, in 1*M* HCl.
4. Solution 2, evaporated, residue heated, and dissolved in 0·1*M* HCl.
5. Ru(II), by reduction of solution 1 with zinc.

hydrochloric acid. The absorption spectrum matched curve 2, and the solution responded to the DABA procedure the same as the solution of curve 2. An ethanol–ruthenium solution in 0·1*M* hydrochloric acid was evaporated to dryness and the residue was heated gently, then dissolved in 0·1*M* hydrochloric acid, giving a green solution (curve 4). The green solution is not stable at room temperature, but it may be kept unchanged for several weeks if refrigerated. When heated with concentrated hydrochloric acid, the green solution turns red; upon addition of ethanol and heating, the green solution becomes colourless. The green and the red solute species have been attributed[17] to the *cis* and *trans* isomers, respectively, of $H[Ru(H_2O)_2Cl_4]$, although efforts to substantiate these formulations were unsuccessful.[18] Reduction of ruthenium solution, 1*M* in hydrochloric acid, with metallic zinc gives an unstable blue solution, attributed to ruthenium(II), having a broad absorption band with peak at 650 nm (curve 5).

*Absorption spectra of the ruthenium–DABA system*

Curve 1 of Fig. 4 represents the spectrum of 1·76 ppm of ruthenium, developed from ruthenium(III) solution by the usual procedure. Curve 2 is the spectrum of the solution developed from 1·76 ppm of ruthenium(IV). Both solutions were

measured against a reagent blank carried through the heating operation. Curve 3 represents the reagent blank carried through the heating step. With increasing concentration of ruthenium(IV) in the starting solution, the absorption band centred at 450 nm increased in intensity, but absorption at this wavelength was at the blank level for all concentrations of ruthenium(III) used. A similar effect was observed in reactions of platinum(IV) and platinum(II) with DABA.[10] These observations suggest that DABA reduces ruthenium(IV) to ruthenium(III) which is then complexed by DABA, and that the absorption at 450 nm is due to an oxidation product of the reagent. The latter assumption was confirmed by measuring the absorption, in the 350–550 nm region, of DABA reagent blanks before heating (no measurable absorption), after heating for 1 hr at 75°, after standing for several days at room temperature, and after treatment with iron(III), platinum(IV), or ammonium peroxydisulphate. In all cases these oxidizing conditions produced a

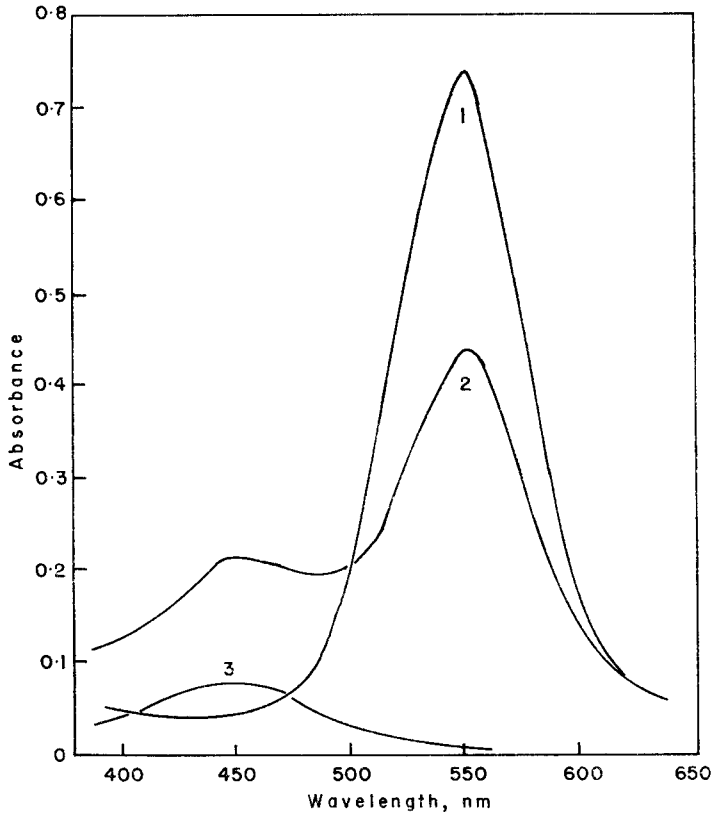


FIG. 4.—Spectral curves of ruthenium—DABA solutions.
1. Ru(III), 1·76 ppm, +DABA.
2. Ru(IV), 1·76 ppm, +DABA.
3. DABA reagent blank.

wide absorption band centered at 450 nm. Oxidation of DABA by iron(III) gives 7-amino-6-hydroxy-phenazine-2-carboxylic acid;[19] presumably the same product is formed from DABA by oxidation by ruthenium(IV).

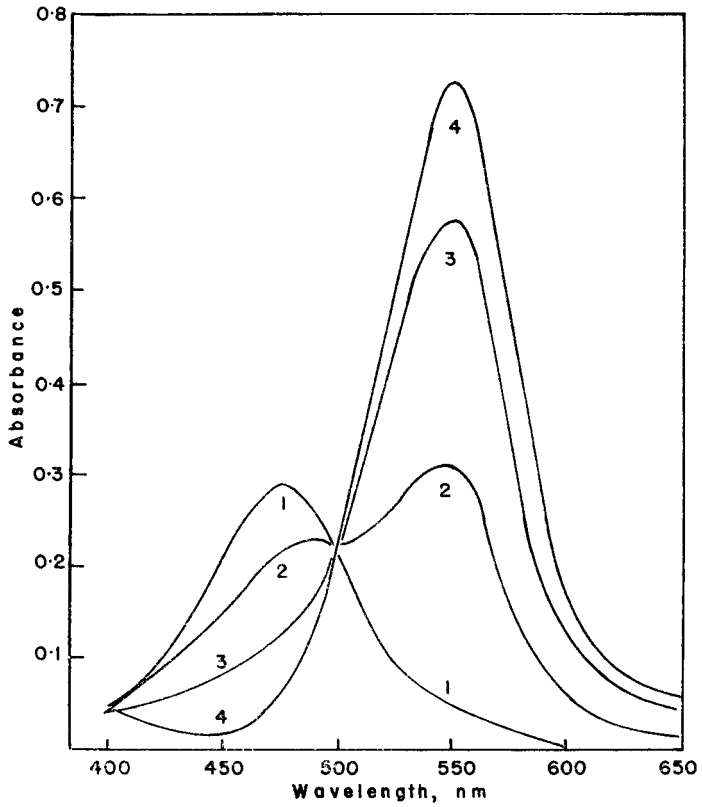


FIG. 5.—Change of absorption spectrum of ruthenium-DABA solution with heating time.
1–4. At times 2·5, 5, 25 and 60 min, respectively.

The reaction between ruthenium(III) and DABA under the conditions of the recommended procedure was monitored by recording the absorption spectrum of the solution at intervals during development. Samples and blanks were taken at the end of 2·5, 5, 25 and 60 min; they were cooled quickly in ice water, and the spectra were recorded. The results for 1·76 ppm ruthenium are shown in Fig. 5. The original amber colour (480 nm absorption) rapidly gave way to purple-red, the absorption at 550 nm increasing with additional development time. It was mentioned earlier that when the DABA-to-ruthenium ratio was less than about 2, the developed solutions were blue-purple. This experiment was repeated with ruthenium(III) and DABA in equimolar amounts. Similar results were obtained, except that the amber colour gave way to blue-purple, and the absorption peak occurred at 565 nm.

## STUDY OF THE COMPLEX

### *Mole-ratio method*

The mole-ratio method[20] is useful for determination of the overall reaction stoichiometry in complex formation. Interpretation of experimental data frequently relies on long and uncertain extrapolations, so that reaction stoichiometry cannot be established unambiguously. Under these circumstances—as in the case considered here—the mole ratio still serves to complement other methods.

A series of solutions each containing 1·05 ppm of ruthenium but varying amounts of DABA was developed by the usual procedure. Absorbance was measured at 565 and 550 nm. Plots of absorbance against mole ratio DABA: Ru were extensively rounded, with no sharp changes of slope. Extrapolation of the initial and the final linear segments of the curves intersected at a mole ratio of 2·2 and 3·1 for the 565 nm and the 550 nm curves, respectively.

The experiment was repeated, with a constant amount of DABA ($7{\cdot}0 \times 10^{-5}M$) and varying ruthenium concentration. Solutions of Ru:DABA ratio less than about 0·3 were red, with absorption maximum at 550 nm; those with ratio greater than 0·5 were blue-purple, with absorption peak at 565 nm. Solutions in the ratio range 0·3–0·5 showed only a single peak at 560 nm. In the mole ratio-plot, changes of slope at 0·33 and 0·50 corresponded to an Ru:DABA ratio of 1:3 and 1:2, respectively. There was also some indication of change of slope at 0·25 mole ratio (1:4 complex), suggesting action of DABA as a monodentate ligand. In reaction with solutions of nickel, cobalt, and zinc, *o*-phenylenediamine is reported to function as a monodentate ligand, giving complexes in which 2, 4, or 6 molecules of ligand are co-ordinated;[21] it is known that *o*-phenylenediamine and DABA are very similar in their reactions with ruthenium. When mole-ratio measurements were made on alcoholic solutions, only a single break occurred at an Ru:DABA mole ratio of 0·50 (1:2 reaction).

*Method of continuous variation*

For application of this method[22,23] the concentration sum, $c_{\mathrm{Ru(III)}} + c_{\mathrm{DABA}}$, was held constant, but the mole fraction of DABA was varied from 0 to 1. In the first series, of concentration sum $1{\cdot}03 \times 10^{-4}M$, with increasing mole fraction of DABA the absorption maximum of the solutions shifted from 565 nm (at mole fraction 0·5) to 550 nm, the shift being complete at DABA mole fraction about 8·5. Of results taken at several wavelengths, the continuous-variation plots in Fig. 6 are typical, and indicate Ru:DABA reaction ratios of 1:2 (mole fraction 0·67) and 1:3 (mole fraction 0·75). Identical indications of reaction ratios were obtained by using a concentration sum of $5{\cdot}06 \times 10^{-5}M$; by using ruthenium sulphate solution (obtained from the stock standard by fuming down with sulphuric acid, then reducing with ethanol); and by acidifying the ruthenium solution with perchloric acid after evaporation of the hydrochloric acid.

Solutions with DABA mole fraction of 0·67, but with the pH varied from 3·3 to 4·8, all showed an absorption maximum at 565 nm, and differed only in absorbance values. Therefore, a wavelength shift of absorption peak is not related to pH variation. Solutions of mole fraction 0·67 and pH 4·5 were treated with oxidizing agents such as iron(III) chloride, ammonium peroxydisulphate, and osmium tetroxide, and with mild reducing agents such as glucose and formic acid, and strong reductants such as zinc powder and titanium(III) sulphate. These reagents merely altered the absorbance value, but caused no change in wavelength of maximum absorption. However, on addition of sufficient DABA to increase its mole fraction to about 0·8, the absorption curve became symmetrical around the 550 nm peak.

Ethanol at a concentration of 50% by volume suppressed the wavelength shift, which became complete only at a DABA mole fraction of 0·95. Higher alcohols were more effective than ethanol in preventing the shift, and dimethylformamide

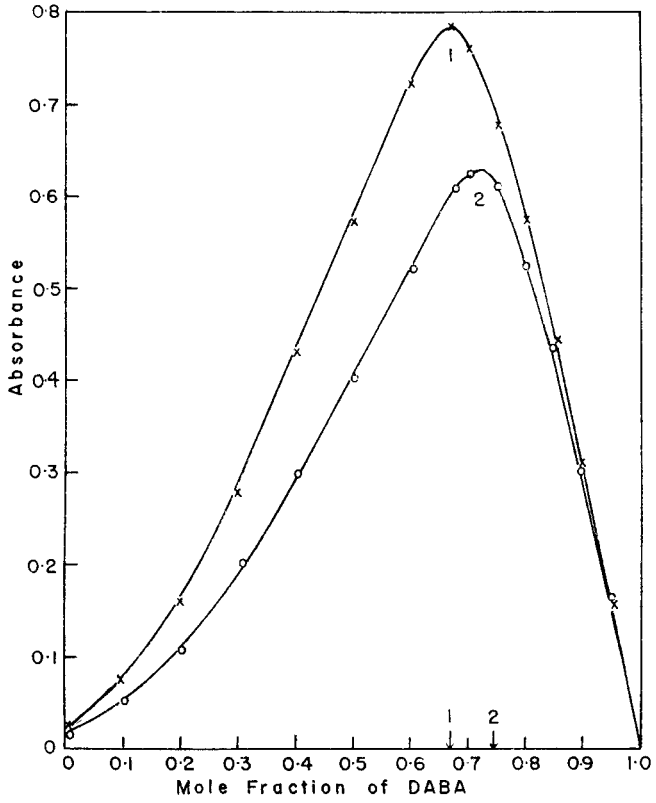


FIG. 6.—Continuous-variation plots, ruthenium–DABA.
1: 570 nm; 2: 530 nm.

(DMF) at 50% v/v entirely prevented the shift, even with very large excesses of DABA. Sodium chloride and sodium bromide slightly suppressed the wavelength shift. In a continuous-variation study of solutions that were $1{\cdot}03 \times 10^{-4}M$ in Ru(III) + DABA, $0{\cdot}27M$ in sodium chloride, and 50% v/v DMF, all solutions exhibited a single sharp peak at 565 nm, and the continuous-variation plot gave a maximum at 0·67 mole fraction of DABA (Ru:DABA = 1:2).

The continuous-variation and the mole-ratio studies give clear evidence for existence of ruthenium–DABA complexes of 1:2 and 1:3 stoichiometry. Ruthenium in the source solution is present as its chloro-complexes; the observed effects of high concentrations of chloride ion and of solvents of low dielectric constant in suppressing the formation of the 1:3 complex are consistent with the role of these substances in the ionic equilibria involving ruthenium(III) and its chloro-complexes.

Mentioned earlier was the fact that formation of amber solutions (absorption maximum at 480 nm) always preceded formation of the blue and the red solutions in the recommended development procedure. Although it was impossible to stabilize the amber solutions, it was found that addition of sodium chloride increased the formation of the amber form and retarded formation of the blue product, as shown in Fig. 7. A continuous-variation study was made with solutions $2{\cdot}58 \times 10^{-4}M$ in Ru(III) + DABA, $1{\cdot}0M$ in sodium chloride, and at pH 4·5. The solutions were

heated at 85° for 2 min, then quenched with ice-water and the absorbance at 480 nm was measured. The continuous-variation plot gave a maximum at DABA mole fraction 0·67. It appears, then, that the amber precursor of the other coloured forms has the same stoichiometry as the blue form. Isolation of an amber solid product is described later.

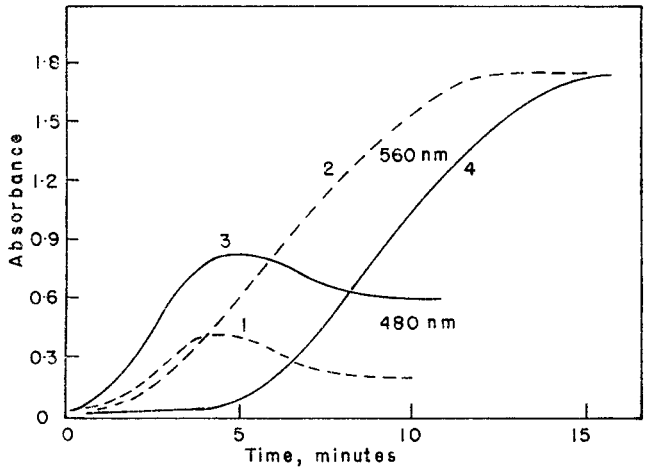


FIG. 7.—Effect of sodium chloride on absorbance developed.
1 and 2: in absence of NaCl; 3 and 4: NaCl added.

*Reactions of similar organic compounds*

The conditions of the general procedure used for DABA were applied to the reaction of ruthenium with several compounds (listed in the second paragraph of this article) possessing the common feature of two adjacent amino groups on an aromatic nucleus; similar colors were produced. In all cases the coloured products were insoluble in non-polar organic solvents. The tertiary amine, TMOPDA, gave a less intense colour than the primary amines; reactions of the latter appear to involve deprotonation. TMOPDA also formed only a 1:1 complex with ruthenium, probably because of steric effects of the methyl groups. In the case of OPDA, which may be considered the parent compound of DABA, a wavelength shift was also observed; in solutions of mole fraction less than 0·67 the absorption peak occurred at 553 nm, while with excess of reagent the peak was at 540 nm. Continuous-variation plots for these regions gave maxima at 0·67 and at 0·75 mole fraction, respectively, corresponding to Ru:OPDA stoichiometry of 1:2 and 1:3, analogous to the ruthenium complexes with DABA.

*Ion-exchange tests*

The ruthenium–DABA complex, in either acidic or alkaline solution, was strongly retained by a cation-exchange resin, from which it was eluted very slowly with concentrated hydrochloric acid. The same behaviour was shown by the ruthenium–OPDA complex. The ruthenium–DABA product, in acid solution, was weakly retained on an anion-exchanger and was easily eluted with water, whereas from alkaline solution the coloured solute was more extensively exchanged

but was readily eluted with dilute hydrochloric acid. By comparison, the ruthenium–OPDA product was very little retained from acidic or from alkaline solution, and was readily eluted with water. These tests indicate that the coloured species in the ruthenium–DABA solutions is predominantly cationic, although in alkaline medium it exhibits some negative character owing to presence of the carboxylate groups. It is also possible that some deprotonation of the amino groups has occurred.

