THE ANALYST

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Two Large Molecules and their Structures

THE bigger any molecule, the harder is it to determine its exact elementary composition, and consequently its exact molecular weight, by the recognised methods of classical analysis. These methods by themselves could never have made it possible for Ryle, Sanger, Smith and Kitai to state, as they have recently done (*Biochem. J.*, 1955, **60**, 541), "It may be safely concluded that the molecular weight of insulin is 5734." Not 5733 or 5735, let it be noted, but 5734! This conclusion is firmly derived from the proof, obtained by the ten years' brilliant work of Dr. F. Sanger and his colleagues at Cambridge, that the empirical formula of insulin is $C_{254}H_{377}O_{75}N_{65}S_6$. The formula, be it noted, is not in any way based on the conventional procedures for determining the percentages of carbon, hydrogen, nitrogen and sulphur in specimens of the pure compound. The formula was established by breaking down the protein into a determinate number of identifiable amino-acid units and then showing how they were joined together. A knowledge of structure was a condition precedent to accurate knowledge of composition—exactly the reverse of what has so often occurred during the pioneer work on structure that has characterised the growth of organic and inorganic chemistry alike.

Knowledge of structure has to precede knowledge of composition; this may be one reason, although it is not the only one, why work of this kind is almost never credited as a triumph to analytical chemistry and why its successful executants are not generally regarded, and do not regard themselves, as analytical chemists. The same consideration applies to the remarkable achievement of Todd, Hodgkin, Lester Smith and their colleagues in consummating seven years' work by announcing (*Nature*, 1955, **176**, 325, 328—August 20th) the structure of cyanocobalamin (vitamin B_{12}), complete in everything but one or two trivial details. True, the vitamin contains a mere 183 atoms and has the barely "macro" molecular weight of 1357, so that elucidation of its structure might appear almost child's play compared with the problems set by insulin, which contains 48 amino-acid residues and 777 atoms in each molecule!

This was far from so. All the amino acids constituting insulin were known and identifiable: there appear to be seventeen of them, arginine, *iso*leucine, lysine, proline and threonine occurring only once each in the molecule, all of the other twelve twice or more often. This was a bad enough knot to unravel, but it did not involve the special difficulties arising with vitamin B_{12}, from which the fragmented portions were chemically diverse, some being previously unknown—at any rate in natural products—and most being hard, if not impossible, to characterise by conventional chemical procedures.

In any event, to establish exactly the empirical formulae and the molecular weights of both insulin and cyanocobalamin, it was necessary first to map their structures. This is apparently held to be no part of analytical chemistry, even though it be indispensable to the attainment of an analytical answer. We doubt if this view is due to anything more than historical "accident" and convention. Certainly there seems little logic in the assertion that to determine the exact stoicheiometric equivalent of an organic acid, without even necessarily knowing or thereby ascertaining its structure, is a process of chemical analysis, whereas to distinguish between molecular weights of 1356 and 1357 is pure organic chemistry and not analysis at all!

However, we have no wish to indulge in territorial disputes or to do more than hint to our colleagues working on molecular structure in the domain of "pure" chemistry that there is at least a case for considering *The Analyst* as a means of publishing their quantitative findings. Rather would we take this occasion, when two triumphs of British chemistry have

been announced within one month, of noting the methodological implications—and importance—of certain facts. It is surely something to ponder over that distinguishing between structures differing by, say, a couple of hydrogen atoms in molecules with weights well over 1000 has involved the use of many recently developed techniques as adjuvants to long-established methods. Indeed, and for reasons adumbrated above, this has perhaps been more striking in the work of cyanocobalamin than in that on insulin. For the latter it was a painstaking application of paper chromatography and, particularly, of electrophoresis, that provided the main weapon for the attack on what only a few years ago would have been thought an unassailable stronghold.

The vitamin-B_{12} studies, on the other hand, have involved the use not only of column and paper chromatography, and of electrophoresis as well, but also of solvent partition methods, of ultra-violet and infra-red spectroscopy, of X-ray crystallography and—to many the most surprising ally of all—of cybernetics. It has been insisted that the immensely complicated and laborious computations necessary for interpreting the crystallographic results in terms of intramolecular structure could never have been done in the time taken—if indeed they could ever have been done at all—without the use of electronic computers. For the analyst, however, all is manipulative grist to his experimental mill. Accustomed as he has been to use the results obtained by procedures as disparate as infra-red spectroscopy and biological assays with the South African clawed toad, he will have no hesitation about continued collaboration with colleagues working in other branches of science, the more heartened to do so by such shining examples as these two recent fine achievements of British chemistry.

A. L. B.

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

EXTRAORDINARY GENERAL MEETING

An Extraordinary General Meeting of the Society was held in London on October 5th, 1955, the President occupying the Chair.

The following Resolution was proposed and carried: "That Article 47 of the Articles of Association of the Society be altered by deleting the word 'three' in the fifth line thereof and substituting therefor the word 'six'."

Experience in recent years has shown that it is not always possible to have the Society's accounts prepared, audited and approved within the three months at present specified in the Articles of Association, and the effect of this new Resolution is to give additional time for their preparation.

ORDINARY MEETING

An Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, October 5th, 1955, in the meeting room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the President, Dr. K. A. Williams, F.R.I.C., A.Inst.P., M.Inst.Pet.

The following papers were presented and discussed: "The Colorimetric Determination of Phosphorus in Steel and Copper-base Alloys," by W. T. Elwell, F.R.I.C., and H. N. Wilson, F.R.I.C.; "The Determination of Small Amounts of Carbon in Steel by Low-pressure Analysis," by R. M. Cook, A.Met., B.Sc. (Eng.), and G. E. Speight, A.Met., B.Sc., F.R.I.C.; "The Determination of Small Amounts of Sulphate by Reduction to Hydrogen Sulphide, and Titration with Mercuric or Cadmium Salts using Dithizone as Indicator," by E. E. Archer, B.Sc.

SCOTTISH SECTION

An Ordinary Meeting of the Section was held at 7.15 p.m. on Friday, September 30th, 1955, at The Royal Technical College, Glasgow. The Chair was taken by Dr. F. J. Elliott.

The subject of the meeting was "The Determination of Traces of Lead." After an introductory talk by N. L. Allport, F.R.I.C., the following papers were presented and discussed: "Lead in Biological Materials," by S. L. Tompsett, B.Sc., Ph.D., D.Sc., F.R.I.C.; "The Determination of Lead by Square-wave Polarography," by D. J. Ferrett, M.A., D.Phil.

WESTERN SECTION

THE summer Meeting of the Section was held in Gloucester on Friday and Saturday, May 20th and 21st, 1955. The Chair was taken by the Chairman of the Section, Mr. H. J. Evans, B.Sc., F.R.I.C.

At 10.30 a.m. on Saturday, May 21st, at the Technical College, Gloucester, Dr. Keith Morgan addressed the meeting on "The Role of Iodine in Analytical Chemistry" (see summary below).

THE ROLE OF IODINE IN ANALYTICAL CHEMISTRY

DR. KEITH MORGAN said that it might be thought that the properties of iodine that made it important in analytical chemistry would be self-evident, but this was not so. Iodine, a mild oxidising agent, was a volatile solid, difficult to purify and sparingly soluble in water. It was hardly surprising that iodine itself was rarely the standard in iodimetry; further, the use of other reagents containing iodine did not entirely avoid the restrictions imposed by these characteristics.

The ease with which iodine could be determined had great practical significance, but the simplicity of the titrations sometimes obscured the concomitant limitations. Arsenite solutions were not always convenient to use, nor were they readily prepared in a state of purity sufficient for use as a standard. Sodium thiosulphate crystallised as a hydrate and although its solutions were readily standardised, on standing they decayed. The use of both these reagents was circumscribed by severe pH restrictions. The end-point in such titrations could be marked with distinction by starch, but the paralysing effect exerted by the colloidal solution on a hitherto homogeneous reaction was only too well known. The nature and composition of the starch was of considerable importance.

The significance of iodine was such that it overcame these inhibitions. Accordingly one had to seek some compelling reason to justify its unique nature. A practical assessment of this was given by the value of the iodide - iodine redox potential, **—0·536** volt. This value was not high (which meant that the iodide - iodine equilibrium was readily affected by such extraneous factors as autoxidation, light and hydrogen-ion concentration), and was considerably smaller than those associated with the familiar analytical oxidising agents. However, iodine was capable of oxidising thiosulphate to tetrathionate (—0·08 volt). It was in the coupling of these reactions—the oxidation of iodide to iodine and the subsequent oxidation of thiosulphate by iodine—that the analytical importance of iodine lay. The picture was not yet quite complete. Mild reagent though iodine was, it was potentially capable of exhibiting those reactions with thiosulphate and tetrathionate that were shown by the more potent oxidising species—oxidation to sulphite (—0·40 and —0·51 volt, respectively). That these reactions did not occur to any appreciable extent was to be ascribed to a prohibitive activation energy; to a happy redox potential iodine added a fortunate series of potential-energy curves. The form adopted by the potential-energy curve, or in other words the mechanism, of the iodine - thiosulphate reaction could not be without interest, and indeed importance, for its application to analysis. By using a constant-flow technique it has been found possible to study this very fast reaction (Awtrey, A. D., and Connick, R. E., *J. Amer. Chem. Soc.*, **1951**, **73**, **1341**). On the iodine being mixed with thiosulphate, the colour of the tri-iodide ion was removed, only to reappear again in the observation tube; a similar effect was to be noticed in the reappearance of the starch - iodine colour near the end of a thiosulphate titration. It was suggested that an intermediate, S_2O_3I', was formed, which could either dissociate, react with more thiosulphate or react with itself—

$$S_2O_3I' \rightleftharpoons S_2O_3'' + I^{\cdot} \qquad 2S_2O_3I' \rightleftharpoons 2S_2O_3' + I_2$$

$$S_2O_3'' + S_2O_3I' \rightarrow S_4O_6'' + I' \qquad 2S_2O_3' \rightarrow S_4O_6''$$

$$I' + I^{\cdot} \rightleftharpoons I_2$$

In certain circumstances a fourth reaction path might be followed: should the ratio of thiosulphate to iodine atoms be less than unity (the situation, it may be noted, present during a titration) a slow side-reaction resulted in the oxidation of some S_2O_3I' to sulphate. It was fortunate that the magnitude of this was normally insignificant.

There was a second group of iodine reactions of considerable importance, although subsidiary to the iodide - iodine reaction system. These were the reactions of the

oxygenated iodine species, reactions that were at once complex and chemically pure. The latter derived from the electrode potentials, which showed firstly, that in acid solution there was no stable state intermediate between iodine and iodate; and secondly, that iodate, unlike chlorate, had no tendency to disproportionate.