*Isolation and analysis of solid products*

Because commercial ruthenium chloride frequently contains nitrosyl complexes,[24] the ruthenium used in these isolations was prepared from the commercial trichloride by distillation from a slurry of sodium bismuthate and sulphuric acid.[14] The ruthenium tetroxide was absorbed in either concentrated hydrochloric acid or hydrobromic acid. By evaporation of the receiving solution the ruthenium halide was obtained.

Addition of acetone to an alkaline solution containing ruthenium(III) chloride and excess of DABA caused separation of a purple solid (presumably the sodium salt) which was very soluble in water; addition of hydrochloric acid to the aqueous solution caused precipitation of a purple solid. Dropwise addition of dilute sodium hydroxide solution to an acidic solution of ruthenium(III) chloride and a 2–3-fold molar amount of DABA produced first a dark blue solution, then a deep amber solution, and when the pH reached approximately 5, a flaky brown precipitate formed. Further addition of sodium hydroxide dissolved the brown precipitate to give a clear amber solution (absorption maximum at 480 nm) followed by a deep purple solution (maximum absorption at 565 nm). At this stage, dropwise addition of hydrochloric acid gave an immediate dark blue precipitate. The brown precipitate first formed was easily filtered off; it was moderately soluble in water, and on exposure to moist air or on washing with water or with DMF it turned blue. The blue precipitate formed as described above, which was very difficult to filter off, was washed with DMF, water and acetone. It was insoluble in water and in the common organic solvents, slightly soluble in concentrated hydrochloric acid, but very soluble in warm concentrated phosphoric acid.

For comparison purposes, ruthenium derivatives of other aromatic *o*-diamines were prepared. The OPDA products were formed by slow addition of ruthenium(III) solution to excess of OPDA at pH about 4. The flaky brown precipitate formed was easily dissolved by warming the mixture, giving a clear amber solution; addition of sodium hydroxide to make the pH about 6 gave a blue precipitate. Brown precipitates were formed from ether solution in the cases of DABA, OPDA and DANp, and from benzene solution with DAPh. The brown precipitates were also formed from ethanol solution. All precipitates were filtered off, washed with the organic solvent, and dried under vacuum. Addition of alcoholic sodium hydroxide to the filtrates from the DANp and DAPh preparations in alcohol developed first an intense green colour, followed by blue and a dense purple precipitate. Filtrates from DABA and OPDA formed purple precipitates, but no intermediate green colour. The filtrate from the TMOPDA preparation gave no colour reaction with sodium hydroxide.

In general, the amber or brown forms of the complexes were moderately to sparingly soluble in water and in DMF, depending on the particular diamine used, the brown product from DABA being the most soluble. They were quite unstable,

readily becoming blue on exposure to moist air; a notable exception was the ruthenium–TMOPDA complex, which was quite stable in solid form and in solution. A marked increase in stability of the brown products was observed when the isolation was performed entirely in anhydrous solvents. The purple or blue precipitates were much more stable, and were very slightly soluble in DMF. X-ray diffraction examination indicated the compounds to be amorphous. Upon heating, all the compounds decomposed without melting; decomposition at high temperature resulted in reduction to ruthenium metal, accompanied by a vivid display of sparks.

Elemental analysis of the amber or brown solid products indicated a composition represented by $RuL_2X_3$ (L = ligand molecule, X = halode), except in the case of the product from TMOPDA, in which the metal to ligand ratio was 1:1, and from which no blue product was obtained. In the blue compounds the 1:2 stoichiometry was preserved, but halide was absent. Conversion of the amber into the blue forms by treatment with alkali indicated a deprotonation. Because of the extreme insolubility of the solid products and the difficulty of obtaining them in high purity, it was not feasible to conduct additional experiments to determine with confidence the number of protons lost per molecule of complex. In describing the nickel complex of OPDA (possibly analogous to the ruthenium–OPDA complex), Feigl and Furth[25] indicated some uncertainty as to the number of hydrogen atoms remaining on the nitrogen atoms, and whether the OPDA acted as a monodentate or a bidentate ligand; recently it has been shown[26] by mass spectrometry measurements that in the reaction with nickel, OPDA serves as a bidentate ligand, and that one hydrogen atom remains on each of the four nitrogen atoms.

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**Zusammenfassung**—Ruthenium(III)chlorid und 3,4-Diaminobenzoesäure reagieren in wäßriger Lösung bei pH 4,0–4,5 langsam bei Zimmertemperatur, schneller beim Erhitzen der Mischung, wobei Lösungen entstehen, die Farbänderungen von bernsteinfarbig bis purpurrot durchlaufen; das Absorptionsmaximum voll entwickelter Lösungen liegt bei 550 nm. Die Einflüsse von Temperatur und Zeitdauer der Erhitzung, pH, Reagenskonzentration und anderer Variabler wurden untersucht. Das System genügt dem Beerschen Gesetz; der optimale Konzentrationsbereich für Messung in 10-mm-Küvetten liegt bei etwa 0,5–2 ppm Ruthenium. Störung durch andere Ionen, besonders andere Elemente der Platingruppe, wird durch destillative Abtrennung von Ruthenium vermieden. Die spektrophotometrischen Methoden des Molverhältnisses und der kontinuierlichen Änderungen zeigten die Gegenwart von Komplexen mit der Stöchiometrie 1:2 und 1:3 (Ruthenium:Reagens). Die Elementaranalyse von aus der Lösung isolierten festen Produkten bestätigte das Reaktionsverhältnis 1:2. Mehrere andere Diamine gaben ähnlich gefärbte Lösungen und ähnliche Stöchiometrie der Reaktion.

**Résumé**—Le chlorure de ruthénium(III) et l'acide 3,4-diaminobenzoïque, en solution aqueuse à pH 4,0–4,5, réagissent lentement à témperature ordinaire, mais plus rapidement quand le mélange est chauffé, donnant des solutions qui vont par des transitions de coloration de l'ambre au pourpre-rouge; l'absorption maximale des solutions entièrement développées se situe à 550 nm. On a étudié les influences de la température et du temps de chauffage, du pH, de la concentration

en réactif, et d'autres variables. Le système se conforme à la loi de Beer; le domaine de concentrations optimal, pour la mesure en cuves de 10 mm, est d'environ 0,5–2 p.p.m. de ruthénium. L'interférence d'ions étrangers, spécialement d'autres éléments du platine, est évitée par une séparation par distillation du ruthénium. Les méthodes spectrophotométriques de rapport de moles et de variations continues indiquent la présence de complexes de stoechiométrie 1:2 et 1:3 ruthénium-réactif. L'analyse élémentaire de produits solides isolés d'une solution a confirmé le rapport de réaction 1:2. Plusieurs autres *o*-diamines donnent des solutions colorées et une stoechiométrie de réaction similaires.

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# SHORT COMMUNICATIONS

## Gas chromatography of metal chelates with carrier gas containing ligand vapour

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THE GAS CHROMATOGRAPHY of volatile metal chelates has received considerable attention in recent years and the studies on volatile $\beta$-diketonates are particularly extensive.[1] With the synthesis of new chelating agents, a number of metals have come to be studied.

However, the chromatographic peaks of metal chelates are often asymmetric and not well defined, showing severe tailing or leading. Therefore, the application of the method is still limited mainly to metals which form thermally stable chelates.

Uden *et al.*[2] have attributed the anomalous peaks to the adsorption of chelates on the solid support, and in some cases the presence of isomers of metal chelates must be considered as a cause. However, we have assumed that the main reason for the anomaly would be the dissociation of metal chelates in the stationary liquid phase. In the present work, therefore, an attempt was made to use a carrier gas containing ligand vapour which would suppress the dissociation. As a typical example, a chromatographic separation of trifluoroacetylacetonates of Th(IV), Fe(III) and U(IV), was examined with the vapour of trifluoroacetylacetone as the carrier gas additive.

Addition of organic solvents[3,4] to the carrier gas has also been examined, but the peak was much more improved by the use of ligand vapour than of solvents.

### EXPERIMENTAL

*Apparatus*

A Shimadzu Model GC-1B gas chromatograph equipped with a thermal-conductivity detector was used as the conventional gas chromatograph. Figure 1 shows a schematic diagram of the gas chromatograph equipped with the vapour generator for supplying the carrier gas additive and the resistance column for smoothing the mixing of helium with the additive.

*Gas chromatographic column.* The columns were of 4-mm bore stainless steel, 0·75 m in length, and were filled with Gas Chrom-CLH (80–100 mesh) as the stationary solid support. The stationary liquid phases were Apiezon L, Silicone SE-30, DC-550, XE-60 and Polyethylene Glycol (PEG)-6000, with 1, 0·5, 5, 2·5 and 10% w/w of coating weight respectively.

*Mobile phase.* Helium gas saturated with the vapour of trifluoroacetylacetone (TFA), isobutyl methyl ketone (IBMK) or cyclohexane at 30° was used as carrier gas.

*Sample.* Trifluoroacetylacetone metal chelates of Be, Al, Cr, Fe(III), U(IV) and Th(IV) were synthesized according to the method of Berg *et al.*[5] and purified by sublimation *in vacuo*. The sample was dissolved in benzene, except for $Th(TFA)_4$, which was dissolved in IBMK, at a concentration of 10% w/v. In general, 10 $\mu$l of the sample solution were injected into the gas chromatograph.

### RESULTS AND DISCUSSION

*Chromatogram of $Th(TFA)_4$*

With the conventional method using helium alone as carrier gas, the IBMK solution of $Th(TFA)_4$ produces an anomalous chromatogram similar to that shown in Fig. 2. The tail of the solvent peak overlaps the $Th(TFA)_4$ peak, even if the experimental conditions such as support material, stationary phase, column temperature, injection temperature and flow-rate are varied. The fact that the anomalous tailing does not appear with the solvent itself and that the area of $Th(TFA)_4$ peak is proportional to the amount present shows that a part of the $Th(TFA)_4$ is dissociated at the beginning of the column and the product (TFA) is gradually eluted right after the solvent peak. Hence it was expected that dissociation could be suppressed by the presence of excess of TFA in the carrier gas. Figure 3 shows the chromatogram of $Th(TFA)_4$ that had been eluted with helium gas saturated with TFA at 30°. It can be seen that the anomalous tailing is completely eliminated and a well-defined peak appears, the retention time of $Th(TFA)_4$ being the same as that in the absence of TFA. The improvement of the peak-shape would also depend on the fact that the adsorption of metal chelates on the solid support is reduced by the scavenging effect with ligand vapour.

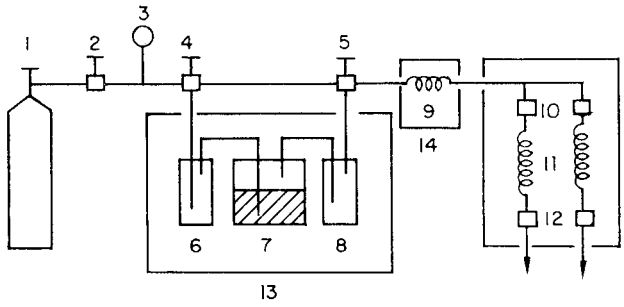


FIG. 1.—Schematic diagram of a gas chromatograph using carrier gas containing the vapour of an organic substance.
1, Helium bomb; 2, valve; 3, pressure gauge; 4, 5 valves; 6, 8, buffer tank; 7, generator of organic vapour; 9, resistance column, 4 mm × 0·75 m, filled with Gas Chrom-CLH (80–100 mesh); 10, injection port; 11, column; 12, detector; 13, water-bath regulated at 30°C; 14, preheater.

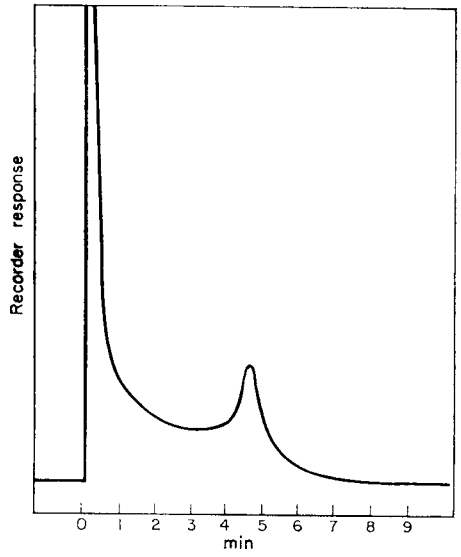


FIG. 2.—Chromatogram of $Th(TFA)_4$.
Carrier gas, helium; sample, 10 $\mu$l of 10% w/v solution of $Th(TFA)_4$ in IBMK; column, 4 mm × 0·75 m stainless-steel U-tube filled with 5% w/w Silicone DC-550/Gas Chrom-CLH (80–100 mesh); column temperature, 180°C; injection temperature, 250°C; detector temperature, 260°C; flow-rate, 56 ml/min at outlet (inlet pressure, 1·07 bar); detector, thermal conductivity; filament current, 150 mA.

*Choice of stationary phase*

Five stationary liquid phases; PEG-6000, Apiezon L, Silicone SE-30, DC-550 and XE-60, were examined, and in use with ligand vapour slightly polar liquids (*e.g.*, Silicone SE-30, DC-550 and XE-60) were found more suitable than strongly polar (*e.g.*, PEG-6000) or non-polar liquids (*e.g.*, Apiezon L).

*Other organic vapours*

Instead of ligand vapour, solvent vapours such as IBMK and cyclohexane were also examined as the carrier gas additive, but yielded practically no improvement in the anomalous tailing.

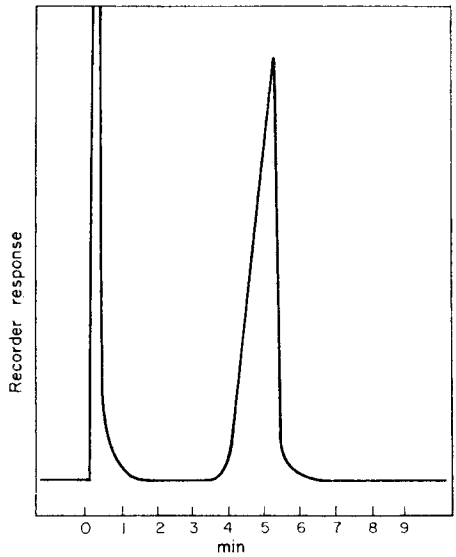


FIG. 3.—Chromatogram of $Th(TFA)_4$.
Carrier gas, helium containing trifluoroacetylacetone vapour; vapour generated at 30°C, other conditions as for Fig. 2.

*Other metal chelates*

The gas chromatography of $Be(TFA)_2$, $Al(TFA)_3$, $Cr(TFA)_3$, $Fe(TFA)_3$ and $U(TFA)_4$ was also investigated by using the TFA vapour as carrier gas additive and it was found that all the gas chromatograms appeared quite normally, in contrast to the conventional method, with which symmetrical peaks were not obtained for U(IV) and Fe(III).

In conclusion, gas chromatography with carrier gas containing the ligand vapour was found extremely useful for the analysis of metal chelates, giving a more reliable chromatographic peak than that in the absence of ligand vapour.

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**Summary**—The vapour of the ligand was used as carrier-gas additive in the gas chromatography of metal chelates. The effect of trifluoroacetylacetone on non-symmetrical peaks was examined for $Be(TFA)_2$, $Al(TFA)_3$, $Cr(TFA)_3$, $Fe(TFA)_3$, $U(TFA)_4$ and $Th(TFA)_4$. All the chromatograms appeared quite normally especially those of $Th(TFA)_4$; $Fe(TFA)_3$ and $Th(TFA)_4$ were improved markedly by using the carrier gas containing ligand vapour.

**Zusammenfassung**—Der Dampf des Liganden wurde als Zusatz zum Trägergas bei der Gaschromatographie von Metallchelaten verwendet. Der Einfluß von Trifluoracetylaceton auf unsymmetrische Peaks wurde für $Be(TFA)_2$, $Al(TFA)_3$, $Cr(TFA)_3$, $Fe(TFA)_3$, $U(TFA)_4$ und $Th(TFA)_4$ untersucht. Sämtliche Chromatogramme erschienen durchaus normal, besonders die des $Th(TFA)_4$, $Fe(TFA)_3$ und $Th(TFA)_4$ wurden wesentlich verbessert durch das Ligandendampf enthaltende Trägergas verbessert.

**Résumé**—On a utilisé la vapeur du ligand comme additif au gaz vecteur dans la chromatographie en phase gazeuse de chélates métalliques. On a examiné l'influence de la trifluoracétylacétone sur des pics non symétriques pour $Be(TFA)_2$, $Al(TFA)_3$, $Cr(TFA)_3$, $Fe(TFA)_3$, $U(TFA)_4$ et $Th(TFA)_4$. Tous les chromatogrammes ont parus tout normal, particulièrement ceux de $Th(TFA)_4$, $Fe(TFA)_3$ et $Th(TFA)_4$ ont été sensiblement améliorés en utilisant le gaz vecteur contenant la vapeur de ligand.

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# Cerimetric determination of dithionate and polythionates

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AMONG the oxy-anions containing sulphur in oxidation states less than +6, dithionate occupies a special position by virtue of its comparative resistance to oxidation. Methods for the determination of dithionate are therefore fewer than, say, for the polythionates. Polythionates can be quantitatively oxidized by hypochlorite[1] and by chloramine-T,[2] but both these reagents have no effect on dithionate. Murthy[3] determined dithionate in the presence of sulphide, sulphite, thiosulphate and other polythionates by first treating these compounds with alkaline permanganate (which oxidizes all these compounds except dithionate) and subsequently oxidizing the dithionate with boiling acidic dichromate, according to the method of Glasstone and Hickling.[4] The dichromate oxidation method seems to be the most widely used procedure for the determination of dithionate, although a vanadatometric method has been developed by Lang and Kurtenacker[5] and a bromate–bromide method has been employed by Mayr and Peyfuss.[6] Since a search through the literature did not reveal any other convenient method, it was considered worthwhile to study the behaviour of dithionate towards other oxidants.

Yost and Pomeroy[7] had observed that the oxidation of dithionate is almost independent of the nature and concentration of the oxidant, but that it depends on the rate of decomposition of the dithionic acid into sulphurous and sulphuric acids, which, in turn, is catalysed by strong acids. Considering this observation along with the fact that all the three oxidimetric methods available at present make use of a boiling medium, it is clear that an oxidant which may be used in strongly acidic medium at its boiling point would be the most suitable. This led our attention to ceric sulphate. The behaviour of dithionate towards cerium(IV) under varying conditions of acidity and temperature was studied and the results are presented in this communication. The success of the cerimetric method with dithionate led us to investigate the behaviour of polythionates towards ceric sulphate. The results of this study are also reported.

## EXPERIMENTAL

Stock solutions of cerium(IV) ammonium sulphate were prepared and standardized with iron(II) ammonium sulphate by the usual procedure.[8] Standard solutions of sodium dithionate were prepared, and standardized by the method of Glasstone and Hickling.[4] Potassium tri-and tetrathionates were prepared from sulphur dichloride and disulphur dichloride respectively, according to the method of Stamm, Goehring and Feldmann.[9] Standard solutions of these were prepared and then standardized by Kurtenacker's degradation procedures[10] as well as by the chloramine-T method.[2] All other reagents employed were of analytical reagent grade purity.

### *Procedures*

Preliminary investigations showed that when treated with ceric sulphate in an acidic medium (*ca.* 2*N*) at room temperature there is practically no oxidation of dithionate or of the polythionates. Increase of temperature as well as increase of acidity was found to be conducive to quantitative oxidation. The reaction is completed in the minimum time in a boiling strongly acidic medium.

(*i*) Measured aliquots (40 ml) of cerium(IV) ammonium sulphate solution were taken in 500-ml conical flasks and were acidified with sulphuric acid to bring about specified overall acidities (see Tables). Known volumes of standard dithionate or polythionate solutions were added and the solutions were boiled for specified time intervals, then cooled, and the unconsumed cerium(IV) was determined by titration against standard iron(II) ammonium sulphate solution, with ferroin as indicator. Blanks were run concurrently. No blank corrections were necessary.

One modification was also attractive. This consisted in acidifying with excess of concentrated

sulphuric acid and utilizing the heat thereby produced to aid the oxidation; no extraneous heating is here necessary. Such a modification has been applied to the vanadatometric method of Lang and Kurtenacker by Crossland and Hofman-Bang.[11]

(*ii*) Measured aliquots of cerium(IV) ammonium sulphate solution (40 ml) were taken in 500-ml conical flasks. Known volumes of standard dithionate or polythionate solutions were added followed by 40 ml of concentrated sulphuric acid, with shaking. The mixtures were allowed to stand for specified time intervals with occasional shaking, then were diluted to 200 ml and allowed to cool to room temperature. The unconsumed oxidant was determined as before. Blanks were run concurrently. No blank corrections were necessary.

## RESULTS AND DISCUSSION

Typical results are presented in Tables I and II. It may be seen from Table I that dithionate is quantitatively oxidized to sulphate by cerium(IV) in a boiling medium of acidity *ca.* 2*N*. The results

TABLE I.—OXIDATION OF DITHIONATE BY Ce(IV)

| Sodium dithionate, *mmol* | Overall acidity, *N* | Other conditions | Time | Ce(IV) consumed *mequiv* | Equivalents of Ce(IV) consumed per mole of dithionate |
|---|---|---|---|---|---|
| 0·942 | 2 | Room temperature (28°) | 30 min | nil | nil |
| 0·942 | 2 | Room temperature (28°) | 24 hr | nil | nil |
| 0·913 | 2 | Boiling | 30 min | 1·831 | 2·005 |
| 1·079 | 2 | Boiling | 30 min | 2·154 | 1·996 |
| 1·162 | 2 | Boiling | 30 min | 2·318 | 1·994 |
| 1·181 | 2 | Boiling | 30 min | 2·359 | 1·997 |
| 1·246 | 2 | Boiling | 30 min | 2·492 | 2·000 |
| 0·928 | 12 | Boiling | 30 min | 1·857 | 2·002 |
| 1·265 | 12 | Boiling | 30 min | 2·533 | 2·002 |
| 0·928 | 18 | *ca.* 80° | 30 min | 1·851 | 1·995 |
| 1·013 | 18 | *ca.* 80° | 30 min | 2·031 | 2·005 |
| 1·096 | 18 | *ca.* 80° | 30 min | 2·185 | 1·994 |
| 1·181 | 18 | *ca.* 80° | 30 min | 2·369 | 2·005 |
| 1·265 | 18 | *ca.* 80° | 30 min | 2·533 | 2·002 |
| 0·742 | 18 | Boiling | 30 min | 1·486 | 2·003 |
| 1·012 | 18 | Boiling | 30 min | 2·024 | 2·000 |

TABLE II.—OXIDATION OF TRI- AND TETRATHIONATES WITH Ce(IV)

| Substance taken, *mmol* | Overall acidity, *N* | Other conditions | Time | Ce(IV) consumed *mequiv* | Equivalents of Ce(IV) consumed per mole of substance |
|---|---|---|---|---|---|
| Potassium tetrathionate | | | | | |
| 0·0865 | 2 | Room temperature (28°) | 30 min | nil | nil |
| 0·0865 | 2 | Room temperature (28°) | 24 hr | nil | nil |
| 0·1089 | 12 | Boiling | 30 min | 1·516 | 13·92 |
| 0·1173 | 12 | Boiling | 30 min | 1·646 | 14·03 |
| 0·1256 | 12 | Boiling | 30 min | 1·756 | 13·97 |
| 0·1341 | 12 | Boiling | 30 min | 1·871 | 13·95 |
| 0·1297 | 18 | *ca.* 80° | 30 min | 1·726 | 13·30 |
| Potassium trithionate | | | | | |
| 0·1892 | 2 | Room temperature (28°) | 30 min | nil | nil |
| 0·1892 | 2 | Room temperature (28°) | 24 hr | nil | nil |
| 0·2193 | 12 | Boiling | 30 min | 1·750 | 7·980 |
| 0·2375 | 12 | Boiling | 30 min | 1·902 | 8·007 |
| 0·2558 | 12 | Boiling | 30 min | 2·048 | 8·005 |
| 0·2741 | 12 | Boiling | 30 min | 2·195 | 8·007 |

are the same when the acidity is increased to 12*N*. The oxidation is also quantitative in an 18*N* acid medium obtained by mixing conc. sulphuric acid and ceric sulphate, in which case no extraneous heating is necessary. The results show that two equivalents of the oxidant are consumed per mole of the dithionate in accordance with the equation

$$2SO_4^{2-} + 4H^+ + 2e^- \rightleftharpoons S_2O_6^{2-} + 2H_2O$$

For tri- and tetrathionates, quantitative oxidation to sulphate can be effected in half an hour by boiling with cerium(IV) in a strongly acidic medium (12*N*). At low overall acidities the time required for quantitative oxidation is found to be longer; hence a strongly acidic medium (12*N*) is recommended. Procedure (*ii*) was not quite successful with tri- and tetrathionates; the reaction mixture had to be boiled even in this case to effect quantitative oxidation. It may be seen from Table II that under ideal conditions (boiling strongly acidic medium), 8 and 14 equivalents of the oxidant are consumed per mole of tri- and tetrathionate respectively, in accordance with the equations

$$3SO_4^{2-} + 12H^+ + 8e^- \rightleftharpoons S_3O_6^{2-} + 6H_2O$$
$$4SO_4^{2-} + 2OH^+ + 14e^- \rightleftharpoons S_4O_6^{2-} + 10H_2O$$

*Interferences*

Other reductants (such as sulphide, sulphite and thiosulphate) would naturally interfere in the cerimetric determination of di- and polythionates described above. But their interference can be eliminated as follows. An iodometric estimation (where di- and polythionates would naturally be unaffected) may be employed to determine these interfering reductants and suitable allowance for the cerium(IV) consumption by these may be made when computing the analytical results.

*Analysis of mixtures of di- and polythionates*

It may be pointed out that the present cerimetric method by itself is suitable only for the determination of individual polythionates or dithionate. However, the cerimetric method could be used along with the well known Kurtenacker degradation procedures for the analysis of mixtures of polythionates. Further, since it is known that dithionate is unaffected by chloramine-T in acid medium whereas the polythionates are oxidized quantitatively to sulphate by this oxidant in acid medium, a combination of the chloramine-T and the cerimetric methods would be useful for determining dithionate in the presence of polythionates.

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**Summary**—Ceric sulphate in a boiling strongly acidic medium oxidizes dithionate as well as tri- and tetrathionates quantitatively to sulphate. These anions may therefore be determined cerimetrically when they are present singly. A combination of the cerimetric method with other known methods (*e.g.* those of Kurtenacker, *etc*) is suggested for the analysis of mixtures of polythionates and dithionate.

**Zusammenfassung**—Cer(IV)sulfat in siedendem, stark saurem Medium oxidiert Dithionat sowie Tri- und Tetrathionat quantitativ zu Sulfat. Wenn diese Anionen einzeln vorliegen, können sie daher cerimetrisch bestimmt werden. Für die Analyse von Gemischen aus Polythionaten und Dithiomat wird eine Kombination des cerimetrischen Verfahrens mit anderen bekannten Methoden, z.B. nach Kurtenacker usw., vorgeschlagen.

**Résumé**—Le sulfate cérique dans un milieu bouillant fortement acide oxyde quantitativement le dithionate ainsi que les tri- et tétrathionates en sulfate. Ces anions peuvent par conséquent être dosés cérimétriquement lorsqu'ils sont présents isolément. On suggère une combinaison de la méthode cérimétrique avec d'autres méthodes connues (par ex celles de Kurtenacker, etc.) pour l'analyse de mélanges de polythionates et de dithionate.

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# Crystal and molecular structure of zinc dithizonate

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THE determination of the molecular structure of zinc dithizonate *via* single-crystal X-ray analysis was prompted primarily by the desire to compare the dihedral angle the proximal angles make with the chelate ring in this compound and in the corresponding nickel chelate[1] because of the unusual response to steric hindrance displayed by these metals with dithizonates.[2] Further, it was hoped to obtain sufficiently precise data to permit an unequivocal assignment, for the first time, of the bond order of the N–N linkage in the chelate ring

The crystals are monoclinic with $a = 0{\cdot}7887 \pm 0{\cdot}0006$ nm, $b = 2{\cdot}2501 \pm 0{\cdot}0017$ nm, $c = 1{\cdot}5327 \pm 0{\cdot}0011$ nm, $\beta = 92{\cdot}58 \pm 0{\cdot}04°$, $Z = 4$, $D_c = 1{\cdot}40$, $D_m = 1{\cdot}40$ g/cm$^3$ (flotation), space group $P2_1/c - C_{2h}^5$. The cell dimensions were obtained by a least-squares refinement from the setting angles of twelve strong X-ray reflections which had been centred on a Picker four-circle automatic diffractometer using Mo$K_{\alpha_1}$ radiation ($\lambda = 70{\cdot}93$ pm)

## EXPERIMENTAL

The intensity data were collected on the Picker diffractometer using Mo$K_\alpha$ radiation monochromated by means of a graphite crystal. The unique data set having $2\theta > 40°$ was gathered; the intensities of independent reflections were recorded. The structure was determined by Patterson and Fourier methods with 1850 independent reflections. Refinement of the structure by full-matrix least-squares techniques for all non-hydrogen atoms led to a conventional $R$ factor of 0·068. Tables I and II contain values of significant bond distances and angles, and Fig. 1 is an ORTEP[1] representation of the structure.

## RESULTS AND DISCUSSION

The crystal structure consists of discrete molecules of zinc(II) dithizonate, $Zn(C_{13}H_{11}N_4S)_2$, in which the co-ordinating atoms lie in a slightly distorted tetrahedral array around the central zinc atom. (The dihedral angle between the planes of the two chelate rings is 83·91°.)

In contrast to what was observed in the mixed ligand chelate formed from nickel(II) dithizonate and bipyridyl,[2] the phenyl rings adjacent to the chelate rings in zinc dithizonate are within a few degrees of coplanarity with the chelate rings, as hypothesized earlier[3] to explain the significant drop in solution stability of the zinc chelate observed when the *o*-tolyl analogue was substituted for dithizone itself. Inasmuch as both the mixed ligand nickel chelate and the zinc dithizonate exhibit a large absorption bond at the same wavelength, it is not too likely that the proximal phenyl ring is conjugated with the rest of the zinc chelate molecule.

The reliability of the interatomic distance parameters in this study permits the assignment, for the first time in a dithizone chelate, of the double bond nature of the N–N bond in the chelate ring. Not only are the distances N(1)–N(2) and N(5)–N(6) significantly shorter than those of N(3)–N(4) and N(7)–N(8) as well as the distances C(1)–N(3) and C(14)–N(7) being shorter than those corresponding

TABLE I.—BOND DISTANCES IN $Zn(C_{13}H_{11}N_4S)_2$, *nm*

| | | | |
|---|---|---|---|
| Zn–S(1) | 0·2285 (2) | C(2)–C(3) | 0·1387 (17) |
| Zn–S(2) | 0·2263 (2) | C(3)–C(4) | 0·1406 (18) |
| | | C(4)–C(5) | 0·1372 (20) |
| Zn–N(1) | 0·2073 (1) | C(5)–C(6) | 0·1391 (20) |
| Zn–N(5) | 0·2078 (1) | C(6)–C(7) | 0·1404 (18) |
| | | (7)C–C(2) | 0·1396 (16) |
| S(1)–C(1) | 0·1756 (1) | | |
| S(2)–C(14) | 0·1758 (1) | C(8)–C(9) | 0·1363 (15) |
| | | C(9)–C(10) | 0·1423 (17) |
| C(1)–N(2) | 0·1377 (1) | C(10)–C(11) | 0·1363 (17) |
| C(14)–N(6) | 0·1373 (1) | C(11)–C(12) | 0·1424 (18) |
| | | C(12)–C(13) | 0·1403 (15) |
| C(1)–N(3) | 0·1324 (1) | C(13)–C(8) | 0·1405 (16) |
| C(14)–N(7) | 0·1312 (1) | | |
| | | C(15)–C(16) | 0·1412 (15) |
| N(1)–N(2) | 0·1281 (1) | C(16)–C(17) | 0·1419 (17) |
| N(5)–N(6) | 0·1274 (1) | C(17)–C(18) | 0·1411 (19) |
| | | C(18)–C(19) | 0·1413(19) |
| N(3)–N(4) | 0·1354 (1) | C(19)–C(20) | 0·1379 (17) |
| N(7)–N(8) | 0·1353 (1) | C(20)–C(15) | 0·1383 (15) |
| N(4)–C(8) | 0·1437 (11) | C(21)–C(22) | 0·1409 (15) |
| N(8)–C(21) | 0·1410 (11) | C(22)–C(23) | 0·1433 (16) |
| | | C(23)–C(24) | 0·1386 (17) |
| N(1)–C(2) | 0·1464 (12) | C(24)–C(25) | 0·1435 (18) |
| N(5)–C(15) | 0·1430 (11) | C(25)–C(26) | 0·1397 (17) |
| | | C(26)–C(21) | 0·1424 (16) |
| N(4)–H(1) | 0·1069 | | |
| N(8)–H(2) | 0·1072 | | |

TABLE II.—SELECTED BOND ANGLES IN $Zn(C_{13}H_{11}N_4S)_2$

| | | | |
|---|---|---|---|
| S(1) Zn N(1) | 86·64° | S(1) C(1) N(3) | 124·99° |
| S(2) Zn N(5) | 86·64° | S(2) C(14) N(7) | 123·81° |
| Zn N(1) N(2) | 118·52° | C(1) S(1) Zn | 91·93° |
| Zn N(5) N(6) | 118·45° | C(14) S(2) Zn | 92·52° |
| N(1) N(2) C(1) | 115·74° | C(1) N(3) N(4) | 116·13° |
| N(5) N(6) C(14) | 115·92° | C(14) N(7) N(8) | 118·33° |
| N(2) C(1) S(1) | 126·59° | N(3) N(4) C(8) | 119·30° |
| N(6) C(14) S(2) | 126·23° | N(7) N(8) C(21) | 119·30° |
| N(2) C(1) N(3) | 108·42° | N(3) N(4) H(1) | 119·79° |
| N(6) C(14) N(7) | 109·96° | N(7) N(8) H(2) | 115·65° |
| | | H(1) N(4) C(8) | 114·94° |
| | | H(2) N(8) C(21) | 121·39° |

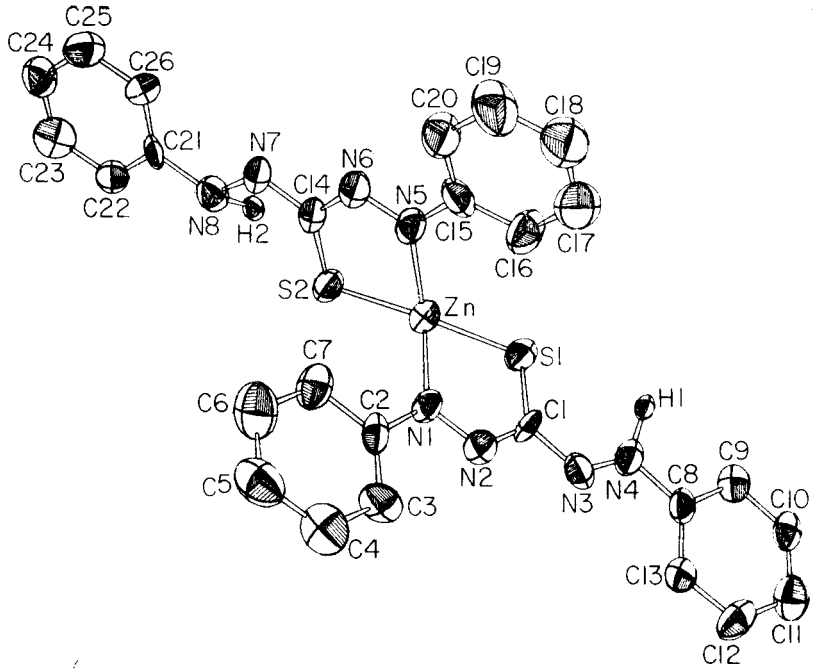


FIG. 1.—Crystal structure of zinc dithizonate.

to C(1)–N(2) and C(14)–N(6), but the two protons attached to nitrogen atoms were found by Fourier difference mapping to be bonded to N(4) and N(8) as required by such an assignment.