Two reactions of this group about which a considerable amount was known were the iodate - iodide reaction and the disproportionation of hypoiodite. The iodate - iodide reaction was the simpler and it had been possible to interpret the observed kinetics in the form of a mechanism, each step of which was sufficiently simple to occur during the lifetime of a molecular collision (Morgan, K. J., Peard, M. G., and Cullis, C. F., *J. Chem. Soc.*, 1951, 1865)—

$$\begin{aligned} HIO_3 &\rightleftharpoons IO_2^{\bullet} + OH' \\ IO_2^{\bullet} + 2I' &\rightarrow I^{\bullet} + 2IO' \\ IO_2^{\bullet} + I' &\rightarrow IO^{\bullet} + IO' \\ IO^{\bullet} + I' &\rightarrow I^{\bullet} + IO' \\ IO' + H^{\bullet} &\rightleftharpoons HIO \\ HIO + H^{\bullet} &\rightleftharpoons H_2IO^{\bullet} \ (= I^{\bullet}) \\ I^{\bullet} + I' &\rightleftharpoons I_2 \end{aligned}$$

The reverse reaction, the hypoiodite disproportionation, was much more complex, and it appeared that there were several competing reactions. The observed kinetics were thus a conglomerate from which at least four distinct expressions had been isolated. A comparison of these led to the suggestion that the initial reaction lay between IO′ and HIO to give an intermediate HI_2O_2', which could subsequently react with every other species present: OH′, IO′, I′ and HIO (Morgan, K. J., *Quart. Rev. Chem. Soc.*, 1954, **8**, 123)—

$$\begin{aligned} HIO + IO' &\rightleftharpoons HI_2O_2' \\ HI_2O_2' + OH' &\rightarrow I' + IO_2' + H_2O \\ HI_2O_2' + IO' &\rightarrow I' + IO_2' + HIO \\ \textit{or} &\rightarrow I_3O_2' + OH' \\ HI_2O_2' + I' &\rightarrow I_3O' + OH' \\ HI_2O_2' + HIO &\rightarrow I_3O_2' + H_2O \\ I_3O' + IO' &\rightarrow I_3O_2' + I' \\ I_3O_2' &\rightarrow IO_2^{\bullet} + 2I' \\ IO_2^{\bullet} + OH' &\rightleftharpoons HIO_3 \\ IO_2' + HIO &\rightarrow HIO_3 + I' \end{aligned}$$

It would be realised that our ability to assign mechanism was restricted. There was an important truism that asserted that no reaction could occur without a mechanism. To discuss reactions in terms of redox potentials and activation energies took analysts one step nearer to the essential answer, but until it was known how and why molecules reacted, their knowledge of reaction was meagre. It was the answers to these questions that would permit the precise assessment of the significance of the role of iodine in analytical chemistry.

MIDLANDS SECTION

An Ordinary Meeting of the Section was held at 7 p.m. on Tuesday, September 13th, 1955, at the University, Edgbaston, Birmingham, 15. The Chairman of the Section, Mr. J. R. Leech, J.P., presided.

The following papers were presented and discussed: "The Use of the Mass Spectrometer in Analysis," by J. C. Robb, B.Sc., Ph.D., A.R.I.C.; "Microwave Spectroscopy," by J. Sheridan, M.A., D.Phil.

The Complexones and their Analytical Application

By G. SCHWARZENBACH

(*Presented at the meeting of the Society on Wednesday, February 2nd*, 1955)

It can be deduced theoretically that certain aminopolycarboxylic acids with nitrogen in the branching positions of the molecule and –COOH as terminal groups should have extraordinary properties as complexing agents. Indeed, such substances are able to form chelates with almost any metal cation, even with lithium and sodium. Ethylenediaminetetra-acetic acid (EDTA) has the best possible structure, its anion being sexadentate and the chelate rings formed with the metal being five-membered. The formation of complexes with EDTA can be used as a basis for titration procedures. The corresponding titration curves [pM = —log (metal-ion concentration) against the volume of complexing agent added] can be calculated theoretically. It can also be shown that there will be a sharp jump of pM at the end-point, corresponding to the jump of pH during the titration of the hydrogen ion by alkali. This jump can be made visible with a suitable metal indicator. The complexones can also be used to buffer metal ions and as masking agents during gravimetric analysis. New separation procedures are made possible, not only through differences in the stabilities of the various metal complexes, but also through differences in the rates of their formation and dissociation.

Let me first express my thanks for the kind invitation to give this lecture to the Society for Analytical Chemistry. I am very pleased to come to London and have been looking forward to spending this evening among you. Certainly, in choosing the lecture theatre of the Royal Institution, you have selected a very good and very famous hall for the occasion. I must confess that I feel very unworthy to speak from the same platform from which Sir Humphrey Davy, Michael Faraday, John Tyndall, James Dewar and many other famous scientists have lectured during the past decades. There is hardly a chemist or a physicist for whom I have a greater admiration than for Davy or Faraday. Let me begin my lecture in this historic hall with an idea that was expressed about 140 years ago by the first chemist of this Institution. Davy worked, around 1810, with the so-called "pungent gas" discovered in 1774 by Scheele, and was able to give evidence that this gas was a chemical element, elementary chlorine as it is known to-day. This finding was most unexpected, because chlorine has strongly acidic properties. A solution of chlorine reacts with acid - base indicators, turning them red before they are oxidised and destroyed, and chlorine can be neutralised to a salt, a mixture of hypochlorite and chloride, as we know to-day. Acidity, however, was believed in those early days to be always connected with a content of oxygen: the German word "*Sauerstoff*" still gives evidence of that belief, which originated from Lavoisier. Davy's findings that the pungent gas did not contain any oxygen and that one of the strongest acids was just a combination of hydrogen and chlorine—called hydrochloric acid to-day—made it necessary to look for a new explanation for acidity, and, in 1814, Davy expressed the idea that acidity probably was not due to any elementary substance, such as oxygen, but was due to a specific arrangement of the atoms within the molecule.[1]

This idea proves Davy's originality and his ability to comprehend quickly the fundamentals of the phenomenon. His idea was not adopted by his contemporaries. For more than another 100 years an elementary substance was believed to be responsible for the phenomenon of acidity—no longer oxygen, but hydrogen. But during the past three decades it has become generally recognised that Davy's idea is much closer to the facts. Lewis reached the conclusion that a certain specific arrangement of electrons was essential.[2] It is my personal belief that it would be still more general to state that acidity and basicity are due to the co-ordination tendency of molecules and ions in a solution. The proton has a particularly strong co-ordination tendency, and therefore is most often the cause of the acidic properties of solutions. But the various metal cations also have co-ordination tendencies similar in nature to the electrophilic properties of the proton. I should like to show you to-day that ordinary metal cations, such as copper ions, nickel ions and even the ions of alkaline-earth metals, can in fact react with indicators in a manner analogous

to hydrogen ions, and can be titrated, just as hydrochloric acid is titrated, in the course of complexometric titrations.[3]

The co-ordination tendency of metal cations is probably the most important phenomenon, at least for the analytical chemist. The majority of analytical procedures for separating and determining metals are based on the co-ordination tendency of the metal cations. Let us first consider an aqueous solution of a simple metal salt. May I remind you that such solution contains the aquo complexes of the metal cation in question. The co-ordination tendency causes the metal cation to be surrounded by a sheath of water molecules. These ligands can then be replaced by others, and Table I gives a survey of various ligands.

TABLE I

COMPLEXING AGENTS

A. *Simple unidentate ligands*—NH_3, CH_3COO', F', CNS', CN', etc.
Complexes are mononuclear and charged: $Cu(NH_3)_4^{\cdot\cdot}$, $AlF_6^{\cdot\cdot\cdot}$, etc.
Complex salts are soluble.

B. *Lattice-building ligands, multidentate*—N_2H_4, OH', CO_3'', PO_4''', etc.
Complexes are infinite lattices: $\{Ni(N_2H_4)_3SO_4\}$, $\{Al(OH)_3\}$, etc.
Complexes are insoluble precipitates.

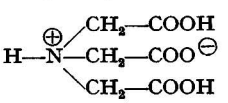
C. *Chelate ligands, multidentate*—8-Hydroxyquinoline, polyamines, polycarboxylic acids, aminocarboxylic acids, etc.

Complexes are mononuclear and—

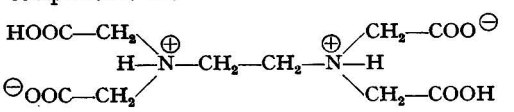
(1) when uncharged are often insoluble in water but soluble in organic solvents: aluminium 8-hydroxyquinolinate, nickel-dimethylglyoxime complex;

(2) when charged are highly soluble in water but insoluble in organic solvents: tartrates, citrates, complexonates.*

* Analytically the most important complexones are—



Nitrilotriacetic acid; NTA; H_3X



Ethylenediaminetetra-acetic acid; EDTA; H_4Y

Adding ammonia to a solution containing metal ions often leads to the formation of ammine complexes, as with copper, with which the deep blue coloured tetrammine complex is formed from the slightly coloured aquo complex. A ligand of this kind is called simple and unidentate because it can satisfy only one single co-ordination site of a metal cation. Other simple and unidentate ligands are the halogen ions, the acetate ion, and many more. On addition of sodium acetate to a solution of a copper salt, a reaction similar to that occurring with ammonia takes place, the tetra-acetato complex, $Cu(CH_3COO)_4''$, being formed; this also has a deep, more greenish blue colour. Simple unidentate ligands form, with very rare exceptions, charged complex ions; for instance, the positively charged ammine and the negatively charged acetato complexes. No precipitation occurs during the process, the corresponding complex salts with rare exceptions being soluble substances.

If now we replace the ammonia molecule by a molecule of hydrazine, we have the bidentate ligand, $H_2N–NH_2$. Each of the two amino groups of this ligand naturally is able to satisfy a co-ordination position of a metal cation; the whole molecule of hydrazine can therefore satisfy two sites. But these two sites cannot be on the same individual metal cation. The three-membered chelate ring that this would entail would not be strain-free and therefore cannot be formed. Hydrazine adds much more readily to two individual metal cations, tying them together, and because each metal cation adds as many hydrazine molecules as there are co-ordination sites, an infinite three-dimensional polynuclear complex is produced. I should therefore like to call hydrazine a lattice-building bidentate ligand. Complex formation leads to precipitation in this case. Hydrazine complexes are insoluble substances, such as, for instance, nickel hydrazine sulphate, $Ni(N_2H_4)_3SO_4$.[4] The lattice structure formed by hydrazine and a metal cation naturally must be charged, and anions have to be taken into the interior of this lattice. But sulphate ions are not essential for the

formation of the lattice. Sulphate can easily be replaced by other anions, and these hydrazine complexes are extremely good anion exchangers.

I am convinced that the majority of precipitates that are so important in analytical chemistry are held together by co-ordination bonds. The hydroxyl ion, for instance, is another bidentate, or possibly terdentate, ligand, again a lattice-forming ligand, producing precipitates, such as aluminium hydroxide. Again, of course, this ligand, OH, is unable to satisfy two or more co-ordination sites on the same individual metal cation, because this, too, would result in the formation of a strained (two-membered) chelate ring. Double bonds like this do not seem to occur in the chemistry of metals. Again, therefore, the hydroxyl ions build up a lattice.[5] Because of the negative charge of the ligand, it is not necessary this time to take other anions from the solution into the interior of the framework.

Other lattice-building ligands are the carbonate ion, the phosphate ion and so on; in fact, all the ordinary, common inorganic precipitating agents that are used so frequently in gravimetric inorganic analysis.

Another series of bi- and terdentate ligands, more generally termed multidentate ligands, are the chelating agents. These consist of a skeleton, most often formed of carbon atoms, and this skeleton carries more than one ligand atom, usually oxygen or nitrogen, which can be donated to the metal cation. The situation has to be such that it is possible to donate several ligand atoms of the complexing agent to the same individual metal cation, so forming strain-free chelate rings. Such rings are strain-free when they are at least five-membered, as in the example of ethylenediamine. In such a case it is much more probable that the various ligand atoms of the complexing agent add to one and the same individual metal cation than that they add to two or more metal cations. Chelating agents therefore do not tie metal cations together—they are not lattice-building. But in spite of this, we may get precipitates with this kind of ligand. This very often happens when the chelating agent has a negative charge of such a magnitude that the requirements of co-ordination of the metal cation lead to the formation of an uncharged complex. A good example is 8-hydroxyquinoline, the anion of which is a bidentate ligand with a single negative charge. If 8-hydroxyquinoline is combined with aluminium, which has six co-ordination sites, three 8-hydroxyquinoline ions are needed; this just compensates the three positive charges of the aluminium ion. The uncharged complex molecule so formed behaves, as far as solubility is concerned, very much like an organic substance, being insoluble in water, but soluble in organic solvents. Chelating agents of this kind are precipitating agents also, and are frequently used by analytical chemists in gravimetric procedures. But they are extracting agents as well, very often used for separation purposes or during a spectrophotometric determination, when the "inner" complex has strong absorption bands, the intensity of which can easily be measured.[6,7,8,9]

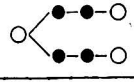
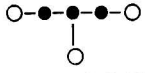
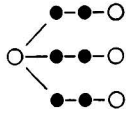
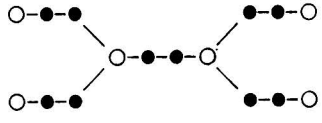
Very often, however, the charge on the metal cation is not compensated by the charge on the chelating agent needed for co-ordination, and the complex formed is a positive or negative ion. Usually in these circumstances there is no precipitation, just as in the case of simple unidentate ligands. In this class of complexing agent we have the polyamines, forming chelate cations, and tartaric acid or citric acid, forming chelate anions. To this group we have added some new substances, the "complexones," which have been described in twenty-seven different articles by the author and his co-workers in *Helvetica Chimica Acta* from 1945 to 1955.

Soluble complexes of groups A and C2 (Table I) have already been in analytical use for a long time; these uses are as follows—

1. The ligands may be used as masking agents. For instance, the cyanide ion, a simple unidentate ligand, is used to mask copper when cadmium sulphide is precipitated, and tartrate is used for masking aluminium when iron is precipitated as sulphide.
2. Coloured soluble complexes may be used during the photometric determination of the metallic constituent; for instance, thiocyanate or *o*-phenanthroline is used for the determination of iron.
3. Complexing agents forming soluble complexes may also be used to stabilise a particular oxidation state of a metal. For instance, *o*-phenanthroline favours the bivalent state for iron and raises the oxidation potential of Fe^{III}/Fe^{II} considerably.
4. Complexing agents have, however, very rarely been used as titrants. This field of analytical application has in fact been opened by the complexones, which are used to-day for the volumetric determination of a great number of metals.

TABLE II

LOGARITHMS OF STABILITY CONSTANTS OF AMMINE COMPLEXES AT 20° C AND AN IONIC CONCENTRATION OF 0·1

Complexing agent (○ = basic nitrogen, ● = carbon)	Nickel						Zinc				Copper			
Ammonia ○	2·8	2·2	1·7	1·2	0·7	−0·01	2·3	2·3	2·4	2·1	4·1	3·5	2·9	2·1
Ethylenediamine ○-●-●-○	7·7		6·5		5·1		5·9		5·2		10·7		9·3	
1:3-Diaminopropane ○-●-●-●-○	6·4		4·3		1·2						9·8			
Diethylenetriamine 	10·7			8·3			8·9		5·5		16·0		5·3	
1:2:3-Triaminopropane 	9·3			6·5			6·8		4·3		11·0		9·0	
Triethylenetetramine ○-●-●-○-●-●-○-●-●-○	14·0						12·1				20·4			
β β′β″-Triaminotriethylamine 	14·8						14·7				18·8			
Pentaethylenehexamine 	19·3						16·2				22·4			

These complexones have some unique properties. They differ in the following respects from the other complexing agents listed under A and C2 in Table I—

1. Complexones form complexes of a specially high stability; only a few cyano complexes are more stable than the ethylenediaminetetra-acetic acid (EDTA) complexes.
2. Complexones are universal complexing agents. They are capable of associating with the cations of almost any metal; the alkaline-earth cations are strongly bound and there is an easily detectable tendency to bind even lithium and sodium. It is because of this universality that these agents have been given the name "complexones."
3. The complexes of the complexones have a very simple composition. Usually only the 1 to 1 complex is important, consisting of one molecule of the complexing agent and a metal cation. The simple stoicheiometry of formation of the complex is especially important when the reaction is to be used as a basis for a titration procedure.

From the experience of complex formation that has been gained during the last 15 years by elucidating the equilibria between the various metal cations and their complexes, we can understand to-day why the anions of certain aminopolycarboxylic acids are such unique complexing agents. The high stability of their complexes is produced by the "chelate effect." The universality of their complex formation is due to the ionic carboxylate groups of the agents. The simple stoicheiometry of the complex formation finally comes from the ability of these agents to furnish ligand atoms for all or nearly all co-ordination sites of the various metal cations.

The "chelate effect" is the stability increase brought about by chelation. This is demonstrated by Table II, in which the stability constants[10] of some complexes with ammonia and polyamines are given. The values are the logarithms of the stability constants. The numbers in the first line are therefore proportional to the free energy gained during the replacement of the first, second, third (and so on) water molecule of the aquo complex by ammonia. Now, if we take one molecule of ethylenediamine instead of two molecules of ammonia, the free energy gained during the addition is proportional to the number shown in the second line, and it can be seen that this energy is larger than the sum of the free energies gained during the addition of two molecules of ammonia. Such an increase in stability is observed with every metal. It is sometimes as large as three units, as in the case of copper. On average the stability increase due to the replacement of two ammonia molecules by one of ethylenediamine is about two units. It is very difficult to give an explanation for this "chelate effect," which accounts quantitatively for the stability increase. However, it is easy to understand qualitatively that a chelating agent binding the metal cation at two or more points will be held very much better than a unidentate ligand. It is important to recognise that the formation of a five-membered chelate ring gives rise to more stable complexes than the formation of a six-membered or even larger ring system. Our knowledge of four-membered chelate rings is still scanty, but most probably they are inferior to the five-membered ring because they are not completely free of strain. Three-membered chelate rings do not form, as mentioned above in the paragraphs on hydrazine, which is not a chelating agent but a lattice-forming bidentate ligand. The lower lines of Table II provide evidence that a triamine capable of forming two condensed five-membered chelate rings is again much superior to three individual molecules of ammonia. Again, of course, the ring system formed during chelation has to be free from strain, which is not the case when 1 : 2 : 3-triaminopropane is used as a complexing agent. Very stable complexes are produced with tetramines, such as $\beta\beta'\beta''$-triaminotriethylamine or triethylenetetramine. Finally, with the hexamine "penten," the highest stability constants have been observed.[11] The stability increase—in other words, the "chelate effect"—amounts to as much as a factor of 10^{10} for the nickel and copper "penten" complexes. As we proceed from ammonia to the diamine, triamine, tetramine and hexamine, the stoicheiometry of formation of the complex becomes simpler and simpler. The metal cations combine only with one single "tren" or "trien" molecule; and the formation of a bis-"tren" complex can hardly be detected. In the case of "penten" there is no reason for the metal to require a second molecule of the complexing agent, because the first molecule in the complexes of zinc, copper or nickel with "penten" has already occupied all the available co-ordination sites of the metal atom.

These polyamines are excellent complexing agents, but they are not universal. They will combine preferentially with cobalt, nickel, copper, zinc, cadmium, mercury and so on, the cations that tend to bind the basic nitrogen of ammonia better than the basic oxygen of water. In order to produce universal complexing agents, we must have oxygen ligand

atoms in the chelating agent. Then, during complex formation, the oxygen of the water molecules in the solvated cations will be replaced by the oxygen ligand atoms of the complexing agent, which of course will be a very nearly universal reaction. What kind of oxygen should be used? Alcoholic hydroxyl groups are not very good ligand atoms and the metal cation will not have much tendency to unite with these instead of with water. Polyalcohols are not very powerful, but they are, in fact, almost universal complexing agents. Negatively charged phenoxide oxygen is an excellent ligand, but it is necessarily attached to an aromatic nucleus, and it is difficult to construct a molecule with several phenolic groups in such a position that they can all combine with the same metal cation. The next possibility is the oxygen of the carboxylate group. The acetate ion, a simple unidentate ligand containing this group, does not form very stable complexes. However, the complex formation is very nearly universal. There is hardly a metal cation that does not combine to some extent with acetate.[12] Every analytical chemist knows that calcium sulphate and lead sulphate can be solubilised with ammonium acetate, and the colour changes of nickel, copper or cobalt solutions when acetate is added prove that complex formation also takes place with these heavy-metal cations. If this weak, but universal, complex-forming tendency of the group –COO′ is enhanced by the "chelate effect," we shall produce strong complexing agents that will react generally with practically all metal cations, a property shown already by tartrate and citrate.

In order to accomplish such an enhancement, carboxylate groups, –COO′, have to be attached to a skeleton of carbon atoms. In doing so, however, we find that the only possibility of forming a five-membered chelate ring with two –COO′ groups is by the direct linking of two such groups, and we obtain the oxalate ion thereby. But oxalate contains two carboxylate groups only and there is no possibility of attaching a third and a fourth ligand atom. If we want to introduce further donors, the carboxylate groups cannot be directly linked together. With malonate and succinate, however, the chelate rings formed are no longer five-membered, and for this reason they are not very effective complexing agents.

Now, it has been found that the introduction of basic nitrogen atoms in a chain of carbon atoms connecting two carboxylate groups makes a further considerable increase in complex stability.