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**Summary**—The structure of zinc(II) dithizonate has been determined by X-ray analysis. The co-ordination round the zinc is slightly distorted tetrahedral and the proximal phenyl rings have been shown to be almost coplanar with the chelate rings.

**Zusammenfassung**—Die Struktur von Zink(II)dithizonat wurde durch Röntgenbeugung ermittelt. Die Koordination um das Zink ist leicht verzerrt tetraedrisch; die außenstehenden Phenylringe liegen fast in der gleichen Ebene wie die Chelatringe.

**Résumé**—On a déterminé la structure du dithizonate de zinc(II) par analyse aux rayons X. La coordination autour du zinc est légèrement déformée tétraédrique et l'on a montré que les cycles phényle les plus proches sont presque coplanaires avec les cycles du chélate.

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# Solvent extraction of the indium-alizarin Red S chelate as its 1,3-diphenylgaunidium salt

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ALIZARIN RED S (ARS) is a rather non-selective reagent which gives strongly coloured chelates or lakes with many metal cations in aqueous solution. The zirconium(IV) chelate of ARS can be extracted into polar solvents such as 1-butanol.[1] The partition coefficient is considerably increased if an organic base is added. In such cases ion-association complexes are usually formed, as shown earlier for the ARS chelates of some rare earth elements in the presence of antipyrine,[2,3] thorium(IV)[4] and uranium(VI)[5] in the presence of tricaprylmethylammonium chloride, molybdenum(VI) in the presence of tetradecyldimethylbenzylammonium chloride,[6] and zirconium(IV) in the presence of tri-n-octylmethylammonium acetate.[7]

The 1,3-diphenylguanidium cation (DPG) forms water-insoluble ion-association complexes with the ARS chelates of many metal cations, and these can be extracted into some aliphatic alcohols and esters and partially halogenated hydrocarbons. In this paper we report the extraction of indium (III) into n-butyl acetate from an aqueous phase containing excess of ARS and DPG.

## EXPERIMENTAL

### *Reagents*

*Indium(III) solution*, $10^{-2}M$. Prepared by dissolving freshly precipitated indium hydroxide in dilute nitric acid, standardized with EDTA (Xylenol Orange as indicator), and diluted as required.

*ARS solution*, $4{\cdot}0 \times 10^{-4}M$. Prepared from chromatographically pure Alizarin Red S recrystallized twice from 50% aqueous ethanol. The acid dissociation constants of ARS were measured spectrophotometrically and found to be $pK_1$ ($\beta$-OH) 5·39, and $pK_2$($\alpha$-OH) 10·72 at 25° and $\mu = 0{\cdot}50$.

*DPG solution.* Prepared by dissolving 10·5 g of 1,3-diphenylguanidine in 12 ml of $6M$ hydrochloric acid and diluting with demineralized water to 100 ml.

### *Standard procedure*

Transfer an aliquot of indium solution to a 50-ml separatory funnel along with 4 ml of $4{\cdot}0 \times 10^{-4}M$ ARS and 10 ml of $7{\cdot}5 \times 10^{-2}M$ DPG buffered with acetic acid/sodium acetate (pH 5·3–5·9). Dilute to 20 ml with demineralized water; equilibrate with 10 ml of n-butyl acetate for 3 min. Measure the absorbance of the extract at 525 nm against a reagent blank.

## RESULTS AND DISCUSSION

Figure 1 shows the absorption spectra in n-butyl acetate. Fifteen water-immiscible organic solvents including alcohols, esters, ketones and partially halogenated hydrocarbons were tested. Acetate esters (ethyl acetate > n-propyl acetate > n-butyl acetate) proved to give the highest absorbance, and halogenated aliphatic hydrocarbons (1,2-dichloroethane > chloroform) the next highest. From considerations of mutual solubility between organic solvent and water, n-butyl acetate was chosen as solvent.

The effect of pH on the extraction is shown in Fig. 2. A reddish violet species is extracted at pH above 4·2 from solutions containing $1{\cdot}2 \times 10^{-4}M$ ARS and $3{\cdot}75 \times 10^{-2}M$ DPG and has an absorption maximum at 525 nm, and maximum extraction is reached at pH 5·2–5·8. This pH range is shifted to 5·3–5·9 for $8{\cdot}0 \times 10^{-5}M$ ARS and to pH 5·5–6·1 for $5{\cdot}0 \times 10^{-5}M$. The shape of the pH-absorbance curves in Fig. 2 may be explained thus: the increased extraction with increase in pH corresponds to formation of the indium ARS chelate and the decrease at high pH is a result of either ionization of the chelate to give a more highly charged anion or of competition between the large chelate anion and hydroxide for the DPG cation. The DPG concentration was without effect provided it was $> 1{\cdot}25 \times 10^{-2}M$.

The shaking time was varied from 0·5 to 11 min, other variables being kept constant. It was found that shaking for 2 min sufficed; for safety, a shaking time of 3 min was selected.

A calibration curve gave a linear relationship over the concentration range of 3–28 $\mu$g of indium per 10 ml of final solution. The molar absorptivity of the extracted species was $2{\cdot}65 \times 10^3$ l.mole$^{-1}$.mm$^{-1}$ at 525 nm, giving a sensitivity about ten times that obtainable by direct reaction in the aqueous phase.[8]

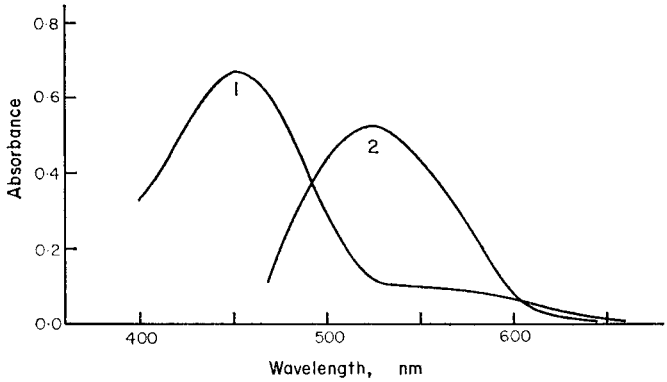



FIG. 1.—Absorption spectra of the systems ARS–DPG and In(III)–ARS–DPG in n-butyl acetate.
[ARS] = $8{\cdot}0 \times 10^{-5}M$, [DPG] = $3{\cdot}75 \times 10^{-2}M$,
[In(III)] = $1{\cdot}0 \times 10^{-5}M$, pH 5·5.
1—ARS-DPG;
2—In(III)-ARS-DPG.

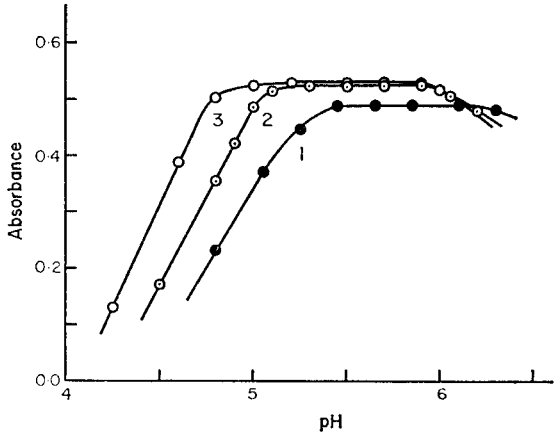



FIG. 2.—Effect of pH on the extraction of In(III)–ARS–DPG.
[In(III)] = $1{\cdot}0 \times 10^{-5}M$, [DPG] = $3{\cdot}75 \times 10^{-2}M$
*1*—[ARS] = $5{\cdot}0 \times 10^{-5}M$; *2*—[ARS] = $8{\cdot}0 \times 10^{-5}M$;
*3*—[ARS] = $1{\cdot}2 \times 10^{-4}M$.

The reproducibility was estimated from the results for 9 sample solutions, each containing 23 $\mu$g of indium. The standard deviation of the absorbance was 0·0024 for a mean value of 0·529, *i.e.*, a relative error of 0.5%.

*Interferences*

These were studied by extracting 23 $\mu$g of indium in presence of the foreign substance under the optimum experimental conditions. Common complexing agents strongly interfered with the determination of indium. A deviation of −5% was given by $2 \times 10^{-2}M$ perchlorate, $1{\cdot}3 \times 10^{-3}M$ phosphate or $5 \times 10^{-4}M$ oxalate. Citrate, NTA and EDTA interfered seriously. A number of metal cations which form ARS chelates under the experimental conditions also interfered with the indium determination, giving positive deviations.

*Composition and structure of the ion-associated species*

The ratio of indium to ARS in the extracted species was determined by Job's method of continuous variations[9] modified for a two-phase system.[10,11] The curves shown in Fig. 3 indicate the formation of a 1:3 indium-ARS chelate. The mole ratio method gave similar results.

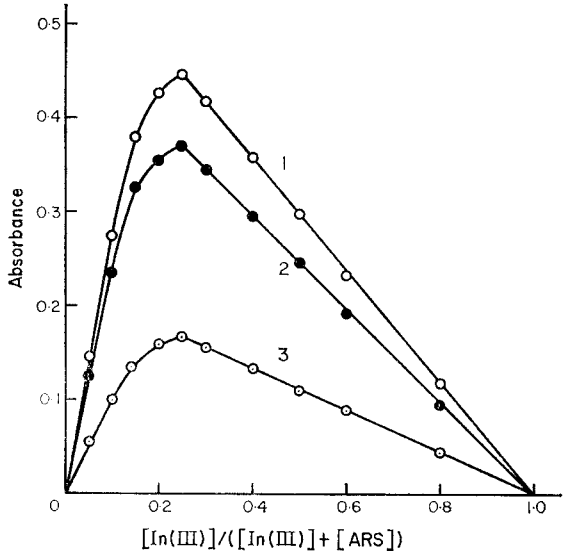



FIG. 3.—Continuous variation method applied to the system In(III)–ARS in the presence of DPG.

[In(III)] + [ARS] = $4{\cdot}0 \times 10^{-5}M$,
[DPG] = $5{\cdot}0 \times 10^{-2}M$, pH 5·6
*1*—525 nm; *2*—550 nm; *3*—430 nm.

The ratio of DPG to indium in the extracted species was deduced by means of a method previously described.[12] A graph of log $D_{In}$ *vs.* log $[DPG]_{aq}$ with fixed concentrations of indium and ARS gave a straight line of slope 3, showing that the ratio indium:DPG was 1:3. Evidently the composition of the extracted species is indium:ARS:DPG = 1:3:3.

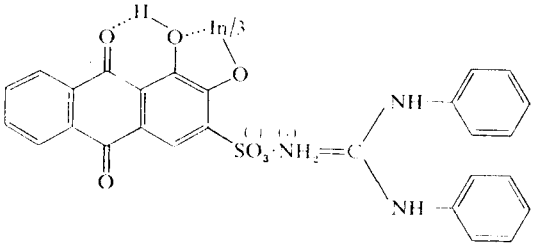
The infrared spectra of ARS, DPG and the associated chelate were recorded. Some characteristic frequencies are listed in Table I. The associated chelate was isolated from aqueous solution as a

TABLE I.—REPRESENTATIVE INFRARED FREQUENCIES OF ARS, DPG AND In(III)–ARS–DPG, $cm^{-1}$

| | $\nu$(N—H) | $\nu$(C=O) | | $\nu$(C=N) | $\delta$(N—H) | $\nu$(S—O) | $\nu$(C—S) | $\delta$(S—O) |
|---|---|---|---|---|---|---|---|---|
| | | free | hydrogen-bonded | | | | | |
| ARS | | 1669 | 1638 | | | 1020 | 642 | 593 |
| DPG(HCl) | 3455 | | | 1654 | 1620<br>1600<br>1499 | | | |
| In(III)–ARS–DPG | ~3460 | 1667 | 1640 | | 1622<br>1597<br>1501 | 1021 | 641 | 602 |

violet solid which contained indium, ARS and DPG in molar ratio 1:3:3. The intense carbonyl peaks at 1638 and 1669 $cm^{-1}$ noted in the infrared spectrum of ARS were also found in that of the associated chelate. This implies that the metal is probably chelated to the two phenolic oxygen atoms of the ARS rather than to a quinoid oxygen and the adjacent phenolic oxygen atoms,[5,6] and that the intramolecular hydrogen bond is scarcely affected by the chelate formation.

In view of this evidence, the structure of the associated chelate may reasonably be written as below.



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**Summary**—The extraction of the 1,3-diphenylguanidium (DPG) salt of the indium–Alizarin Red S (ARS) chelate with n-butyl acetate has been investigated. The extracted species has an absorption maximum at 525 nm, and Beer's law is obeyed over the range from 0·3 to 2·8 ppm of indium. The molar absorptivity is $2{\cdot}65 \times 10^3$ l.mole$^{-1}$.mm$^{-1}$ at 525 nm. The 1:3 indium(III)–ARS chelate is extracted from aqueous solution in a 1:3 molar ratio with DPG. The infrared spectrum implies that the metal ion is probably chelated by the two phenolic oxygen atoms of ARS.

**Zusammenfassung**—Die Extraktion des 1,3-Diphenylguanidium(DPG)-Salzes des Indiumchelats mit Alizarinrot S (ARS) durch n-Butylacetat wurde untersucht. Die extrahierte Spezies hat ein Absorptionsmaximum bei 525 nm das Beersche Gesetz gilt bei 0,3 bis 2,8 ppm Indium. Der molare Extinktionskoeffizient beträgt $2{,}65{\cdot}10^3$ l.mol$^{-1}$.mm$^{-1}$ bei 525 nm. Das 1:3-Chelat aus Indium(III) und ARS wird im Molverhältnis 1:3 mit DPG aus wäßriger Lösung extrahiert. Das Infrarotspektrum legt nahe, daß das Metallion mit den beiden Phenolsauerstoffatomen von ARS einen Chelatring bildet.

**Résumé**—On a étudié l'extraction du sel de 1,3-diphénylguanidinium (DPG) du chélate indium-Rouge d'Alizarine S (ARS) par l'acétate de n-butyle. L'espèce extraite a un maximum d'absorption à 525 nm, et la loi de Beer est suivide dans le domaine 0,3 à 2,8 p.p.m. d'indium. Le coefficient d'absorption molaire est de $2{,}65 \times 10^3$ l.mole$^{-1}$.mm$^{-1}$ à 525 nm. Le chélate 1:3 indium (III)–ARS est extrait de la solution aqueuse dans un rapport molaire 1:3 avec le DPG. Le spectre infrarouge implique que l'ion métallique est probablement chélaté par les deux atomes d'oxygène phénoliques de ARS.

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# Spectrophotometric determination of cadmium in nuclear-grade Zircaloy-2 after selective extraction with a liquid anion-exchanger

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NUCLEAR TECHNOLOGY often requires structural materials strictly controlled in their content of certain elements. Nowadays, specifications for nuclear-grade zirconium alloys such as Zircaloy-2 generally demand cadmium contents lower than 0·5 ppm. Only emission spectrographic procedures readily allow detection of such low concentrations,[1–5] but in the case of cadmium the lack of reproducibility makes the method peculiarly unsatisfactory.

The accurate and precise determination of tenths of ppm of cadmium in Zircaloy-2 can be carried out by atomic-absorption spectroscopy[6] or by polarography,[7] after a preliminary solvent extraction step. A spectrophotometric method has also been published,[8] involving a very long and cumbersome separation procedure.

In this work, a new spectrophotometric method is presented that allows determination of tenths of ppm of cadmium in Zircaloy-2 with satisfactory accuracy and precision. Cadmium is first isolated by extraction with tri-n-octylamine, and then determined by the dithizone procedure.[9]

The proposed method is quite simple and rapid, does not require any special instrumentation, and avoids toxic reagents such as the potassium cyanide used as a masking agent in the polarographic and spectrophotometric procedures mentioned above.

## EXPERIMENTAL

### *Reagents*

All reagents and chemicals were analytical grade unless otherwise specified.

*Stock cadmium solution* (10 $\mu$g/ml). Accurately weighed high-purity cadmium metal powder (1 g) was treated with 2 ml of conc. hydrochloric acid and a few drops of conc. hydrofluoric acid and heated to dryness in a Teflon beaker. The cooled melt was dissolved in conc. hydrochloric acid, and diluted with water and more acid so as to yield a 1*M* hydrochloric acid solution containing 1 mg of cadmium per ml. The solution was further diluted 100-fold with 1*M* hydrochloric acid to 10 $\mu$g/ml. The 1 mg/ml solution was standardized by EDTA titration.

*Tri-n-octylamine* (TOA) (*equilibrated 0·1M solution*). The technical grade amine (22·2 ml) dissolved in cyclohexane (500 ml) was equilibrated by shaking for 5 min with an equal volume of 1*M* hydrochloric acid.

*Tartaric acid solution*, 10%.

*Hydrochloric acid*, 1*M*.

*Sulphuric acid*, 2*M*.

*Dithizone solution*. 0·002% *in chloroform*.

*Potassium hydroxide solution*, 25% *w/v*. Prepared at least 24 hr before use.

*Hydrochloric acid*, ~ 6*M*.

### *Calibration*

Prepare 1*M* hydrochloric acid solutions containing up to 1 $\mu$g of Cd per ml by suitable dilutions of the stock cadmium solution.

*Separation procedure.* In a separating funnel, shake for 5 min 10 ml of a standard cadmium solution in 1*M* hydrochloric acid with 10 ml of equilibrated 0·1*M* TOA solution, and discard the aqueous (lower) layer. Shake the organic layer with 5 ml of 10% tartaric acid solution for 3 min, and discard the aqueous (lower) layer. Repeat twice more. Shake the organic layer with 10 ml of 2*M* sulphuric acid for 5 min, and collect the aqueous (lower) layer in another separating funnel. Repeat and combine the aqueous phases.

*Colorimetric procedure.* To the separating funnel containing the sulphuric acid extracts add 10 ml of 0·002% dithizone solution and 50 ml of 25% potassium hydroxide solution, and shake for 3 min. Add 50 ml of water and collect the organic (lower) layer in a 25-ml calibrated flask, through a paper filter (Whatman No. 42) previously saturated with chloroform.

Dilute to volume with chloroform and measure the absorbance at 520 nm in 40-mm glass cuvettes, within 15 min after the development of the colour, against a reagent blank obtained by applying the whole procedure to 50 ml of 1*M* hydrochloric acid.

*Analysis of Zircaloy-2*

Weigh accurately about 1 g of Zircaloy-2 (the sample may advantageously be degreased with trichloroethylene, and pickled by a 2-min immersion in an aqueous solution containing 32·5% conc. nitric acid and 2·5% conc. hydrofluoric acid) and heat it in a Teflon beaker together with 15 ml of 6*M* hydrochloric acid. Add 2 ml of conc. hydrofluoric acid in 0·5-ml portions, and evaporate to dryness. Add 5 ml of conc. hydrochloric acid, and evaporate to dryness. Repeat this last step twice more.

Add 4 ml of conc. hydrochloric acid and 20–40 ml of water. Heat gently to assist dissolution, cool, and transfer into a 50-ml calibrated flask. Dilute to volume with water, transfer to a separating funnel and treat the solution as described under *Calibration*.

## RESULTS AND DISCUSSION

*Separation procedure*

Zircaloy-2 is a zirconium alloy containing about 1·5% tin, 0·15% iron and chromium, and 0·05% nickel. Taking into account the different elements in the oxidation states expected after a conventional hydrochloric + hydrofluoric acid dissolution of the alloy, it is known from the literature[10–12] that the extraction step with an anion-exchanger from 1*M* hydrochloric acid allows separation of cadmium from bulk constituents zirconium, tin(IV), chromium(III), nickel, and those elements—such as cobalt, copper(II), and manganese(II)—which are likely to be present as impurities in the alloy and which normally interfere in the spectrophotometric determination. Iron(III), however, is extracted together with cadmium, and therefore requires an additional separation step.

Several experiments were performed with TOA to check these statements and to find a suitable agent to separate cadmium from iron, which appeared to be the only interfering ion brought into the organic phase. Mineral acids and complexing agents were tried as stripping agents. Sulphuric and perchloric acids stripped both iron and cadmium; citric acid stripped all the iron but also some cadmium; oxalic acid gave rise to a milky third phase; salicylic acid did not extract iron or cadmium. Tartaric acid gave satisfactory results for stripping iron: it is interesting to note that tartaric acid separates the cadmium and iron only if they are present in the organic phase as chloro-complexes; there is no separation if TOA is used as extractant for an aqueous tartaric acid phase containing cadmium and iron.

As the final step, $\geqslant 2M$ sulphuric acid was used to strip cadmium. To minimize the use of alkali in the subsequent colorimetric determination, the lowest suitable acid concentration was adopted.

The recovery of cadmium in the separation procedure was checked with 1*M* hydrochloric acid containing 0·5 $\mu$g of cadmium per ml; the amount of cadmium left behind in the extraction (1:1 phase-volume ratio) was determined spectrophotometrically and so was the amount left in the organic phase after stripping. The results showed that 0·7 $\pm$ 1·7% cadmium was left in the original solution and 4·1 $\pm$ 1·1% in the organic phase; 95·2 $\pm$ 2·0% (95% confidence level) of the original cadmium was therefore recovered in the sulphuric acid stripping solution. The figures were essentially the same whether the cadmium was extracted from 10 or 50 ml of original solution.

*Colorimetric determination*

Since the separation procedure eliminated all interfering substances, the colorimetric procedure was made as simple as possible. When the colorimetric determination described under *Experimental* was applied to sulphuric acid solutions containing known amounts of cadmium, the molar absorptivity obtained was $6{\cdot}85 \times 10^3$ l.mole$^{-1}$.mm$^{-1}$. When it was applied to cadmium extracted from hydrochloric acid and recovered by stripping, the apparent molar absorptivity was $6{\cdot}50 \times 10^3$ l.mole$^{-1}$.mm$^{-1}$, in agreement with the 5% loss in the separation process. In both cases, Beer's law was obeyed for up to 10 $\mu$g of cadmium.

The molar absorptivity is rather low when compared to those usually found in the literature for the cadmium-dithizone complex in chloroform.[9] However, since the same procedure, when applied to hydrochloric acid solutions of cadmium, gave a molar absorptivity satisfactorily close to the one reported by Saltzman ($7{\cdot}4 \times 10^3$ and $7{\cdot}8 \times 10^3$ l.mole$^{-1}$.mm$^{-1}$ respectively), the value obtained with sulphuric acid was considered correct and the discrepancy was ascribed to the experimental conditions.

Since stripping with tartaric acid leaves an organic solution practically free from ions able to interfere in the colorimetric determination of cadmium with dithizone, the direct spectrophotometric determination of cadmium in the organic phase was also considered. Experiments were carried out with synthethic samples containing cadmium and iron. After extraction and stripping of iron, the cadmium dithizonate colour was developed by adding 10 ml of a 0·002% solution of dithizone in chloroform to the 10 ml of TOA solution; the mixture was diluted to 25 ml with chloroform and the colour was measured at 520 nm, immediately after development, against a reagent blank. A straight calibration curve was obtained for cadmium up to 10 $\mu$g: the molar absorptivity was $6{\cdot}0 \times 10^3$

$l.mole^{-1}.mm^{-1}$. The precision was independent of the amount of cadmium present: the standard deviation was 1·1 μg of cadmium (15 variates) for reproducibility tests made at the 2, 4, 6, 8 and 10 μg levels. Such a poor reproducibility, attributable to the observed instability of the colour obtained in the organic phase, was considered unacceptable, and the additional extraction step with sulphuric acid was confirmed to be necessary.

*Precision and accuracy*

Table I shows the standard deviations of results obtained when the colorimetric determination was applied to cadmium directly added to 2*M* sulphuric acid and to cadmium originally present in 1*M* hydrochloric acid and taken through the entire procedure. It appears that the separation steps do not affect the precision obtainable.

TABLE I.—PRECISION OF RESULTS WITH SAMPLES CONTAINING ONLY CADMIUM

| Cd, μg | Cd in $H_2SO_4$ | | Cd in 10 ml of HCl | | Cd in 50 ml of HCl | |
|---|---|---|---|---|---|---|
| | *s*, μg | *n* | *s*, μg | *n* | *s*, μg | *n* |
| 0 | 0·037 | 8 | 0·054 | 12 | 0·033 | 4 |
| 2 | 0·038 | 1 | 0·054 | 5 | 0·064 | 4 |
| 4 | 0·098 | 4 | 0·091 | 5 | 0·071 | 4 |
| 6 | 0·069 | 4 | 0·060 | 5 | 0·075 | 4 |
| 8 | 0·052 | 1 | 0.072 | 5 | — | — |
| 10 | — | — | 0·058 | 5 | 0·079 | 4 |

The second column refers to cadmium in 20 ml of 2*M* $H_2SO_4$; the third and fourth columns to cadmium in 10 and 50 ml of 1*M* HCl, respectively, taken through the entire procedure; *n* is the number of variates for the calculation of the standard deviation *s*.

Table II shows results obtained by applying the procedure to 10 ml of hydrochloric acid containing 1·0 μg of cadmium together with each of the elements that may be present in Zircaloy-2. The oxidation states were the ones expected after the hydrochloric + hydrofluoric acid dissolution. The alloying constituents were taken in amounts comparable to those present in 1 g of sample, and the impurities in amounts considerably greater than the highest generally accepted in 1 g of nuclear-grade alloy. None of the elements, in the amounts considered, appears to affect the accuracy and the precision of the cadmium determination.

TABLE II.—EFFECT OF DIVERSE IONS ON THE DETERMINATION OF CADMIUM

| Foreign ion | Amount, μg | Cd found, μg | *s* μg of Cd |
|---|---|---|---|
| — | — | $1·00_7$ | 0·070 |
| Al(III) | 350 | $0·95_9$ | 0·059 |
| B(III) | 5 | $1·00_8$ | 0·054 |
| Co(II) | 30 | $0·97_4$ | 0·061 |
| Cr(III) | $2 \times 10^3$ | $0·99_4$ | 0·055 |
| Cu(II) | 100 | $0·98_9$ | 0·072 |
| Fe(III) | $3 \times 10^3$ | $0·94_9$ | 0·072 |
| Mn(II) | 100 | $0·99_2$ | 0·065 |
| Ni(II) | $1·5 \times 10^3$ | $1·08_4$ | 0·059 |
| Mo(VI) | 100 | $0·97_2$ | 0·056 |
| Pb(II) | 50 | $1·00_3$ | 0·041 |
| Sn(II) | $20 \times 10^3$ | $0·93_8$ | 0·058 |
| Ti(IV) | 200 | $1·00_0$ | 0·056 |
| $UO_2^{2+}$ | 5 | $0·98_9$ | 0·058 |
| W(VI) | 200 | $0·98_9$ | 0·066 |
| Zr(IV) | $1 \times 10^6$ | $1·06_3$ | 0·054 |

Ten ml of 1*M* HCl, containing the foreign ion and 1 μg of Cd were run through the whole procedure. The "cadmium found" figures are averages of four results.

The effect of fluoride was also checked. Although most of the hydrofluoric acid is likely to be fumed off during the dissolution step, and although the remaining fluoride is likely to be strongly bound to the zirconium present, the tests were made with 10 ml of 1*M* hydrochloric acid containing 0·46 g of hydrofluoric acid (*i.e.*, the total amount added in the procedure). The results obtained, at 0 and 5 $\mu$g cadmium levels, showed that the presence of such large amounts of hydrofluoric acid does not affect the precision or the accuracy of the method.

The final experiments were accomplished with actual samples of Zircaloy-2, with known amounts of cadmium added during the dissolution step. Nuclear-grade alloys from different sources were used. The results obtained are collected in Table III, where the amount of cadmium found is corrected for

TABLE III.—ACCURACY AND PRECISION OF THE METHOD

| Cadmium added, $\mu$g | Cadmium found, $\mu$g | *s*, *ppm* of Cd | *n* |
|---|---|---|---|
| 0 | 0 | 0·054 | 7 |
| 2·00 | 1·89 | 0·039 | 4 |
| 4·00 | 4·16 | 0·11 | 2 |
| 6·00 | 6·04 | 0·11 | 2 |
| 8·00 | 7·80 | 0·054 | 2 |
| 10·00 | 9·95 | 0·054 | 2 |

Known amounts of cadmium were added to 1 g of Zircaloy-2 during dissolution. The figures referring to cadmium found are corrected for the cadmium originally present in the alloy.

the amount originally present in the alloy, as determined by the same method applied to samples with no cadmium added. Since there was no proof that the absorbance figures obtained in these blank determinations were due to cadmium actually present in the alloy (and not—for instance—to some unknown interference), some sulphuric acid solutions obtained at the end of the separation procedure were analysed by atomic absorption (air–hydrogen flame, total consumption HETCO burner, 228·8 nm wavelength resonance line). The results obtained spectrophotometrically were confirmed: as an example, one alloy was found to contain 0·32 $\pm$ 0·03 ppm of cadmium by the proposed procedure, and 0·30 ppm by atomic absorption.

In conclusion, the results reported in Table III show that the proposed method has precision and accuracy comparable to those of the polarographic method,[7] the only one of the more precise methods[6–8] for which reproducibility data were found in the literature.

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**Summary**—A method is presented for the spectrophotometric determination of cadmium in Zircaloy-2 at the tenths of ppm level. The method involves the extraction of Cd with tri-n-octylamine from 1*M* HCl, its separation from Fe(III) by scrubbing with tartaric acid, its recovery with 2*M* sulphuric acid, and the final development of the Cd-dithizonate colour. The method is simple and rapid, does not require any special instrumentation, and avoids the use of toxic reagents. Cadmium at the 0·3-ppm level has been determined with standard deviation 0·054 ppm.

**Zusammenfassung**—Ein Verfahren zur spektrophotometrischen Bestimmung von Cadmium in Zircaloy-2 im Zehntel-ppm-Bereich wird mitgeteilt. Cd wird dabei mit Tri-n-oktylamin aus 1*M* HCl extrahiert, durch Waschen mit Weinsäure von Eisen getrennt, mit 2*M* Schwefelsäure rückextrahiert und schließlich mit Dithizon angefärbt. Das

Verfahren geht einfach und schnell, bedarf keiner besonderen instrumentellen Ausrüstung und vermeidet die Verwendung toxischer Reagentien. Cadmium wurde im Bereich um 0,3 ppm mit einer Standardabweichung von 0,054 ppm bestimmt.

**Résumé**—On présente une méthode pour la détermination spectrophotométrique du cadmium dans le Zircaloy-2 au niveau de fractions de ppm. La méthode comprend l'extraction du Cd avec la tri-n-octylamine de HCl 1*M*, sa séparation du Fe(III) par lavage avec acide tartrique, son recouvrement avec $H_2SO_4$ 2*M* et le développement final de la couleur Cd-dithizonate. La méthode est assez simple et rapide, ne demande aucune instrumentation particulière et ne comporte pas l'usage de réactifs toxiques. Cadmium au niveau de 0,3 ppm a été déterminé avec une déviation standard de 0,054.

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# Spectrophotometric determination of EDTA

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SEVERAL colorimetric procedures have been reported for determination of EDTA. They are mostly based on forming an EDTA–metal chelate and measuring its amount either directly[1–5] or indirectly.[6–8] The decrease in absorbance of a suitable metal complex on addition of EDTA has also been used.[9–11] Recently[12] a method based on the inhibitory effect of EDTA on Mn(II)-catalysed oxidation of Malachite Green by periodate has been reported. Most of these procedures require higher pH values and are subject to interferences. It seems preferable to select a metal ion that forms a highly stable EDTA complex and use it at a pH at which it will displace other metal ions from their EDTA complexes. Iron(III) appears to be very suitable since its EDTA complex has a very high stability constant[13] and is stable[14] even at pH 1. It has been used before, but only for indirect determinations.[8,15,16] As iron(III) has an absorption maximum at 304 nm,[17–19] it appeared probable that a direct method would be possible, based on measurement of the Fe–EDTA complex and the excess of iron(III).

## EXPERIMENTAL

To an aliquot of EDTA solution the requisite amount of ferric alum solution in sulphuric acid was added. The solution was then diluted to give an acidity of about 0.1*N* and Fe–EDTA complex concentration of 1–7 $\times$ $10^{-5}$ *M*. Absorbances were then measured at 258 and 305 nm.

## RESULTS AND DISCUSSION

The absorption spectra of ferric alum and the Fe–EDTA complex in 0.1*N* sulphuric acid are shown in Fig. 1. The Fe(III)–EDTA absorbs less strongly at 305 nm, but iron(III) has roughly equal absorption at the two wavelengths of interest, 258 and 305 nm. Both the systems obey Beer's law at

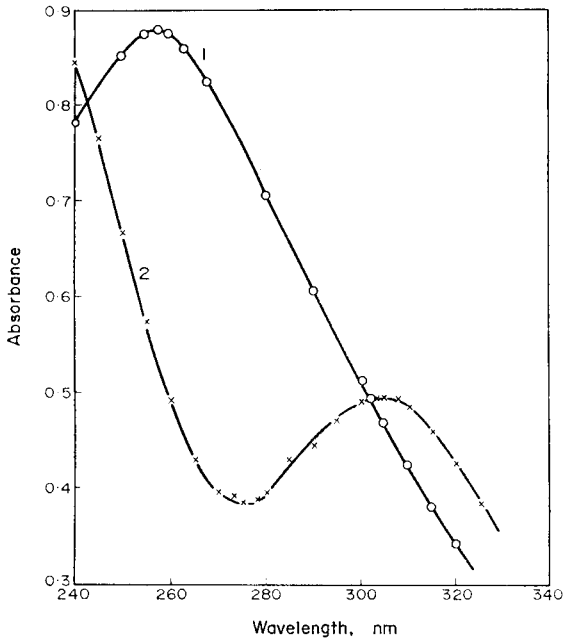


FIG. 1—Absorption spectra of *1*-1·03 × $10^{-4}M$ Fe(III)–EDTA, *2*-2·28 × $10^{-4}M$ ferric alum.

the two wavelengths, and if the absorbances are additive, the concentration of Fe–EDTA can be found from measurements made at 258 and 305 nm. It can readily be shown that if the ratios of the molar absorptivities at 258 and 305 nm are $\alpha$ and $\beta$ for iron(III) and Fe–EDTA respectively, then

$$A_{258} - \alpha A_{305} = [\text{Fe–EDTA}]\varepsilon_{258}(1 - \alpha/\beta)$$

where $A_{258}$ and $A_{305}$ are the total absorbances at 258 and 305 nm, and $\varepsilon_{258}$ is the molar absorptivity of Fe–EDTA at 258 nm. $\varepsilon_{258}$ was found to be 856 l.mole$^{-1}$. mm$^{-1}$ and $\alpha$ and $\beta$ to be 1·039 and 1·901 respectively. A plot of $(A_{258} - \alpha A_{305})$ *vs.* [Fe–EDTA] should yield a straight line passing through the origin; experiment verified this and for a 10-mm cell gave a slope of 3880 l.mole$^{-1}$. Then [Fe–EDTA] = $(A_{258} - 1{\cdot}039A_{305})/3880$ mole/l. for measurements made in 10-mm cells.