The anion of glutaric acid is not much better as a complexing agent than is acetate itself. By replacing the central $>CH_2$ group by $>NH$, we get iminodiacetic acid, which, in contrast, is a very good and general complexing agent.[13] Although this central basic nitrogen is only a very weak donor, say for calcium and lead, it helps to bring the two carboxylate groups into the correct position and thereby greatly enhances the stability of the complex. After having recognised the fact that even a weak donor atom in the correct position in a chain connecting two strong or fairly strong ligand groups has considerable effect on complex stability, it is easy to understand the principles by which the complexone substances are constructed. Nitrogen atoms can be used as links between carboxylic acid groups. They have to be in such a position that five-membered chelate rings can be formed during the complex formation, which means that these basic nitrogen atoms have to be in the α-position to the carboxyl. The best imaginable constitutions, therefore, are nitrilotriacetic acid and ethylendiaminetetra-acetic acid (for structures, see Table I).

TABLE III

LOGARITHMS OF STABILITY CONSTANTS OF 1 TO 1 ZINC COMPLEXES AT 20° C AND AN IONIC CONCENTRATION OF 0·1

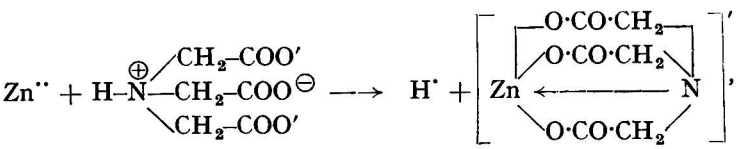
Ammonia	NH_3	2·27
Aminoacetate	$NH_2–CH_2–COO'$	4·80
Iminodiacetate	$NH(CH_2–COO')_2$	7·77
Nitrilotriacetate	$N(CH_2–COO')_3$	10·45
Ethylenediaminetetra-acetate	$CH_2–N(CH_2–COO')_2$ $\vert$ $CH_2–N(CH_2–COO')_2$	16·12

Table III shows the increase of complex stability when the hydrogen atoms of ammonia are replaced successively by acetate groups. The very much increased complex-forming ability of nitrilotriacetate in comparison with that of ammonia can be demonstrated by a simple experiment. If ammonium chloride is added to a solution of zinc sulphate, the following reaction takes place—

$$Zn^{\cdot\cdot} + NH_4^{\cdot} \rightarrow H^{\cdot} + Zn(NH_3)^{\cdot\cdot} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

A 0·1 *M* solution of zinc sulphate and a *M* solution of ammonium chloride are used and methyl red is added as indicator. Both solutions should have a pH value of about 5. If the pH is lower, a drop of dilute ammonium hydroxide solution is added. On mixing the two solutions, a drop in pH to 4·2 takes place and can easily be observed by the colour change of the indicator.

In a second similar experiment, the solution of ammonium chloride is replaced by a neutral solution of 0·1 *M* sodium nitrilotriacetate, again of pH 5. If this solution is added to the zinc sulphate solution, the pH effect is very much greater and amounts to about 2·5 pH units, the indicator turning bright red. The neutral solution of nitriloacetate contains a substituted ammonium ion and the reaction analogous to that in equation (1) therefore has to be formulated in the following manner—



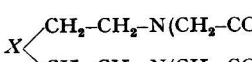
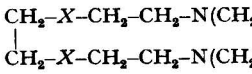
$$\quad .. \quad .. \quad (2)$$

the larger pH drop in reaction (2) in comparison with that in reaction (1) demonstrating the much larger complex-forming tendency of the substituted ammonia.[14,15]

The data in Table IV illustrate the influence on complex stability of a weak ligand atom like oxygen, sulphur or nitrogen in a carbon chain connecting two iminodiacetate groups. This influence is very great indeed and gives us an understanding of the strongly enhanced complex stability of iminodiacetate in comparison to glutarate.

TABLE IV

LOGARITHMS OF STABILITY CONSTANTS OF 1 TO 1 CALCIUM COMPLEXES AT 20° C AND AN IONIC CONCENTRATION OF 0·1

	X =	CH_2	O	S	$N(CH_3)$
	log K_{MZ} =	4·60	10·01	6·21	9·60
	X =	CH_2	O	S	$N(CH_3)$
	log K_{MZ} =	4·50	11·00	4·87	9·45

The formation constants shown in Table V permit a comparison of the complex forming ability of triaminotriethylamine with nitrilotriacetate and pentaethylenehexamine with ethylendiaminetetra-acetate.[16] The values demonstrate that the complexone substances react with many more metal cations than do the polyamines.

Having seen the reasons why the complexones are such strong and universal complexing agents and why the stoicheiometry of their reactions with metal cations is so simple, we will look now at their analytical application. Let me deal first with complexometric titration.

As I have mentioned, complex formation has up to the present been only very rarely used as a basis for titration procedures. Such titration is not possible because the free energy of complex formation is usually too small and because complex formation takes place in steps, as is demonstrated in Fig. 1 for cadmium and ammonia.[17]

The hydrogen ion, of course, can be titrated with ammonia, the equivalence point being marked by a jump in pH, as shown in Fig. 2. Now we will replace the hydrogen ion by a metal cation. Instead of pH, we will then have to plot the value pM, the negative logarithm of the metal-ion concentration, against the number of equivalents of amine base added. Fig. 3 shows the titration curves we obtain when we do so.[3] The metal is zinc in this

example, and when pZn is plotted against the number of equivalents of ammonia added, during the formation of the ordinary ammine complex, the lowest curve is obtained. There is no jump this time after the addition of four molecules of ammonia, the free energy of complex formation being much too small. More correctly, I should say that the free energy of formation of $Zn(NH_3)_4^{\cdot\cdot}$ is very much the same as the free energy of formation of the ammonium ion, $NH_4^{\cdot}$. But in the formation of the metal complex, four molecules of ammonia are needed and these four molecules are added stepwise, and it is the free energy of each individual step that counts and that determines the general form of the titration curve.

TABLE V

LOGARITHMS OF STABILITY CONSTANTS OF 1 TO 1 COMPLEXES AT 20° C AND AN IONIC CONCENTRATION OF 0·1

Metal cation	$N(CH_2-CH_2-NH_2)_3$	$N(CH_2-COO')_3$	$CH_2-N(CH_2-CH_2-NH_2)_2$ | $CH_2-N(CH_2-CH_2-NH_2)_2$	$CH_2-N(CH_2-COO')_2$ | $CH_2-N(CH_2-COO')_2$
$Li^{\cdot}$	< 1	~ 1·5	< 1	2·8
$Na^{\cdot}$	< 1	~ 1	< 1	1·7
$Mg^{\cdot\cdot}$	< 1	5·4	< 1	8·7
$Ca^{\cdot\cdot}$	< 1	6·4	< 1	10·6
$Sr^{\cdot\cdot}$	< 1	5·0	< 1	8·6
$Ba^{\cdot\cdot}$	< 1	4·8	< 1	7·8
$V^{\cdot\cdot}$	—	—	—	12·7
$Cr^{\cdot\cdot}$	—	—	—	~ 13
$Mn^{\cdot\cdot}$	5·8	7·4	9·4	13·5
$Fe^{\cdot\cdot}$	8·8	8·8	11·2	14·3
$Co^{\cdot\cdot}$	12·8	10·4	15·8	15·9
$Ni^{\cdot\cdot}$	14·8	11·5	19·3	18·2
$Cu^{\cdot\cdot}$	18·8	12·9	22·4	18·4
$Zn^{\cdot\cdot}$	14·7	10·7	16·2	16·1
$Cd^{\cdot\cdot}$	11·7	9·8	16·2	16·1
$Pb^{\cdot\cdot}$	small	11·4	small	17·6
$Hg^{\cdot\cdot}$	~ 25	~ 19	29·6	20·4
$Sc^{\cdot\cdot\cdot}$	< 1	—	< 1	21·3
$Y^{\cdot\cdot\cdot}$	< 1	11·4	< 1	17·7
$La^{\cdot\cdot\cdot}$	< 1	10·5	< 1	15·1
$Ce^{\cdot\cdot\cdot}$	< 1	10·7	< 1	15·6
$Pr^{\cdot\cdot\cdot}$	< 1	10·9	< 1	16·0
$Nd^{\cdot\cdot\cdot}$	< 1	11·1	< 1	16·2
$Sm^{\cdot\cdot\cdot}$	< 1	11·4	< 1	16·8
$Eu^{\cdot\cdot\cdot}$	< 1	—	< 1	16·9
$Gd^{\cdot\cdot\cdot}$	< 1	11·4	< 1	17·0
$Tb^{\cdot\cdot\cdot}$	< 1	—	< 1	17·5
$Dy^{\cdot\cdot\cdot}$	< 1	11·6	< 1	18·0
$Ho^{\cdot\cdot\cdot}$	< 1	—	< 1	18·3
$Er^{\cdot\cdot\cdot}$	< 1	—	< 1	18·6
$Ta^{\cdot\cdot\cdot}$	< 1	—	< 1	19·1
$Yb^{\cdot\cdot\cdot}$	< 1	12·1	< 1	19·4
$Lu^{\cdot\cdot\cdot}$	< 1	—	< 1	19·6
$V^{\cdot\cdot\cdot}$	—	—	—	25·9
$Cr^{\cdot\cdot\cdot}$	—	—	—	~ 24
$Fe^{\cdot\cdot\cdot}$	—	—	—	25·1
$Co^{\cdot\cdot\cdot}$	—	—	—	~ 36
$Al^{\cdot\cdot\cdot}$	—	—	—	15·5
$Ga^{\cdot\cdot\cdot}$	—	—	—	19·9
$In^{\cdot\cdot\cdot}$	—	—	—	26
$VO^{\cdot\cdot}$	—	—	—	18·4
$Th^{\cdot\cdot\cdot\cdot}$	—	—	—	23·2

From this curve it becomes apparent that the zinc ion cannot be titrated with ammonia, the end-point of such a titration not being characterised by a jump in pM.

The other curves in Fig. 3 demonstrate how matters can be improved tremendously by chelation. If we replace the four individual ammonia molecules by a single molecule of the tetramine "tren" ($\beta\,\beta'\beta''$-triaminotriethylamine), the titration curve that appears looks very much like the neutralisation curve of hydrochloric acid with a strong base.

This effect of chelation on the titration curve is used in the complexometric titration procedures. Instead of a polyamine we use a solution of the disodium salt of EDTA as the

titrant, a much more general complexing agent, the complex formation of which is also less dependent on pH. In order to understand quantitatively what happens during such a titration, we have to know something about the acidity of ethylenediaminetetra-acetic acid.