Table I shows some typical results, and it is evident that a final EDTA concentration as low as

TABLE I.—DETERMINATION OF EDTA IN 0·1*N* $H_2SO_4$ MEDIUM

| EDTA taken, $10^{-6}M$* | $Fe^{3+}$ added, $10^{-6}M$* | Molar ratio, $Fe^{3+}$/EDTA | EDTA found, $10^{-6}M$* | Error, % |
|---|---|---|---|---|
| 90·0 | 110·0 | 1·22 | 90·8 | +0·9 |
| 90·0 | 132·0 | 1·37 | 91·7 | +1·9 |
| 90·0 | 154·0 | 1·71 | 90·5 | +0·6 |
| 70·0 | 150·0 | 2·14 | 70·5 | +0·7 |
| 54 0 | 66·0 | 1·22 | 53·7 | −0·6 |
| 44·0 | 55·0 | 1·25 | 43·6 | −0·9 |
| 44·0 | 66·0 | 1·50 | 43·8 | −0·5 |
| 44·0 | 77·0 | 1·75 | 44·2 | +0·5 |
| 36·0 | 44·0 | 1·22 | 36·1 | +0·3 |
| 28·8 | 55·0 | 5·22 | 28·9 | −0·4 |
| 8·45 | 200·0 | 23·7 | 8·18 | −3·2 |
| 54·1† | 67·5 | 1·25 | 53·9 | −0·4 |
| 54·1§ | 67·5 | 1·25 | 55·2 | +2·0 |

* After dilution to 100 ml.
† 0·05*N* $H_2SO_4$ medium.
§ 0·01*N* $H_2SO_4$ medium.

$10^{-5}$ $M$ can be measured with fair accuracy. The concentration of EDTA is determined by the difference of the absorbances measured at the two wavelengths. The larger the difference, the more accurate will be the result. Since $\alpha$ is approximately unity, the difference will be largely determined by the amount of Fe(III)–EDTA that is formed in the system. The accuracy is further governed by the relative error of the instrument used. Generally[20] absorbances should be in the range 0·2–0·7 for precision and accuracy. The amount of iron(III) to be added should be such that the measured absorbances lie within this range. Table I shows that wide variation in the molar ratio of iron(III) to EDTA does not affect the result. However, an excess of iron(III) must always be added. It will be obvious whether insufficient iron(III) has been added, since in that case $A_{258}/A_{305} = \beta = 1{\cdot}901$.

The effect of changing the acidity was examined, and the results are given in Table II. The absorbance for Fe(III)–EDTA remains almost unchanged, but that of iron(III) decreases with decrease in

TABLE II.—EFFECT OF ACID CONCENTRATION ON THE ABSORBANCES DUE TO $Fe^{3+}$ AND Fe(III)–EDTA

| $[H_2SO_4]$, $N$ | $Fe^{3+}$ | | Fe(III)–EDTA | |
|---|---|---|---|---|
| | $A_{258}$ | $A_{305}$ | $A_{258}$ | $A_{305}$ |
| 0·10 | 0·535 | 0·515 | 0·555 | 0·293 |
| 0·05 | 0·528 | 0·489 | 0·560 | 0·296 |
| 0·01 | 0·519 | 0·455 | 0·561 | 0·298 |

acidity. Even so, if the excess of iron(III) is kept to a minimum, the error is small (see Table I).

The Fe–EDTA complex is photosensitive,[10,21] and in sunlight undergoes photodecomposition, but in work done under normal diffuse room lighting there is no significant analytical error, since the concentrations are low, the time taken is short, and in any case the calibration curve is determined under the same conditions.

Some experiments on interferences are reported in Table III. Ions with EDTA complexes that are

TABLE III.—INTERFERENCE OF VARIOUS IONS

| Ion added | Molar ratio ion:EDTA | EDTA taken,* $10^{-6}M$ | EDTA found,* $10^{-6}M$ | Error % |
|---|---|---|---|---|
| $Ca^{2+}$ | 2·00 | 50·2 | 50·6 | +1·0 |
| | 4·00 | 50·2 | 48·9 | −2·4 |
| $Sr^{2+}$ | 2·00 | 50·2 | 49·3 | −1·6 |
| $Co^{2+}$ | 1·86 | 54·0 | 54·7 | +1·3 |
| | 3·72 | 54·0 | 54·5 | +0·9 |
| $Ni^{2+}$ | 1·86 | 54·0 | 52·5 | −2·8 |
| | 3·72 | 54·0 | 52·9 | −2·0 |
| $Cu^{2+}$ | 1·00 | 22·2 | 22·0 | −0·5 |
| | 1·00 | 44·3 | 44·0 | −0·7 |
| $Cr^{3+}$ | 1·00 | 50·2 | 51·7 | +3·2 |
| | 2·00 | 50·2 | 50·3 | +0.4 |
| $Sc^{3+}$ | 0·39 | 51·7 | 51·5 | −0·4 |
| | 0·77 | 51·7 | 50·7 | −1·9 |
| $Th^{4+}$ | 1·15 | 52·2 | 51·5 | −1·3 |
| | 2·30 | 52·2 | 51·0 | −2·2 |
| $In^{3+}$ | 1·15 | 52·2 | 19·2 | −63 |
| | 2·30 | 52·2 | 5·9 | −89 |

* After final dilution to 100 ml; $[Fe^{3+}]$ 67·5 × $10^{-6}M$.

not stable at pH 1 do not interfere, nor do the ter- and quadrivalent ions which are easily displaced by iron(III) from their EDTA complexes at pH 1 and do not show any significant absorbance at the wavelengths used. Indium(III), however, interferes appreciably; probably the stability of its EDTA complex is similar to that of Fe–EDTA.[13]

An advantage over the method of Brady and Gwilt[8] is that it is not necessary to know whether iron(III) is initially present in the EDTA solution.

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**Summary**—Both iron(III) and its EDTA complex in 0·1*N* $H_2SO_4$ medium absorb in the ultraviolet region of the spectrum, with absorption maxima at 305 and 258 nm respectively. EDTA can thus be determined by addition of excess of Fe(III) and measurement of the absorbance at the two wavelengths. An EDTA concentration as low as $\sim 10^{-6}M$ can be determined with a fair degree of accuracy. The method is free from interferences from most common metal ions, and is simple and rapid.

**Zusammenfassung**—Eisen(III) und sein EDTA-Komplex absorbieren beide in 0,1*N* $H_2SO_4$ im ultravioletten Bereich des Spektrums mit Absorptionsmaxima bei 305 bzw. 258 nm. EDTA kann demnach durch Zugabe eines Überschusses von Eisen(III) und Messung der Extinktion bei diesen beiden Wellenlängen bestimmt werden. Eine EDTA-Konzentration von etwa $10^{-6}M$ kann noch mit ausreichender Genauigkeit bestimmt werden. Das Verfahren wird durch die meisten häufig vorkommenden Metallionen nicht gestört und geht einfach und schnell.

**Résumé**—Le fer(III) et son complexe EDTA en milieu $H_2SO_4$ 0,1 *N* absorbent tous deux dans la région ultra-violette du spectre, avec maximums d'absorption à 305 et 258 nm respectivement. On peut ainsi doser l'EDTA par addition d'excès de Fe(III) et mesure de l'absorption aux deux longueurs d'onde. On peut déterminer une concentration en EDTA aussi faible que $\sim 10^{-6}$ *M* avec un assez bon degré de précision. La méthode est exempte d'interférences de la plupart des ions métalliques communs et elle est simple et rapide.

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# A borax fusion technique for quantitative X-ray fluorescence analysis

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THE BORAX FUSION technique is a well known sample preparation method in quantitative X-ray analysis. The advantages of the method are that standards can be easily prepared and that no particle-size problem arises. Several variations of the method have been published.[1–9] The need for these has arisen because of lack of reproducibility.

We report a variation of the borax fusion technique which is reproducible and does not require a skilled operator. The method is based on the observation of Zuurbier and Thomson[10] that molten borax poured on "Degussa Geräteplatin II" (a 95:5 Pt/Au alloy) will loosen after its solidification and yield an intact glass disc with a specular surface.

## EXPERIMENTAL

### *Platinum casting moulds*

These were formed from polished sheet "Degussa Pt II" (50 mm diameter × 0.2 mm thick). A die of the form shown in Fig. 1 is clamped in a lathe and a circular platinum sheet is concentrically

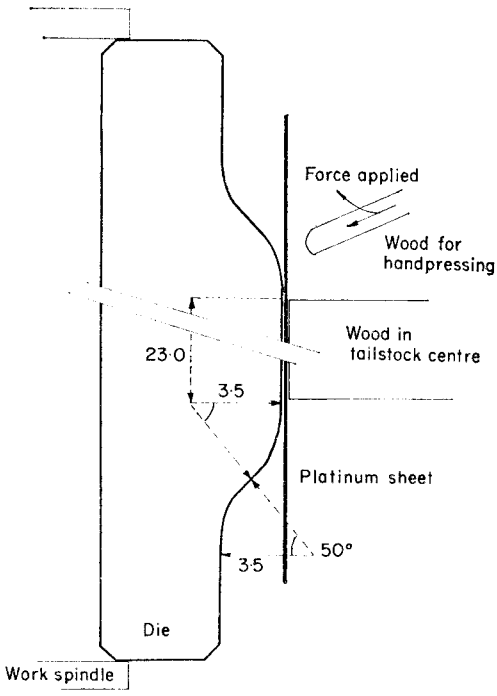


FIG. 1.—Form of the die and arrangement for the forming process.

held against the die by a piece of wood in the tailstock centre. While the lathe is spinning the platinum is manually pressed against the die by means of another piece of wood. After forming, the platinum sheet is removed from the lathe and heated to reduce strain. The process is repeated four times until the fit of mould to die is satisfactory. Then the inner surface of the mould is slightly sand-blasted and next polished with cotton and some household silver polish. Instead of sand-blasting a satisfactory procedure is to press the mould between the die and a counter die of such form as to leave exactly the space for the thickness of the platinum.

The dimensions of the die are not critical, but it has appeared essential for loosening of the glass discs that the radius should not be appreciably below 3.5 mm and the angle above 50°.

*The flux*

This was prepared in batches of about 1½ kg by mixing in a cube mixer for 3 hr the following chemicals: 45 parts by weight anhydrous sodium tetraborate, 7 parts by weight lithium hydroxide powdered to approximately 80-mesh, 16 parts by weight of orthoboric acid; when kept over silica gel in a desiccator the flux is stable for at least 6 months. The quantitative composition is not very critical, but for standards and samples the same composition should be used.

*Heavy absorbers*

Lead(II) oxide.
Tungsten(VI) oxide.
Barium sulphate.
Lanthanum oxide.
Cadmium oxide.

*Procedure (molybdenum in alumina catalyst)*

Accurately weigh 100 ± 5 mg of powdered sample (Note 1), add 1200 ± 1 mg of barium sulphate (Note 2) and an amount of flux up to 8000 ± 1 mg (Notes 3 and 4).

Mix the powders in a platinum crucible by stirring with a spatula and heat over a Méker burner at 1200° until molten. Protect flame and crucible with a firebrick heat-shield. Keep the sample mixture molten for another 2–5 min with occasional swirling to promote homogeneity. Take care that all gas bubbles are removed. Meanwhile heat a casting-mould until nearly red hot; the temperature of the mould is not critical.

Pour the contents of the crucible into the mould and allow to cool. The glass disc will loosen from the mould during cooling. Remove the glass disc from the mould. Sand-paper the rim of the glass disc, if necessary, so that it fits in a sample holder for the fluorescence measurement. Use a sample holder with a mask of $\leqslant$23 mm diameter.

Prepare standards from the appropriate oxides or salts of the element to be determined. They are stable for at least one year, if kept in a desiccator with silica gel.

*Notes*

1. Metallic samples should be previously converted into oxides or salts.
2. Instead of barium sulphate, other heavy absorbers may be used. Those mentioned above are satisfactory.
3. The relative amounts of the sample, the heavy absorber and the flux may be changed, although fracturing of the glass discs may occur when the amount of absorber and sample is increased.
4. If the sample contains a large amount of copper the addition of 200–500 mg of sodium or potassium chloride prevents fracturing of the glass discs. This addition also guards against fracturing in the case of certain combinations of elements (*e.g.*, Ti and Zr with PbO as heavy absorber).

## RESULTS AND DISCUSSION

With homogeneous samples the relative standard deviation[11] can be as low as 0.2%. These deviations include the deviations due to sampling and weighing and those due to instrumental short-time drift and unequal sample positions (0.1%).

Most elements give good clear discs when present in amounts of 100 mg. Some may be present in such amounts that they can be used as the heavy absorber. The following elements give intact glass discs, but the glass is not clear and may not be homogeneous: Zn, Nb, Ru, Rh, Pd, Sn, Te, I, Ir. The following elements either do not give intact discs or cause other difficulties: As, Ag, Au. It should be noted that the compound used may be of importance, *e.g.*, chromium(III) oxide gave a "cloudy" disc, chromium(VI) oxide a clear one.

In this method the samples are diluted substantially. A loss in sensitivity has therefore to be accepted. The method is, however, advantageous for an accurate determination of major constituents.

The method is also relatively rapid. Two hours is ample time for a duplicate determination if the standards have already been prepared. When several elements can be determined in one glass disc and/or when the sample is brought into solution with difficulty this time compares favourably with the time needed for wet methods.

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**Summary**—A borax fusion technique to cast glass discs for quantitative X-ray analysis is described in detail. The method is based on the "non-wetting" properties of a Pt/Au alloy towards molten borax, on the favourable composition of the flux and finally on the favourable form of the casting mould. The critical points of the technique are stressed, resulting in a method which could be carried out successfully by inexperienced workers. In general the method compares favourably in speed and accuracy with wet-chemical methods.

**Zusammenfassung**—Ein Schmelzverfahren mit Borax wird im Detail beschrieben, um Glasscheiben für die quantitative Röntgenanalyse zu gießen. Das Verfahren beruht darauf, daß eine Pt/Au-Legierung von geschmolzenem Borax nicht benetzt wird, ferner auf einer günstigen Zusammensetzung des Schmelzflusses und auf der günstigen Gestaltung der Gußform. Es wird Nachdruck auf die kritischen Punkte des Verfahrens gelegt; daher sollte es auch von Unerfahrenen mit Erfolg ausgeführt werden können. Im allgemeinen schneidet das Verfahren bezüglich Geschwindigkeit und Genauigkeit im Vergleich mit naßchemischen Methoden günstig ab.

**Résumé**—On décrit en détail une technique de fusion au borax pour couler des disques de verre pour l'analyse aux rayons X quantitative. La méthode est basée sur les propriétés "non-mouillantes" d'un alliage Pt/Au vis-à-vis du borax fondu, sur la composition favorable du fondant et finalement sur la forme favorable du moule de coulée. On fait ressortir les points critiques de la technique, avec pour résultat une méthode qui pourrait être exécutée avec succès par des travailleurs inexpérimentés. En général, la méthode est favorablement comparable en rapidité et précision avec les méthodes chimiques par voie humide.

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# ANNOTATION

## The stability of gold solutions

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THE STABILITY of dilute gold solutions has become increasingly important as the techniques of chemical analysis have become more sensitive and have required more dilute standards. Although instability of dilute gold solutions has been assumed by some experimenters and ignored by others, a survey of the literature indicates a variety of reports and conclusions on this problem.

Witzmann and Helmshauss[1] have reviewed some of the past literature dealing with gold stability and note the first references to the light sensitivity of gold solutions were by Hellot in 1737[2] and by Scheele in 1777.[3] However, most references deal with the use of gold solutions for the preparation of colloidal gold and the effect of various forms of irradiation (ultraviolet,[1,4–11] visible,[12–14] sunlight,[4,15,16] X-ray[14] and ultrasonic[17]) prior to the use of reducing agents. These reports indicate a decided accelerating effect on the formation of gold particles, dependent on the length of exposure to irradiation. Thiesson[8,9] was able to produce a series of monodispersed sols by reduction after ultraviolet irradiation for fixed times. Bjerrum[18] indicated that the hydrolysis of gold chloride in solutions 0·1–0·001*M* in gold, caused by the addition of alkali, proceeded by the four-step replacement of $Cl^-$ by $OH^-$. Sonstadt[19,20] reported the formation of colloidal gold and precipitation when aqueous gold solutions containing 0·04% and 0·007% gold chloride were exposed to sunlight for several days or simply were heated for several hours.

### RESULTS AND DISCUSSION

The gold solutions containing 4 ppm or more showed a variety of effects at different acidities by the end of 400 days. Solutions at pH above 3 eventually exhibited colour changes from the normal yellow to pink for the range pH 3–7 and to blue or purple for pH > 7. In some cases, a gold precipitate was gradually coagulated in the bottles. No major change occurred in the ultraviolet–visible region spectra of the solutions other than the decrease in absorbance. A few trials with sodium chloride content varying from 0·15 to 15 g/250 ml showed that there was no effect on stability.

From the results presented in Table I it can be seen that the gold solutions at pH 0–2 are quite stable for extended periods and especially stable the more concentrated the gold solution, with the maximum loss being 4% over 400 days for the range 12–258 ppm. Gold solutions in 6*M* hydrochloric acid show 93 to 100% stability for at least 6 days with concentrated gold solutions again exhibiting more extended stability. Above pH 3 the gold solutions show a variety of stabilities, depending on pH and gold concentration, perhaps indicating some reaction with the glass, incomplete cleaning or a reaction with air introduced during sampling. However, in all but one sample, the solutions showed a loss of only 6% or less for the first 6 hr, and except for four samples, only 16% or less over the first 6 days. In only five of the samples was the gold content less then 10% at the end of 400 days

Though extraordinary precautions might ensure longer stability of gold solutions, the results obtained indicate the stability to be expected under normal laboratory conditions. The best stability was obtained with 12–258 ppm solutions at pH 0–2, where the loss was less than 4% over 400 days. Outside these ranges the gold stability was less reliable and more dependent on the acidity and gold content. Svedburg[5] indicated that in alkaline solutions $10^{-3}$–$10^{-5}M$ in gold chloride, a number of colloidal gold particles were formed during irradiation with ultraviolet light, dependent on the duration of irradiation and on the concentration of solution.

Very few reports have been made regarding the stability of gold solutions under conditions which are common for standard solutions of gold. Leutwein[21] reported that 0·001% solutions of gold in nitric acid showed a 90% loss of the original strength after 230 days storage in Jena glass but showed only 10% loss when stored in quartz. Hummel[22] has shown that gold in sea-water is strongly absorbed by polyethylene but not by silica containers.

A preliminary note by Chow and Beamish[23] indicated the unexpected stability of 2·5–25 ppm gold solutions in 0·12*M* hydrochloric acid when stored in glass bottles in the dark. The present report deals with an extensive survey of the stability of gold solutions, covering the range 0·4–258 ppm gold in solutions ranging from 6*M* hydrochloric acid to pH 11, for a period of 400 days.

TABLE I

| Original concentration *μg/ml* | pH | Gold left in solution, %, after | | | | |
|---|---|---|---|---|---|---|
| | | 6 hr | 6 d | 30 d | 100 d | 400 d |
| 258 | 6*M* HCl | — | 100 | 100 | 97 | 97 |
| | 2·7 | — | 100 | 100 | 97 | 97 |
| | 4·9 | — | 100 | 95 | 91 | 90 |
| | 5·1 | — | 100 | 92 | 91 | 89 |
| | 5·5 | — | 100 | 94 | 91 | 89 |
| 103 | 6*M* HCl | 100 | 100 | 100 | 100 | 97 |
| | 1·7 | 100 | 100 | 100 | 100 | 100 |
| | 4·5 | 94 | 89 | 89 | 82 | 76 |
| | 5·7 | 95 | 87 | 86 | 78 | 73 |
| | 10·7 | 95 | 89 | 87 | 71 | 68 |
| 41·3 | 6*M* HCl | 100 | 99 | 98 | 94 | 92 |
| | 0 | 100 | 100 | 100 | 100 | 100 |
| | 1·0 | 100 | 100 | 100 | 100 | 100 |
| | 2·1 | 100 | 99 | 99 | 96 | 96 |
| | 3·6 | 98 | 91 | 89 | 76 | 62 |
| | 4·5 | 98 | 84 | 80 | 75 | 54 |
| | 5·6 | 95 | 74 | 72 | 61 | 54 |
| | 9·6 | 89 | 86 | 81 | 61 | 24 |
| | 11·4 | 96 | 87 | 62 | 40 | 25 |
| 12·4 | 6*M* HCl | 100 | 96 | 83 | 78 | 67 |
| | 0 | 100 | 100 | 100 | 98 | 98 |
| | 1·1 | 100 | 100 | 100 | 100 | 97 |
| | 2·1 | 100 | 100 | 100 | 96 | 96 |
| | 3·2 | 100 | 93 | 92 | 70 | 55 |
| | 3·8 | 90 | 76 | 65 | 37 | 23 |
| | 6·5 | 100 | 84 | 75 | 45 | 0 |
| | 9·5 | 100 | 100 | 100 | 88 | 23 |
| 4·13 | 6*M* HCl | 100 | 93 | 50 | — | 40 |
| | 0 | 100 | 98 | 89 | 73 | 73 |
| | 1·0 | 100 | 98 | 98 | 90 | 83 |
| | 2·0 | 100 | 98 | 90 | 83 | 81 |
| | 2·9 | 100 | 85 | 76 | 59 | 37 |
| | 4·2 | 100 | 88 | 71 | 49 | 0 |
| | 6·1 | 100 | 100 | 98 | 82 | 10 |
| | 8·0 | 100 | 100 | 49 | 42 | 24 |
| | 10·1 | 98 | 97 | 47 | 30 | 12 |
| 1·24 | 6*M* HCl | 100 | 100 | 74 | 20 | 10 |
| | 0 | 100 | 100 | 98 | 59 | 55 |
| | 1·0 | 100 | 100 | 100 | 90 | 86 |
| | 2·2 | 100 | 100 | 87 | 65 | 51 |
| | 2·9 | 98 | 58 | 51 | 32 | 0 |
| | 4·4 | 98 | 94 | 80 | 24 | 0 |
| | 6·1 | 98 | 97 | 92 | 88 | 73 |
| | 9·8 | 100 | 100 | 96 | 95 | 55 |
| 0·413 | 6*M* HCl | 100 | 100 | 66 | 64 | 54 |
| | 0 | 100 | 100 | 100 | 100 | 100 |
| | 1·0 | 100 | 83 | 40 | 30 | 30 |
| | 2·0 | 100 | 47 | 12 | 5 | 0 |
| | 2·9 | 100 | 20 | 12 | 5 | 0 |
| | 4·5 | 100 | 100 | 70 | 40 | 25 |
| | 6·2 | 100 | 98 | 97 | 51 | 24 |

## EXPERIMENTAL

*Apparatus*

*Glass bottles:* "Vitro-400" 250-ml glass bottles (Wheaton Glass Co., Millville, N.J.) with ground-glass stoppers. Vitro-400 is a borosilicate glass with high resistance to chemical attack.

*Reagents*

The hydrobromic acid used was the colourless, constant-boiling fraction from a distillation of reagent grade acid. The concentration is about 48% hydrobromic acid.

A standard gold solution was prepared by dissolving a weighed quantity of Johnson Matthey "Specpure" gold in *aqua regia*, adding 50 mg of sodium chloride and removing the nitric acid by several evaporations just to dryness after addition of a few drops of hydrochloric acid. The final residue was dissolved in 10 ml of hydrochloric acid and 40 ml of water, the solution was filtered through Whatman No. 541 paper and the filter was washed thoroughly. The filter paper was charred and ignited in the dissolution beaker and the dissolution step repeated. These solutions were combined in a volumetric flask and diluted to the mark. The gold content was determined gravimetrically by hydroquinone precipitation[24] and found to be $1{\cdot}032 \pm 0{\cdot}002$ mg/ml. A dilute gold standard solution was prepared by diluting 2 ml of the original stock solution to 100 ml.

*Procedure*

New bottles were obtained to avoid any unknown contamination. These bottles were cleaned with chromic acid, then twice with soap and water and finally rinsed with doubly distilled water. The bottles were finally cleaned with hot *aqua regia*, rinsed thoroughly and then allowed to dry.

Gold solutions for stability tests were prepared by dilution of one of the standard gold solutions to 250 ml. The following concentrations were investigated: 0·40, 1·2, 4·0, 12, 40, 100, 250 $\mu$g/ml. The acidity of these test solutions was adjusted by addition of hydrochloric acid or sodium hydroxide solution to provide acidities ranging from 6*M* acid to pH 11.

The storage bottles were rinsed with the gold solution, the test solutions were transferred and the bottles were stoppered and then stored away from light in the laboratory bench without special precautions.

The amount of gold in solution was determined by the direct hydrobromic acid method previously described.[23] This method requires the addition of 2·5 and 5 ml of 48% hydrobromic acid to aliquots of gold solution and dilution to final volumes of 25 and 50 ml respectively. The gold solutions that were 6*M* in hydrochloric acid required twice the stated volume of hydrobromic acid to produce the full colour. The bromoaurate produced by this method is stable for at least 1 hr and is measured spectrophotometrically at 382·5 nm.

The gold content of the solutions was determined within 1 hr after preparation of the test solutions and then at 6 hr and 6, 30, 100, and 400 days. The acidities of the solutions were measured with a pH-meter during the first day of the test. The results of the gold determinations are reported in Table I, and show the amount of gold remaining in solution, the value at one hour being taken as reference, i.e., 100%.

*Acknowledgement*—The author wishes to thank the National Research Council of Canada for its financial support and Johnson Matthey and Mallory Ltd. (Toronto, Canada) for providing the gold used.

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**Summary**—The stability of gold solutions was studied over the concentration range of 0·41–258 ppm at acidities ranging from 6*M* hydrochloric acid to pH 1, the solutions being stored in glass-stoppered borosilicate bottles. The best conditions were found to be 12–258 ppm solutions at pH 0–2, for which the loss of gold was 4% or less over a period of 400 days.

**Zusammenfassung**—Die Stabilität von Goldlösungen wurde im Konzentrationsbereich 0,41–258 ppm bei Aciditäten zwischen 6*M* Salzsäure und pH 1 untersucht, wobei die Lösungen in Borosilikatflaschen mit Glasstopfen aufbewahrt wurden. Die besten Ergebnisse wurden bei Lösungen mit 12–258 ppm bei pH 0–2 gefunden; der Goldverlust betrug bei ihnen 4% oder weniger in einem Zeitraum von 400 Tagen.

**Résumé**—On a étudié la stabilité de solutions d'or dans le domaine de concentrations 0,41–258 p.p.m. à des acidités comprises entre l'acide chlorhydrique 6*M* et pH 1, les solutions étant conservées dans des flacons au borosilicate bouchés à l'émeri. On a trouvé que les meilleures conditions sont des solutions à 12–258 p.p.m. à pH 0–2, pour lesquelles la perte d'or a été de 4% ou moins pour une période de 400 jours.

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# LETTER TO THE EDITOR

## The HALTAFALL program—some corrections, and comments on recent experience

SIR,

HALTAFALL, a general program for calculating the composition of equilibrium mixtures, published by Sillén *et al.* in 1967,[1] has since been applied, rather extensively, to a variety of problems. Analytical chemists,[2–5] in particular, have used it for the calculation of titration curves, distribution diagrams, solubility curves, *etc.* By including a suitable plotting routine in the program, one may, of course, have such curves plotted directly by the computer; this has been done by Ginstrup[6] (Umeå University, Sweden) and others. A FORTRAN version[7] of Haltafall is now available on a time-sharing computer system in the U.S.A.[8].

In the meantime, and largely in response to difficulties experienced in a few "unusual" cases not encountered at the time of the original paper,[1] we have found and corrected a (small) number of additional errors (Table I).

TABLE I.—ADDITIONAL CORRECTIONS TO HALTAFALL, SEPTEMBER 1970*

1. The statements just before "BEFALL", p. 1280, and Errata,[1b] should read:
*if not* Singfall *then begin for* i := 1 *step* 1 *until* Nfall *do* ifspar [i] := ifall [i] ;
Nfspar := Nfall ; Nut := 0 ;
*if* Nfall > Nva *then begin*
Nut := Nfall—Nva—1 ; Singfall := *true* ; *goto* UPPNUT *end end* ;
*if* Nfall ≥ Nva *and* Singfall *then begin* i := 0; Nfall := Nfspar—Nut ; *goto* HOPPFUT
*end* ;

2. Page 1280, the line just before "SING" should read:
Invert(Nfall, ruta, $1_{10}$-14, det, SING) ; *goto* ANFALL ;

3. Page 1280, line 22 from bottom (= line just before UPPNUT) should read:
*if* Nfall = 0 *then goto* ANFALL ; *goto* BEFALL ;

4. Page 1275, *procedure* Totber, after "w := abs(y—y0) ;" add:
*if* Nfall > 0 *and* tol[ivar] > 0 *and*
abs(Totva[ivar]) > 1 *and* w < abs(tol[ivar] × Totva[ivar]) *then* w := 0 ;

5. Page 1279, lines 9 and 10 should read:
Lnbas ;
Totber ;
*i.e.*, these two procedure calls should change places.

* Page references are to Ref. 1*a*.

In addition, the FORTRAN version[7] of Haltafall from the Department of Analytical Chemistry, Göteborg University, Sweden, contained, until recently, a significant error in the solid phases section of the program: in the FORTRAN DO statement corresponding to the Algol statement

*for j* := i *step* 1 *until* Nfall *do* .....

on p. 1280, line 4 (Ref. 1*a*) the "i" had been accidentally replaced by "1".

In order to improve further (*a*) the speed and (*b*) the reliability of the program, particularly in certain cases of very complicated systems, we have also found it useful (*a*) to rewrite the iteration loops (p. 1279 of Ref. 1*a*) somewhat, as in Ref. 9; (*b*) to give very small tolerance values [*tol*[*ia*] or abs(*tol*[*ia*] × *Tot*[*ia*])]—not smaller than the rounding error of the computer, of course—for those reactants for which equations [Eq. (5) of Ref. 1*a*] are solved in the "inner" iteration loops. These are the reactants which are assigned the highest "Nober" values in the program,[1a] *i.e.*, those which complex the least with other reactants, and those which are mononuclear. (The reactants in the outer loops may then be assigned successively larger tolerance values as a precaution against accumulated rounding errors.)

We wish to express our gratitude to the late Professor Lars Gunnar Sillén for the privilege, among many other things, of working with him in the development of Haltafall and related computer programs.

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*continued overleaf*

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37 **Analytical and Experimental Aspects of Molecular-Sieve Chromatography** by D. M. W. Anderson, I. C. M. Dea and A. Hendrie

# SUMMARIES FOR CARD INDEXES

**Analytical and experimental aspects of molecular-sieve chromatography:** D. M. W. ANDERSON, I. C. M. DEA and A. HENDRIE, *Talanta*, 1971, **18**, 365. (Chemistry Department, The University, Edinburgh, U.K.)

**Summary**—The object of this Review is to give analytical chemists a general introduction to molecular-sieve chromatography, a form of liquid chromatography in which molecular size forms the primary basis for separation, although other effects are also frequently involved. The technique can be used for inorganic and organic molecules, both monomeric and polymeric, in either aqueous or non-aqueous systems. The range of xerogel and aerogel molecular-sieves available at present is described, and the experimental techniques involved in their use are emphasized rather than mechanistic and theoretical considerations. The references cited have been selected critically to form a balanced, up-to-date review and also to indicate the general analytical potential and scope for future development of the technique. An Appendix lists the commercial sources of molecular-sieves and calibration standards.

**Contributions to the basic problems of complexometry—XXIV. Determination of aluminium in the presence of very large amounts of manganese:** RUDOLF PŘIBIL and VLADIMÍR VESELÝ, *Talanta*, 1971, **18**, 395. (Analytical Laboratory, Polarographic Institute of J. Heyrovský, Czechoslovak Academy of Sciences, Prague 1, Jilská 16, Czechoslovakia.)

**Summary**—A simple complexometric determination of aluminium in the presence of a large amount of manganese has been developed. For such determinations only triethylenetetraminehexa-acetic acid (TTHA) can be used. In slightly acidic medium TTHA forms with aluminium a binuclear complex $Al_2L$. The complex is formed almost immediately at room temperature. The excess of TTHA is back-titrated with copper sulphate at pH about 5·3, with Glycinecresol Red as indicator. Reliable results were obtained for Mn:Al ratios up to at least 20. The sum of Al + Fe can be determined by the same method. Very large amounts of calcium and magnesium do not interfere.

**Cation-exchange behaviour of several elements in formic acid solution:** MOHSIN QURESHI and WAQIF HUSAIN, *Talanta*, 1971, **18**, 399. (Chemical Laboratories, Aligarh Muslim University, Aligarh, India.)

**Summary**—The cation-exchange characteristics of 20 elements towards the strongly acidic cation-exchange resin Dowex 50 × 8 in media containing varying concentrations of formic acid and mixtures of formic acid with aqueous dioxan were investigated. Possible separations are indicated and discussed. Bismuth may be quantitatively separated from copper.

## АНАЛИТИЧЕСКИЕ И ОПЫТНЫЕ ХАРАКТЕРИСТИКИ ХРОМАТОГРАФИИ С ИСПОЛЬЗОВАНИЕМ МОЛЕКУЛЯРНЫХ СИТ:

D. M. W. Anderson, I. C. M. Dea and A. Hendrie, *Talanta*, 1971, **18**, 365.

**Резюме**—Предлежащий обзор дает химикам-аналитикам общее введение в хроматографию с использованием молекулярных сит, вид жидкостой хроматографии в котором разделение основывается на величине молекулы, хотя часто другие факторы также влияют на разделение. Методом можно пользоваться для разделения неорганических и органических молекул—мономерных и полимерных—в водных и неводных системах. Приведен обзор имеющихся в настоящее время молекулярных сит тип ксерогела и аерогела, придавая особенное внимание экспериментальным методам, больше чем механистическим и теоретическим взглядам. Критически отобраны ссылки на опубликованные данные с целью создать уравновешенный, вполне современный обзор, а также указать на общую аналитическую характеристику и возможность развития метода. В приложении приведены коммерческие источники молекулярных сит и эталонов.

## ВКЛАД В ОСНОВНЫЕ ПРОБЛЕМЫ КОМПЛЕКСОНОМЕТРИИ—XXIV. ОПРЕДЕЛЕНИЕ АЛЮМИНИЯ В ПРИСУТСТВИИ ОЧЕНЬ БОЛЬШИХ КОЛИЧЕСТВ МАРГАНЦА:

Rudolf Přibil and Vladimir Vesely, *Talanta*, 1971, **18**, 395.