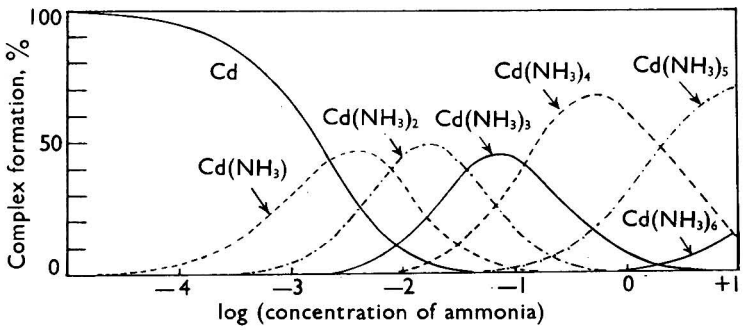


Fig. 1. Formation of complexes between cadmium and ammonia

The acid itself is a double betaine, as indicated by the formula in Table I. Only two carboxylic acid groups are present in the molecule and these two groups are strongly activated because of the charges on the two immonium groups, having pK values of about 2 and 3. These positively charged immonium groups have, of course, been formed by proton transfer from the other two carboxylic acid groups to the basic nitrogen atoms. These nitrogen

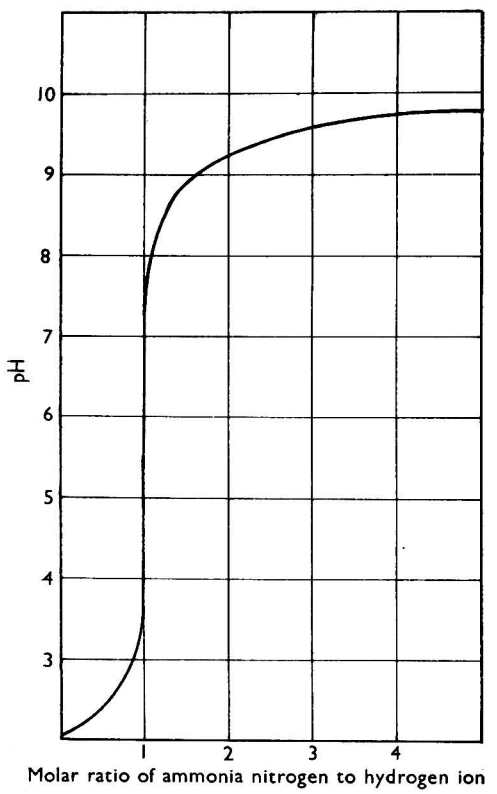


Fig. 2. Titration of hydrochloric acid with ammonia.

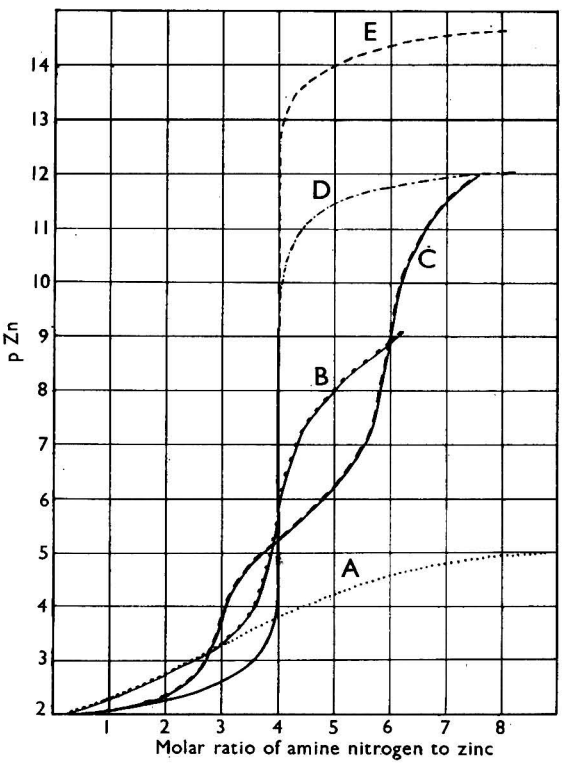


Fig. 3. Titration of zinc ions with amines: curve A, ammonia; curve B, ethylenediamine; curve C, diethylenetriamine; curve D, triethylenetetramine; curve E, triaminotriethylamine

atoms hold the protons so much better that they can be taken away from the molecule only at higher pH values, the first of the two at pH 6·2, this number being identical with the third pK value. The last proton leaves the molecule at about pH 10, the value of pK_4 being 10·26 exactly.[18]

Only above pH 10·5 or 11 does the complex formation correspond to the simple reaction—

$$\text{pH} > 11 : M^{+\nu} + Y^{-4} > MY^{\nu-4}.$$

At lower pH regions a protonated complexing agent is reacting with the metal cation and during the formation of the complex, protons must therefore be liberated. Between pH 7 and 10 we have—

$$10 > \text{pH} > 7 : M^{+\nu} + HY^{-3} \rightarrow MY^{\nu-4} + H^{+}$$

and between pH 6 and 4—

$$6 > \text{pH} > 4 : M^{+\nu} + H_2Y^{-2} \rightarrow MY^{\nu-4} + 2H^{+}.$$

These equations explain the pH effects that can be observed during the reaction analogous to the one described with nitrilotriacetic acid and zinc. The liberation of hydrogen ions can be used for an alkalimetric titration procedure for various metal cations, *i.e.*, the acid liberated during the reaction can be titrated with sodium hydroxide. This was the first form of titration with complexones, developed by us in 1945.[19]

Later we found much more accurate procedures. We now work in a buffered solution —for example, in an acetate buffer—which keeps the pH constant during the complex formation. If we do so, we must not forget, however, that the free energy of complex formation will depend on the pH range chosen. It will be higher at high pH values and lower at low pH values. This is reflected in the titration curves in Fig. 4, which can be calculated from

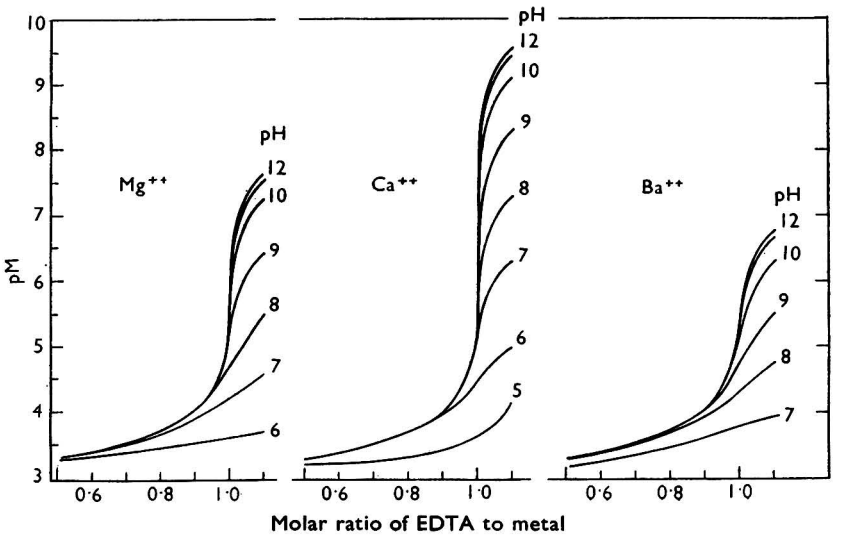


Fig. 4. Titration of alkaline-earth metals with EDTA at various pH values

our measurements of the stability constants of the EDTA complexes (Table V). The pM jump is larger at higher pH values (marked on the individual curves). From these curves it follows that the alkaline-earth metals have to be titrated in alkaline solution. Below pH 8 or 7, the free energy of complex formation is too small and we no longer get a pM jump.

A similar dependence on pH of course must exist for the heavy-metal cations. A further complication arises with these, however, because of the precipitation of hydroxides as soon as we exceed a certain limit, *viz.*, pH 7 for bivalent cations or pH 5 for tervalent ions. Such precipitation must be prevented by addition of auxiliary complexing agents. With bivalent cobalt, nickel, copper, cadmium or zinc, we may use ammonia to keep the metals in solution, and if added together with ammonium ion, this substance will at the same time serve as

a buffer to keep the pH constant. In such a solution the metal is present in the form of a series of ammine complexes (A = NH_3)—

$$\left\{\begin{array}{c} M^{+\nu} \\ + \\ MA^{+\nu} \\ + \\ MA_2^{+\nu} \\ + \\ MA_3^{+\nu} \\ \vdots \end{array}\right\} + HjY^{j-4} \rightarrow MY^{\nu-4} + j\mathrm{HA} + n\mathrm{A}$$

and these will be converted during the titration into the complex with EDTA, the ion of which is designated by the letter Y^{-4}. Because of the presence of these ammine complexes, the pM of the solution will be higher at the starting point of the titration. This is illustrated in Fig. 5. In order to calculate these curves, where again pM is plotted against number of

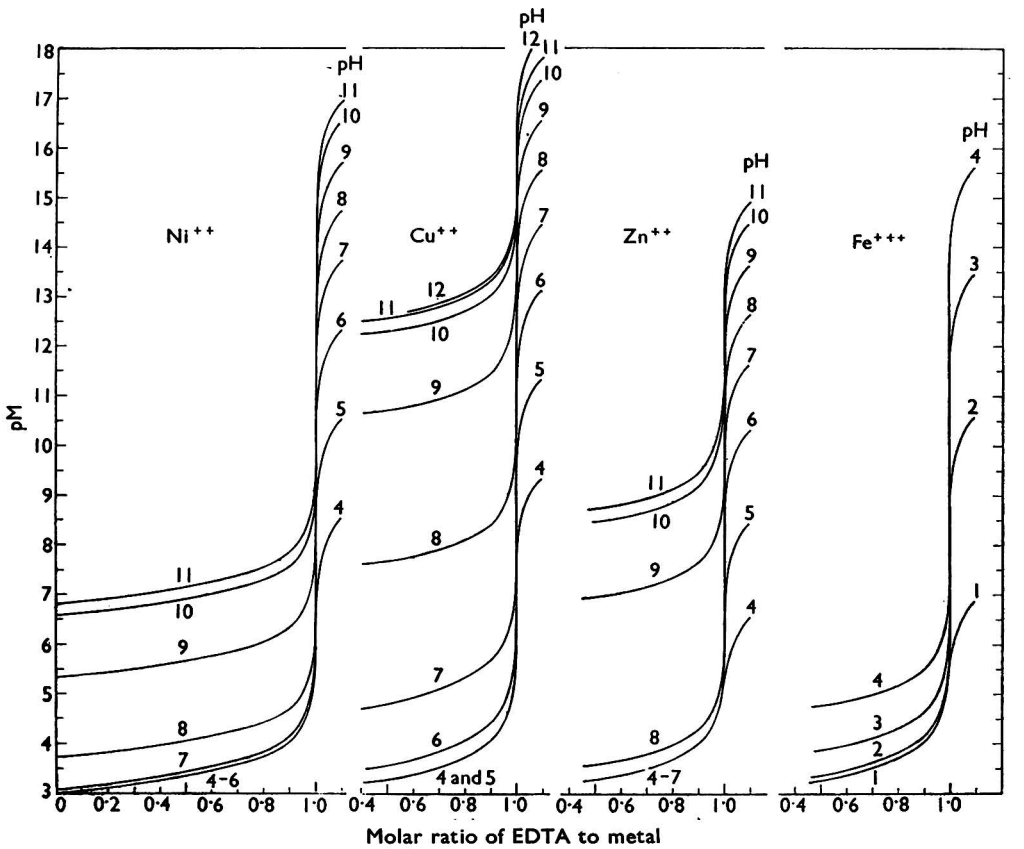


Fig. 5. Titration of nickel, copper, zinc and iron with EDTA at various pH values

moles of EDTA added per mole of metal, it was assumed that the sum of the concentrations of ammonia and ammonium ion would have the constant value of 0·1. A rise in pH will therefore be accompanied by a rise in concentration of free ammonia, and a greater percentage of the metal will then be present in the form of ammine complexes, corresponding to a higher value of pM at the starting point of the titration.

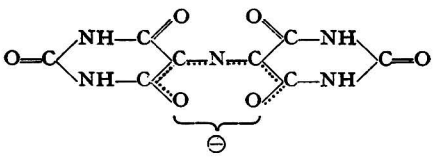
These curves illustrate clearly that the conditions during a complexometric titration have to be chosen carefully. A rise in pH is an advantage, because it produces greater jumps in pM at the equivalence point. Above pH 6 or 7, however, a further rise in pH is followed

by a lift of the level from which the titration curve starts, which results, of course, in a diminution of the height of the end-point jump. The auxiliary complexing agent therefore should be as weak as possible, although it must be strong enough to prevent precipitation of the metal hydroxide.

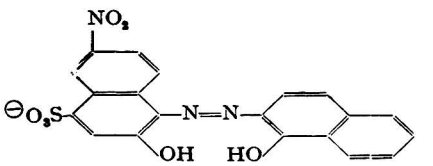
In the curve for tervalent iron, the lift of the starting level is due to the formation of the well known hydroxy complexes of iron, mainly $FeOH^{\cdot\cdot}$.

These pM jumps taking place after the introduction of the exact amount of EDTA required to complex the metal may be detected by several methods. We can use an electrode responding to the metal ion in question and carry out a potentiometric titration. However, for only a few metal cations do such electrodes exist, namely for copper, zinc, cadmium and lead, the amalgams of these metals being fairly good electrodes. They respond rather sluggishly, however, to changes of pM.

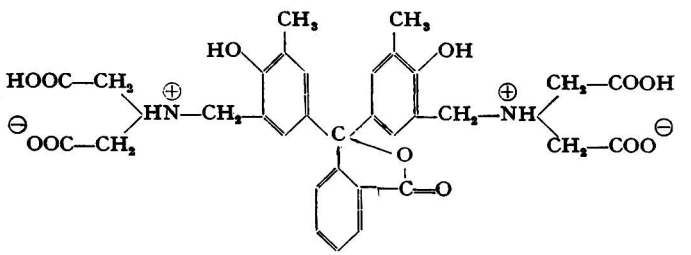
TABLE VI



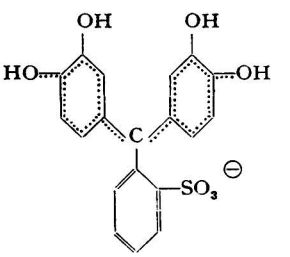
I. Purpureate anion (from murexide)



II. Eriochrome black T



III. Metalphthalein



IV. Pyrocatechol violet

Sometimes a simple bright platinum-foil electrode can be used as indicator of the end-point. Tervalent iron may be so titrated, the electrode responding to the ratio of ferric to ferrous ions in the solution, and this ratio changes abruptly at the end-point of the titration because the ferric iron is complexed preferentially by EDTA. The oxidation - reduction potential jumps to more negative values at the end-point of the titration.[20] This jump can also be made visible by adding an oxidation - reduction indicator. Flaschka[21] describes such a titration with Variamine blue B (4-amino-4'-methoxydiphenylamine), which is oxidised to an indophenol blue by ferric iron and reduced again when the ferric iron complex with EDTA is formed.

A very good and generally applicable method for the detection of the pM jumps makes use of the diffusion current at a dropping-mercury electrode during an amperometric titration with EDTA. We have to apply a potential to the mercury drop such that it is somewhat more negative than the polarographic half-wave potential of the metal ion in question, but more positive than the half-wave potential of its EDTA complex. These two half-wave potentials in practice lie far apart from each other in most cases.[22,23]

Most frequently, however, dyestuff indicators are used for the detection of the end-points of complexometric titrations. We have found some dyestuffs that change colour when the pM value is changed in just the same way as an ordinary indicator changes colour when the pH value is changed, and we therefore have named them "metal indicators."[24] Table VI gives the formulae of the best of these metal indicators. All of these molecules and ions are capable of forming complexes with calcium, copper, nickel and other metals; with murexide (I), for example, the metal cation probably takes the central nitrogen and two of the carbonyl oxygen atoms as ligand atoms. Formation of such a complex is accompanied by a colour

change. In certain pH regions protons from the imido groups are released when the metal cation enters the complex and, just as with EDTA, complex formation is dependent on pH.

Our most widely used metal indicator is Eriochrome black T(**II**),[25] which forms red complexes with many metal cations in a pH range where the metal-free dye itself is blue. A most brilliant colour change from red to blue is observed when the pM value jumps to such high values that the metal is extracted from the dye at the end-point of a titration. The metal

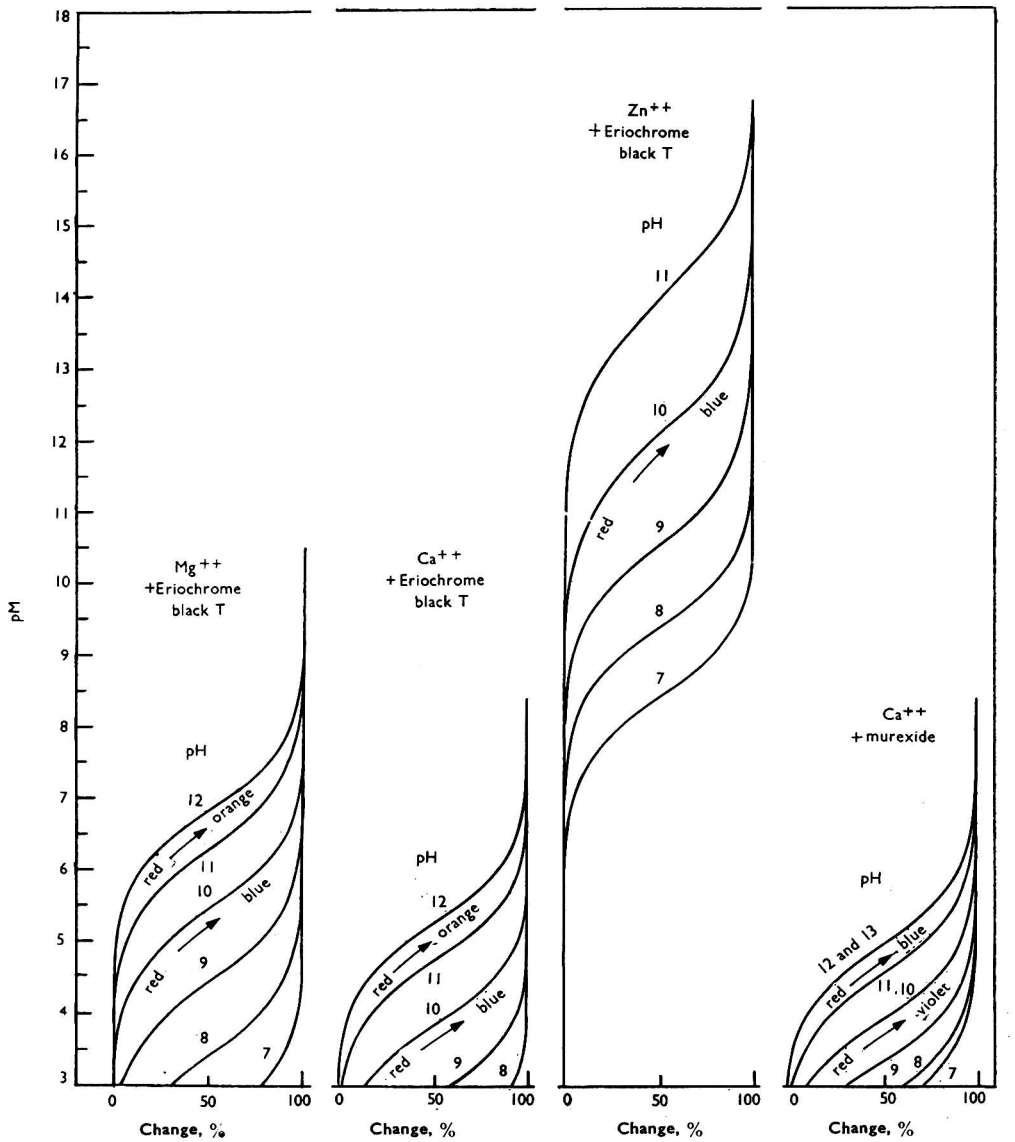


Fig. 6. Colour change of metal indicators

certainly is held by the oxygen atoms of the phenolic groups and the nitrogen atoms of the azo group of the dye molecule. You will recognise that five-membered chelate rings again can be formed in this way. Again the free energy of formation of the complex is dependent on pH.

Substance **III** is a derivative of phenolphthalein.[26] Two iminodiacetate groups have been introduced and these are capable of holding the metal cations. At the same time the metal cation will accept the phenoxide oxygens as ligand atoms, causing colour effects. The molecule deprived of all six of its acidic protons, the two carboxylic acid protons, the two

immonium protons and the two phenolic protons, is deep red, like phenolphthalein. Deep red also are the complexes of barium, strontium and calcium. The magnesium complex, however, is only pink, and all the heavy-metal complexes are colourless. The complexes of these last-named metals are similar, therefore, to the protonated forms of the anion, which also are pink or colourless. Between pH 10 and 11, addition of a cation of calcium, strontium or barium causes a colour change from pink to deep red, because of the transformation of the pink-coloured proton complex to the deeply coloured metal complex.

The metalphthalein is a good example of the transformation of an acid - base indicator into a metal indicator by the introduction of groups that are capable of binding metal cations. A second example is the sulphonphthalein of pyrocatechol (IV). This dye has been synthesised by Malát and his co-workers.[27] The pyrocatechol part of the molecule is capable of binding metal cations, as is pyrocatechol itself, and colour changes are produced thereby. The indicator is especially suitable for the titration of bismuth and thorium in acid solutions.