**Резюме**—Разработан несложный метод комплексонометрического определения алюминия в присутствии больших количеств марганца. В этих определениях можно применять только триэтилентетраминтетрауксусную кислоту (ТТГА). В слабокислой среде ТТГА образует с алюминием двухъядерный комплекс $Al_2L$. Комплекс образуется почти немедленно при комнатной температуре. Избыток ТТГА оттитрируют сульфатом меди при pH 5,3, с использованием глицинкрезолового красного в качестве индикатора. Получены надёжные результаты при отношениях Mn:Al до больше чем 20. Тот же метод позволяет определять сумму Al + Fe. Очень большие количества кальция и магния не мешают определению.

## КАТИОНООБМЕННЫЕ ХАРАКТЕРИСТИКИ НЕКОТОРЫХ ЭЛЕМЕНТОВ В МУРАВЬИНОКИСЛИХ РАСТВОРАХ:

Mohsin Qureshi and Waqif Husain, *Talanta*, 1971, **18**, 399.

**Резюме**—Исследованы катионообменные характеристики 20 элементов на сильнокислой катионообменной смоле Дауекс 50 × 8 в средах, содержащих различные концентрации муравьиной кислоты и смесей муравьиной кислоты с водным раствором диоксана. Указаны и обсуждены проведимые разделения. Метод позволяет отделять количественно висмут от меди.

**Extraction with long-chain amines—IV. Separation and colorimetric determination of gold:** JIŘÍ ADAM and RUDOLF PŘIBIL, *Talanta*, 1971, **18**, 405. (Analytical Laboratory, Institute of Geological Sciences, Charles University, Prague 2, Albertov 6, and Analytical Laboratory, J. Heyrovský Polarographic Institute, Czechoslovak Academy of Sciences, Prague 1, Jilská 16, Czechoslovakia.)

**Summary**—A highly selective method, almost free from interferences for extraction of gold(III) from sulphuric acid into a chloroform solution of trioctylamine (TOA) is described. The yellow extract is then measured at 325–330 nm. Gold can be determined in the presence of platinum. The method has been applied to the determination of gold in waste dumps. A more sensitive method is based on reaction of the gold with diphenylcarbazide after the separation of the gold from the TOA-chloroform extract. The violet colour is stable and is measured at 560 nm.

**Spectrophotometric determination of ruthenium with 3,4-diaminobenzoic acid:** GILBERT H. AYRES and JAMES A. ARNO, *Talanta*, 1971, **18**, 411. (Department of Chemistry, The University of Texas at Austin, Austin, Texas 78712, U.S.A.)

**Summary**—Ruthenium(III) chloride and 3,4-diaminobenzoic acid, in aqueous solution at pH 4·0–4·5, react slowly at room temperature, but more rapidly when the mixture is heated, giving solutions that go through colour transitions from amber to purple-red; maximum absorbance of fully developed solutions occurs at 550 nm. The effects of heating temperature and time, pH, reagent concentration, and other variables have been studied. The system conforms to Beer's law; optimum concentration range, for measurement in 10-mm cells, is about 0·5–2 ppm of ruthenium. Interference from foreign ions, especially other platinum elements, is avoided by a distillation separation of ruthenium. The spectrophotometric mole-ratio and continuous-variation methods indicated the presence of complexes of 1:2 and 1:3 ruthenium-to-reagent stoichiometry. Elemental analysis of solid products isolated from solution confirmed the 1:2 reaction ratio. Several other *o*-diamines gave similar coloured solutions and reaction stoichiometry.

**Gas chromatography of metal chelates with carrier gas containing ligand vapour:** T. FUJINAGA, T. KUWAMOTO and S. MURAI, *Talanta*, 1971, **18**, 429. (Department of Chemistry, University of Kyoto, Kyoto, Japan.)

**Summary**—The vapour of the ligand was used as carrier-gas additive in the gas chromatography of metal chelates. The effect of trifluoroacetylacetone on non-symmetrical peaks was examined for $Be(TFA)_2$, $Al(TFA)_3$, $Cr(TFA)_3$, $Fe(TFA)_3$, $U(TFA)_4$ and $Th(TFA)_4$. All the chromatograms appeared quite normally especially those of $Th(TFA)_4$; $Fe(TFA)_3$ and $Th(TFA)_4$ were improved markedly by using the carrier gas containing ligand vapour.

## ЭКСТРАГИРОВАНИЕ АМИНАМИ С ДЛИННОЙ ЦЕПЬЮ—IV. ОТДЕЛЕНИЕ И КОЛОРИМЕТРИЧЕСКОЕ ОПРЕДЕЛЕНИЕ ЗОЛОТА:

JIŘÍ ADAM and RUDOLF PŘIBIL, *Talanta*, 1971, **18**, 405.

**Резюме**—Описан высокоизбирательный, почти свободный от мешающих влияний метод экстрагирования золота(III) из серной кислоты раствором триоктиламина (ТОА) в хлороформе. Светопоглощение желтого экстракта измеряют при 325–330 нм. Метод позволяет определять золото в присутствии платины, а был применен в определении золота в отбросах. Более чувствительный метод основан на реакции золота с дифенилкарбазидом после отделения золота от экстракта ТОА в хлороформе. Устойчивую фиолетовую окраску измеряют при 560 нм.

## СПЕКТРОФОТОМЕТРИЧЕСКОЕ ОПРЕДЕЛЕНИЕ РУТЕНИЯ 3,4-ДИАМИНО-БЕНЗОЙНОЙ КИСЛОТОЙ:

GILBERT H. AYRES and JAMES A. ARNO, *Talanta* 1971, **18**, 411.

**Резюме**—Хлорид рутения(III) и 3,4-диаминобензойная кислота в водном растворе при pH 4,0–4,5 реагируют медленно при комнатной температуре, но быстрее при нагревании смеси, с одновременной переменой цвета раствора от янтарного до пурпурнокрасного. Растворы с вполне проявленным цветом показывают максимум светопоглощения при 550 нм. Изучены влияния температуры и продолжительности нагревания, pH, концентрации реагента и других переменных Система повинуется закону Бёра; оптимальные предели концентрации для измерения в кюветках 10 ми—0,5–2 мкг/мл рутения. Влияние других ионов, особенно ионов платиновых элементов, избегнуто применением дистилляционного метода отделения рутения. Применение спектрофотометрических методов молярного отношения и непрерывной вариации показало присутствие комплексов с отношением рутений: реагент 1:2 и 1:3. Отношение реакции 1:2 подтверждено элементарным анализом твердих продуктов изолированных из раствора. Ряд других *о*-диаминов дали подобные окрашенные растворы и стехиометрию реакции.

## МЕТОД ГАЗОВОЙ ХРОМАТОГРАИИ ДЛЯ РАЗДЕЛЕНИЯ ХЕЛАТОВ МЕТАЛЛОВ С ИСПОЛЬЗОВАНИЕМ ГАЗА-НОСИТЕЛЯ СОДЕРЖАЩЕГО ПАРЫ ЛИГАНДА:

T. FUJINAGA, T. KUWAMOTO and S. MURAI, *Talanta*, 1971, **18**, 439.

**Резюме**—Пары лиганда использованы как присадка газуносителю в разделении хелатов металлов методом газовой хроматографии. Испытано влияние трифторацетилацетона на несимметрические пики $Be(TФА)_2$, $Al(TФА)_3$, $Cr(TФА)_3$, $Fe(TФА)_3$, $U(TФА)_4$ и $Th(TФА)_4$. Хроматограммы $Th(TФА)_4$, $Fe(TФА)_3$ и $U(TФА)_4$ улучшены использованием паров лиганда в газе-носителе.

**Cerimetric determination of dithionate and polythionate:**: V. R. NAIR and C. G. R. NAIR, Talanta, 1971, **18**, 432. (Department of Chemistry, University of Kerala, Trivandrum, Kerala, India.)

**Summary**—Ceric sulphate in a boiling strongly acidic medium oxidizes dithionate as well as tri- and tetrathionates quantitatively to sulphate. These anions may therefore be determined cerimetrically when they are present singly. A combination of the cerimetric method with other known methods (*e.g.* those of Kurtenacker, *etc*) is suggested for the analysis of mixtures of polythionates and dithionate.

**Crystal and molecular structure of zinc dithizonate:** KUMAR S. MATH and HENRY FREISER, *Talanta*, 1971, **18**, 435. (Department of Chemistry, University of Arizona, Tucson, Arizona, U.S.A.)

**Summary**—The structure of zinc(II) dithizonate has been determined by X-ray analysis. The co-ordination round the zinc is slightly distorted tetrahedral and the proximal phenyl rings have been shown to be almost coplanar with the chelate rings.

**Solvent extraction of the indium–Alizarin Red S chelate as its 1,3-diphenylguanidium salt:** MAKOTO OTOMO and KOICHI TONOSAKI, *Talanta*, 1971, **18**, 438. (Department of Chemistry, Faculty of Science, Hirosaki University, Hirosaki, Japan.)

**Summary**—The extraction of the 1,3-diphenylguanidium (DPG) salt of the indium–Alizarin Red S (ARS) chelate with n-butyl acetate has been investigated. The extracted species has an absorption maximum at 525 nm, and Beer's law is obeyed over the range from 0·3 to 2·8 ppm of indium. The molar absorptivity is $2{\cdot}65 \times 10^3$ l.mole$^{-1}$.mm$^{-1}$ at 525 nm. The 1:3 indium(III)–ARS chelate is extracted from aqueous solution in a 1:3 molar ratio with DPG. The infrared spectrum implies that the metal ion is probably chelated by the two phenolic oxygen atoms of ARS.

**Spectrophotometric determination of cadmium in nuclear-grade Zircaloy-2 after selective extraction with a liquid anion-exchanger:** G. GHERSINI and S. MARIOTTINI, *Talanta*, 1971, **18**, 442. (CISE, Via Redecesio, 12, 20090 Segrate (Milano), Italy.)

**Summary**—A method is presented for the spectrophotometric determination of cadmium in Zircaloy-2 at the tenths of ppm level. The method involves the extraction of Cd with tri-n-octylamine from 1*M* HCl, its separation from Fe(III) by scrubbing with tartaric acid, its recovery with 2*M* sulphuric acid, and the final development of the Cd-dithizonate colour. The method is simple and rapid, does not require any special instrumentation, and avoids the use of toxic reagents. Cadmium at the 0·3-ppm level has been determined with standard deviation 0·054 ppm.

## ЦЕРИМЕТРИЧЕСКОЕ ОПРЕДЕЛЕНИЕ ДИТИОНАТА И ПОЛИТИОНАТОВ:

V. R. Nair and C. G. R. Nair, *Talanta*, 1971, **18**, 432.

**Резюме**—Сульфат церия в кипящей сильнокислой среде окисляет дитионат и три- и тетратионаты количественно в сульфат. Поэтому можно определить эти анионы, если они присутствуют отдельно, церимет рическим методом. Комбинация церимет рического метода с другими знакомыми методами (на пример, методом Куртенакера и т.д.) предложена для анализа смесей политионатов с дитионатом.

## КРИСТАЛЛИЧЕСКАЯ И МОЛЕКУЛЯРНАЯ СТРУКТУРА ДИТИЗОНАТА ЦИНКА:

Kumar S. Math and Henry Freiser, *Talanta*, 1971, **18**, 435.

**Резюме**—Структура дитизоната цинка(II) определена методом рентгеновского анализа. Координация около цинка незначительно искривленная тетраэдрическая, а ближайшие фениловые кольца почти копланарны с кольцами хелата.

## ЭКСТРАКЦИЯ ХЕЛАТА ИНДИЯ С АЛИЗАРИНОВЫМ КРАСНЫМ S В ФОРМЕ ЕГО СОЛИ 1,3-ДИФЕНИЛГВАНИДИНИЯ:

Макото Отомо and Koichi Tonosaki, *Talanta*, 1971, **18**, 438.

**Резюме**—Исследована экстракция соли 1,3-дифенилгванидиния (ДФГ) хелата индия с ализариновым красным S (AKS) н-бутилацетатом. Экстрагированный комплекс показывает максимум светопоглощения при 525 нм, а Бера закон почитается в пределах 0,3 до 2,8 мкг/мл индия. Молярный коэффициент светопоглощения равен $2{,}65 \times 10^3$ л. моль$^{-1}$. мм$^{-1}$ при 525 нм. 1:3 хелат индия(III) с AKS экстрагируют из водного раствора с ДФГ в молярном отношении 1:3. Инфракрасный спектр указывает что ион металла может быть хелирован двумя фенолэ̀ными атомами кислорода в AKS.

## СПЕКРОФОТОМЕТРИЧЕСКОЕ ОПРЕДЕЛЕНИЕ КАДМИЯ В ЦИРКАЛОЙ-2 ЯДЕРНОЙ ЧИСТОТЫ ПОСЛЕ СЕЛЕКТИВНОГО ИЗВЛЕЧЕНИЯ ЖИДКИМ АНИОНООБМЕННИКОМ:

G. Chersini and S. Mariottini, *Talanta*, 1971, **18**, 442.

**Резюме**—Предложен метод спектрофотометрического определения кадмия в циркалой-2 при концентрациях меньше частей на миллион. Метод основан на извлечении кадмия три-н-октиламином из раствора 1*M* HCl, его отделении от Fe(III) промывкой винной кислотой, его регенерации с 2*M* серной кислотой и, конечно, на проявлении окраски дитизоната кадмия. Метод является несложным и быстрым, не потребует специального прибора и не использует токсические реагенты. Метод позволил определить 0,3 частей на миллион кадмия, с стандартной ошибкой 0,054 частей на миллион.

**Spectrophotometric determination of EDTA:** S. N. BHATTACHARYYA and K. P. KUNDU, *Talanta*, 1971, **18**, 446. (Nuclear Chemistry Division, Saha Institute of Nuclear Physics, 92, Acharya Prafulla Chandra Road, Calcutta-9, India.)

**Summary**—Both iron(III) and its EDTA complex in 0·1*N* $H_2SO_4$ medium absorb in the ultraviolet region of the spectrum, with absorption maxima at 305 and 258 nm respectively. EDTA can thus be determined by addition of excess of Fe(III) and measurement of the absorbance at the two wavelengths. An EDTA concentration as low as $\sim 10^{-6}M$ can be determined with a fair degree of accuracy. The method is free from interferences from most common metal ions, and is simple and rapid.

**A borax fusion technique for quantitative X-ray fluorescence analysis:** J. H. H. G. VAN WILLIGEN, H. KRUIDHOF and E. A. M. F. DAHMEN, *Talanta*, 1971, **18**, 450. (Twentey University of Technology, P.O. Box 217, Enschede, Holland.)

**Summary**—A borax fusion technique to cast glass discs for quantitative X-ray analysis is described in detail. The method is based on the "non-wetting" properties of a Pt/Au alloy towards molten borax, on the favourable composition of the flux and finally on the favourable form of the casting mould. The critical points of the technique are stressed, resulting in a method which could be carried out successfully by inexperienced workers. In general the method compares favourably in speed and accuracy with wet-chemical methods.

**The stability of gold solutions:** A. CHOW, *Talanta*, 1971, **18**, 453. (Department of Chemistry, University of Manitoba, Winnipeg 19, Canada.)

**Summary**—The stability of gold solutions was studied over the concentration range of 0·41–258 ppm at acidities ranging from 6*M* hydrochloric acid to pH 1, the solutions being stored in glass-stoppered borosilicate bottles. The best conditions were found to be 12–258 ppm solutions at pH 0–2, for which the loss of gold was 4% or less over a period of 400 days.

## СПЕКТРОФОТОМЕТРИЧЕСКОЕ ОПРЕДЕЛЕНИЕ ЭДТА:

S. N. Bhattacharyya and K. P. Kundu, *Talanta* 1971, **18**, 446.

**Резюме**—Железо(III) и его комплекс с ЭДТА в 0,1*N* $H_2SO_4$ поглощают в ультрафиолетовой области спектра, показывая максимумы светопоглощения при 305и 258 нм,соответственно. Поэтому можно определять ЭДТА добавлением избытка Fe(III) и измерением светопоглощения при этих двух длинах волн. Метод позволяет определять концентрации ЭДТА до $\sim 10^{-6}M$ с удовлетворительной точностью. Метод является несложным и быстрым, а большинство обыкновенных ионов металлов не мешают определению.

## МЕТОД ПЛАВЛЕНИЯ БУРОЙ ДЛЯ КОЛИЧЕСТВЕННОГО АНАЛИЗА МЕТОДОМ РЕНТРЕНОВСКОЙ ФЛУОРЕСЦЕНЦИИ:

J. H. H. G. van Willigen, H. Kruidhof and E. A. M. F. Dahmen, *Talanta*, 1971, **18**, 450.

**Резюме**—Описан в детале метод плавления бурой для получения стеклянных дисков, применимых в рентгеновском флуоресцентном анализе. Метод основан на «несмачивании» Pt/Au сплава расплавленной бурой, на оптимальном составе флюса и, конечно, на оптимальной форме литейной формы. Подчеркнуты критические точки метода в результате чего даже неопытный оператор может хорошо пользоваться методом. Быстрота и точность метода хороши в сравнении с классическими химическими методами.

## УСТОЙЧИВОСТЬ РАСТВОРОВ ЗОЛОТА:

A. Chow, *Talanta*, 1971, **18**, 453.

**Резюме**—Изучена устойчивость растоворов золота в области концентраций 0,41–258 частей на миллион при кислотностях от 6*M* соляной кислоты до pH 1, при хранении растворов в склянках, закрытых стеклянными пробками. Оптимальные условия представляют собой растворы 12–258 частей на миллион при pH 0–2. В этом случае потеря золота была меньше чем 4 % в течение 400 дней.

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