We have investigated quantitatively many of these colour changes. Figs. 6 and 7 record these measurements. Just as an acid - base indicator changes its colour within two pH units, a metal indicator has a corresponding colour-transition area of about two pM units. For example, at pH 10, murexide changes colour from yellow to purple between pCu 12·5 and 14·5. Figs. 6 and 7 show that all these transition areas are dependent on pH; this dependence is caused by proton release during complex formation.

A combination of the titration curves (Figs. 4 and 5) and the indicator curves (Figs. 6 and 7) leads us to curves giving the colour change as a function of the amount of titrant added (Fig. 8). These combination curves will tell us exactly at which pH values the best titration results can be expected. For instance, for the titration of copper with EDTA and murexide, pH values of 7 and 8 will be excellent. At lower pH values and at values above pH 9, the indicator will no longer change abruptly at the equivalence point. Such curves, therefore, are excellent for finding the best conditions for a titration.

Eighteen different metal cations can now be titrated directly with EDTA and a suitable metal indicator,[3] namely: Mg, Ca, Sr, Ba, Zn, Cd, Pb, Cu, Ni, Co, Fe, Mn, Hg, Tl, In, Bi, Th and all the rare-earth metals. Some other metal cations can be determined only by a back-titration method or by a special procedure, such as, for instance, that applied to silver. This metal does not form a sufficiently stable EDTA complex, and therefore cannot be titrated directly. It forms a very stable cyano complex, however, and if we treat an ammoniacal solution of the tetracyanonickelate ion with silver, the following exchange of ligand takes place—

$$2\ Ag^{\cdot} + Ni(CN)_4'' \rightarrow 2\ Ag(CN)_2' + Ni^{\cdot\cdot},$$

and we can titrate the equivalent amount of nickel liberated by this reaction.[28] Instead of silver ion, we can take any silver compound such as silver chloride, bromide or thiocyanate. Therefore the halogens also can be determined by first precipitating the silver halide, treating it with tetracyanonickelate ion and titrating the nickel, which is liberated in amounts equivalent to the halogen to be determined, murexide being used as indicator.

Sodium can be determined by precipitating it first with zinc uranyl acetate, dissolving the precipitate and then titrating the zinc that is present in it in amount equivalent to the sodium.[29] This type of procedure can be applied in many other cases. It consists in precipitating the ion to be determined, dissolving the precipitate and titrating one of the metallic constituents of the precipitate. Sometimes the complexones can be used as masking agents during such a precipitation in order to obtain a quantitative separation from other constituents of a mixture. Phosphate, for instance, can be precipitated in the form of magnesium ammonium phosphate in presence of almost any metal, if EDTA is first added to form complexes with all the heavy-metal cations.[30] A solution containing magnesium and an excess of EDTA and ammonia is added, resulting in the precipitation of magnesium ammonium phosphate, even in presence of aluminium, iron or whatever you like. The precipitate is then dissolved in acid, an EDTA titrating solution is added in slight excess, and the excess is titrated with a standard magnesium or zinc chloride solution.

Now in giving these examples I have already started to describe the use of complexones as masking agents. There are many other such examples, for instance—

Precipitation of calcium oxalate in presence of all other metals, which are masked with EDTA.[31]

With a mixture of thallium and lead, EDTA will mask lead during the precipitation with iodide, so that only thallium iodide will be precipitated. The thallium iodide

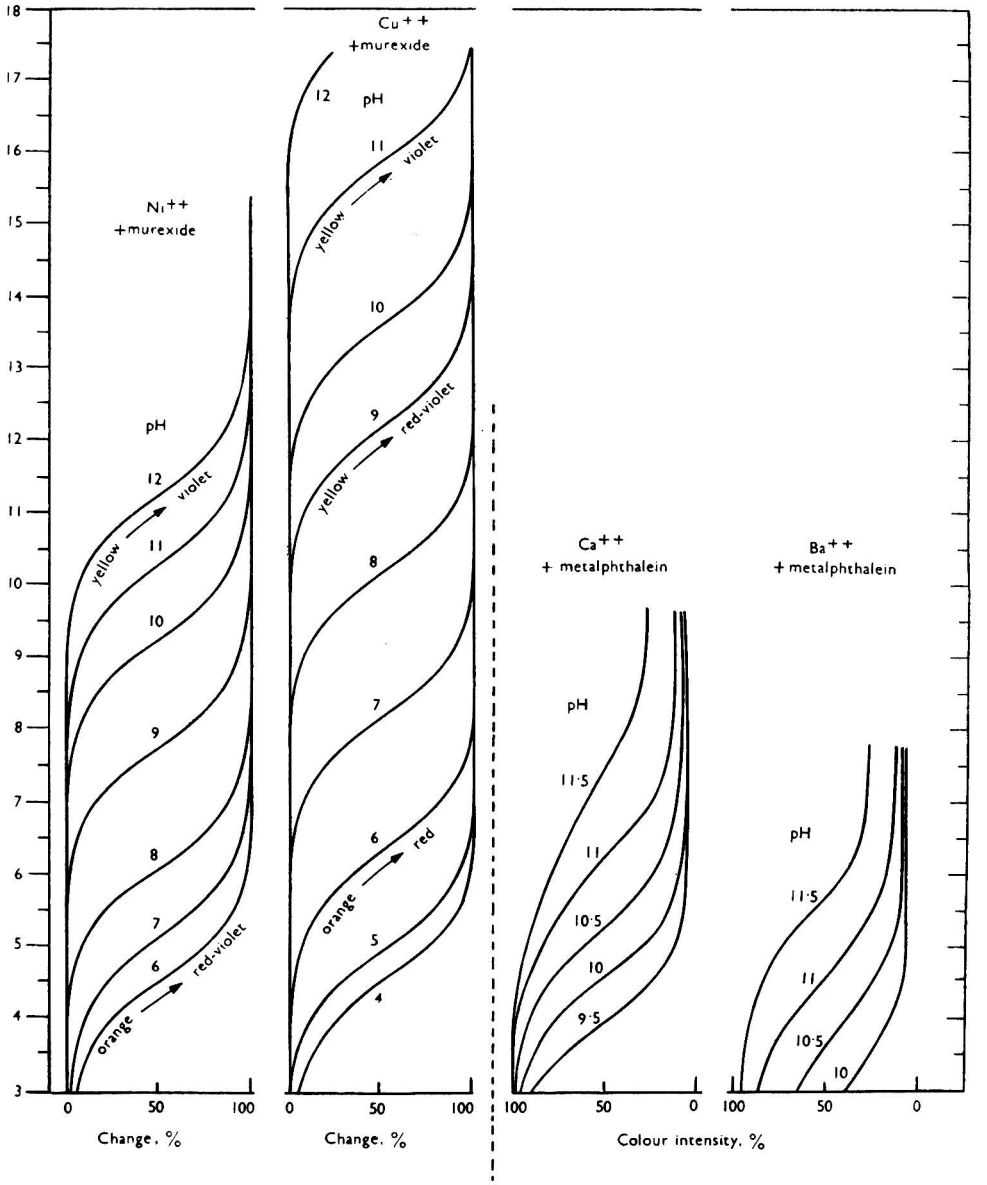


Fig. 7. Colour change of metal indicators

can then be dissolved in nitric acid and oxidised to tervalent thallium, which can be titrated with standard EDTA solution.[32]

Bismuth may be separated from most bivalent metals by the addition of EDTA as a masking agent. Bismuth hydroxide will be precipitated when calcium chloride and ammonia are added.[33] This precipitate can afterwards be dissolved and the bismuth titrated with standard EDTA solution, as previously described.

With a mixture of cobalt and nickel, the following procedure may be applied: introduce an excess of EDTA and add calcium chloride and ammonium sulphide; only cobalt will then be precipitated, owing to the slow decomposition of the nickel - EDTA complex.[34] The precipitated cobalt sulphide can be dissolved in nitric acid and titrated complexometrically. In a second portion of the mixture the nickel - dimethylglyoxime

complex is precipitated, filtered off and dissolved, and the nickel is titrated with standard EDTA solution.

EDTA may be used also as a masking agent during the iodimetric determination of chromate in presence of copper or iron, because the EDTA complexes, CuY'' and FeY', are so stable that no iodine is liberated by these metals from potassium iodide. Similarly, gold may be titrated iodimetrically in presence of copper when the latter metal is masked with EDTA, a method frequently used in the analysis of gold alloys.

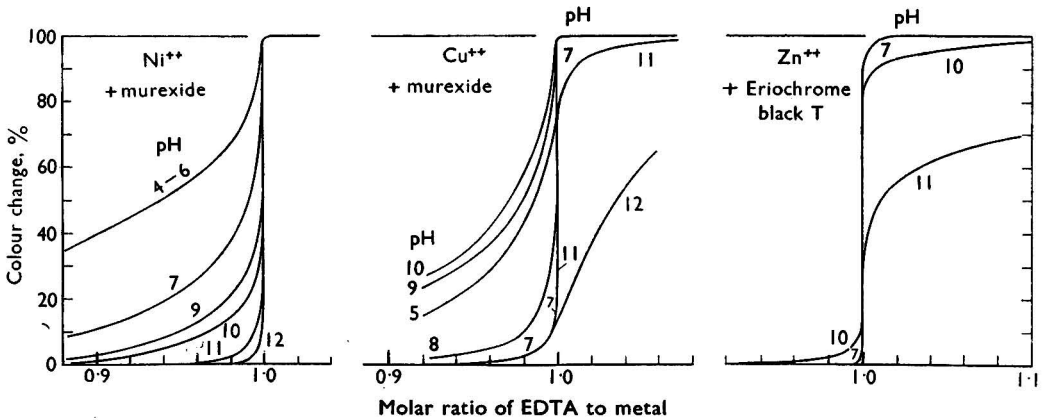


Fig. 8. The colour change of murexide during the complexometric titration of nickel and during the titration of copper, and the colour change of Eriochrome black T during the titration of zinc

Of course these last-named procedures are based on the lowering of the oxidation - reduction potential of the ferric - ferrous and cupric - cuprous systems. In presence of EDTA, tervalent iron is no longer an oxidising agent.[35] At the same time bivalent iron has become a strong reducing agent. On this fact Přibil has based a method for the detection of silver. The silver cation, Ag^+, is not reduced by ferrous ions. But when EDTA is added, elementary silver is produced.[36]

These are some examples of the use of EDTA as a masking agent and its further use to change oxidation - reduction potentials. EDTA can also be used in colorimetry. The complexes of manganese^{III}, chromium^{III} and cobalt^{III} are strongly coloured and can be used in a spectrophotometric determination of these metals.[37]

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Studies on Oxine and its Derivatives: The Selectivity and Sensitivity of 7-Allyl-8-hydroxyquinoline (7-Allyloxine) and 7-Allyl-8-hydroxy-5-nitrosoquinoline (7-Allyl-5-nitroso-oxine) Towards Certain Metals

BY R. G. W. HOLLINGSHEAD

7-Allyloxine and 7-allyl-5-nitroso-oxine have been prepared and their reactions with copper, magnesium, nickel, zinc and aluminium examined. The reagents follow closely the behaviour of oxine, being "unselective" in their action. A possible explanation for the non-selectivity of these derivatives, particularly 7-allyl-5-nitroso-oxine, is discussed. The effect of selectivity on sensitivity and the limiting sensitivity of other oxine derivatives are considered.

MANY attempts have been made to modify the oxine structure[1] in the hope of obtaining a reagent that is more specific or "selective" in its action, *i.e.*, a reagent that will chelate with fewer metals than the parent compound. Stemming from the observation[2] that a methyl group in position 2 of the oxine structure caused an increase in selectivity, in that 2-methyloxine would not cause the precipitation of aluminium, many derivatives with various substituents in position 2 have been prepared[3] and the reactions of certain of these with various metals examined.[4] Irving, Butler and Ring[4] found that some increase in sensitivity was obtained with 7-methyloxine, owing, in part, to steric-hindrance phenomena.

More recently[5] it has been observed that the introduction of a nitroso group in position 5 of the oxine structure causes a remarkable increase in selectivity, coupled with a marked decrease in sensitivity. Further investigation with 2-methyl-5-nitroso-oxine[6] has shown that a combination of steric hindrance and increased acidity of the reagent causes still greater selectivity. It therefore seemed of interest to examine the behaviour of further derivatives containing substituents in position 7 and a nitroso group in position 5. To this end 7-allyl-oxine and 7-allyl-5-nitroso-oxine have been prepared and examined. 7-Dibromoallyl-5-bromo-oxine has also been prepared, but its extreme insolubility has mitigated against its complete investigation.

EXPERIMENTAL

PREPARATION OF REAGENTS—

7-Allyloxine—7-Allyloxine was prepared by the method of Mander-Jones and Trikojus,[7] the modification of Long and Schofield being employed.[8] The crude material so obtained was recrystallised three times from chloroform before further use.

7-Allyl-5-nitroso-oxine—A solution of 5 g of purified 7-allyloxine in 50 ml of 5 *N* hydrochloric acid was cooled to 5° C and treated with 3 g of sodium nitrite, added in very small portions to the well stirred solution. The hydrochloride of 7-allyl-5-nitroso-oxine began to separate after the addition of about two-thirds of the required amount of sodium nitrite. The mixture was set aside for 1 hour after the addition of all the nitrite and then the solid was separated by filtration on a sintered-glass disc of porosity No. 2 and dissolved in the minimum amount of saturated sodium carbonate solution at 60° C. This solution was filtered to remove the small amount of tarry material and then treated with glacial acetic acid until precipitation of the free base was complete. The mixture was set aside overnight, then the precipitate was filtered off and washed *in situ* with 100 ml of ice-cold water. The buff-coloured precipitate was recrystallised repeatedly from ethanol, but a final material with a definite melting point was not obtained. Paper chromatography showed that the sample was homogeneous (compare with 5-nitroso-oxine[5]).

Key to tables of results

V_1 and V_2 are, respectively, the volumes of metal solution of known molarity M (column 2) within which the limit of detection of precipitate formation lies.
M is the molarity of the metal solution.
pL is defined as $-\log_{10}$ of the limiting concentration in gram-equivalents per litre for V_1 and V_2, respectively.
NP = No precipitation.

Table I

Sensitivity reactions with 7-allyloxine in buffers A, B and C

Buffer A, pH = 5·3; buffer B, pH = 8·35; buffer C, pH = 13·1; and total volume = 7 ml

Metal	M	Range V_1 to V_2	pL	Buffer	Colour of precipitate	Tube examined
Al^{III}	0·01	0·4 to 0·04	3·2 to 4·2	A	yellow	hot
Al^{III}	0·01	0·4 to 0·04	3·2 to 4·2	A	yellow	cold
Al^{III}	0·001	0·2 to 0·1	4·5 to 4·8	B	yellow	hot
Al^{III}	0·001	0·2 to 0·1	4·5 to 4·8	B	yellow	cold
Al^{III}		NP		C	see Note	hot
Al^{III}		NP		C	see Note	cold
Cu^{II}	0·001	0·1 to 0·04	4·8 to 5·2	A	green-yellow*; yellow†	hot
Cu^{II}	0·001	0·1 to 0·04	4·8 to 5·2	A	green-yellow*; yellow†	cold
Cu^{II}	0·001	0·1 to 0·04	4·8 to 5·2	B	green-yellow*; yellow†	hot
Cu^{II}	0·001	0·1 to 0·04	4·8 to 5·2	B	green-yellow*; yellow†	cold
Cu^{II}	0·001	0·1 to 0·04	4·8 to 5·2	C	yellow	hot
Cu^{II}	0·001	0·1 to 0·04	4·8 to 5·2	C	yellow	cold
Zn^{II}	0·001	0·4 to 0·3	4·2 to 4·4	A	yellow	hot
Zn^{II}	0·001	0·1 to 0·04	4·8 to 5·2	A	yellow	cold
Zn^{II}	0·001	0·1 to 0·04	4·8 to 5·2	B	yellow	hot
Zn^{II}	0·0001	0·3 to 0·2	5·4 to 5·5	B	yellow	cold
Zn^{II}	0·0001	0·1 to 0·04	5·8 to 6·2	C	yellow	hot
Zn^{II}	0·0001	0·3 to 0·2	5·4 to 5·5	C	yellow	cold
Mg^{II}		NP		A		hot
Mg^{II}		NP		A		cold
Mg^{II}		NP		B		hot
Mg^{II}		NP		B		cold
Mg^{II}	0·01	0·4 to 0·04	3·2 to 4·2	C	yellow	hot
Mg^{II}	0·01	0·4 to 0·04	3·2 to 4·2	C	yellow	cold
Ni^{II}	0·001	0·1 to 0·04	4·8 to 5·2	A	yellow	hot
Ni^{II}	0·001	0·1 to 0·04	4·8 to 5·2	A	yellow	cold
Ni^{II}	0·001	0·1 to 0·04	4·8 to 5·2	B	yellow	hot
Ni^{II}	0·001	0·1 to 0·04	4·8 to 5·2	B	yellow	cold
Ni^{II}	0·001	0·1 to 0·04	4·8 to 5·2	C	yellow	hot
Ni^{II}	0·001	0·1 to 0·04	4·8 to 5·2	C	yellow	cold

Note—

The metal is held up in solution at this pH. (See under discussion of results (p. 733).)
* In concentrated solution.
† In dilute solution.

TABLE II

SENSITIVITY REACTIONS WITH 7-ALLYL-5-NITROSO-OXINE IN BUFFERS A, B AND C

Buffer A, pH = 5·3; buffer B, pH = 8·35; buffer C, pH = 13·1; and total volume = 7 ml

Metal	M	Range V_1 to V_2	pL	Buffer	Colour of precipitate	Tube examined
Al^{III}	0·001	0·3 to 0·2	4·4 to 4·5	A	yellow	hot
Al^{III}	0·001	0·3 to 0·2	4·4 to 4·5	A	yellow	cold
Al^{III}	0·01	0·3 to 0·2	3·4 to 3·5	B	yellow	hot
Al^{III}	0·01	0·4 to 0·3	3·2 to 3·4	B	yellow	cold
Al^{III}		NP		C	see Note	hot
Al^{III}		NP		C	see Note	cold
Cu^{II}	0·0001	0·1 to 0·04	5·8 to 6·2	A	olive-yellow*; yellow†	hot
Cu^{II}	0·0001	0·2 to 0·1	5·5 to 5·8	A	olive-yellow*; yellow	cold
Cu^{II}	0·0001	0·1 to 0·04	5·8 to 6·2	B	olive-yellow*; yellow†	hot
Cu^{II}	0·0001	0·2 to 0·1	5·5 to 5·8	B	olive-yellow*; yellow†	cold
Cu^{II}		NP		C		hot
Cu^{II}		NP		C		cold
Zn^{II}	0·001	0·3 to 0·2	4·4 to 4·5	A	yellow	hot
Zn^{II}	0·001	0·4 to 0·3	4·2 to 4·4	A	yellow	cold
Zn^{II}	0·001	0·3 to 0·2	4·4 to 4·5	B	yellow	hot
Zn^{II}	0·001	0·1 to 0·04	4·8 to 5·2	B	yellow	cold
Zn^{II}		NP		C		hot
Zn^{II}		NP		C		cold
Mg^{II}	0·001	0·4 to 0·3	4·2 to 4·4	A	yellow	hot
Mg^{II}	0·001	0·4 to 0·3	4·2 to 4·4	A	yellow	cold
Mg^{II}	0·001	0·4 to 0·3	4·2 to 4·4	B	yellow	hot
Mg^{II}	0·001	0·4 to 0·3	4·2 to 4·4	B	yellow	cold
Mg^{II}	0·01	0·2 to 0·1	3·5 to 3·8	C	yellow	hot
Mg^{II}	0·01	0·2 to 0·1	3·5 to 3·8	C	yellow	cold
Ni^{II}	0·0001	0·2 to 0·1	5·5 to 5·8	A	yellow	hot
Ni^{II}	0·0001	0·2 to 0·1	5·5 to 5·8	A	yellow	cold
Ni^{II}	0·0001	0·2 to 0·1	5·5 to 5·8	B	yellow	hot
Ni^{II}	0·0001	0·1 to 0·04	5·8 to 6·2	B	yellow	cold
Ni^{II}		NP		C		hot
Ni^{II}		NP		C		cold

NOTE—

The metal is held up in solution at this pH. (See under discussion of results (p. 733).)

* In concentrated solution.

† In dilute solution.

The melting point of the recrystallised material was indefinite, being about 105° C. Analysis gave: found, N = 13·2 per cent.; $C_{12}H_{10}O_2N_2$ requires N = 13·08 per cent.

7-Dibromoallyl-5-bromo-oxine—When a solution of 7-allyloxine in chloroform is treated with one mole fraction of bromine in the same solvent, no precipitation occurs. Addition in the allyl chain is obviously favoured in preference to substitution in the phenolic ring. Only on the addition of the third mole fraction of bromine is precipitation complete, with the precipitation of the dibromo-addition monobromo-substitution product.

A 5·65-g portion of 7-allyloxine is dissolved in 10 ml of chloroform and treated with a total of 7·2 g of bromine in 30 ml of chloroform. A heavy yellow precipitate is obtained. This is set aside overnight in a refrigerator, then filtered off, pressed as dry as possible, transferred to a beaker and dissolved in 250 ml of boiling ethanol. The bright yellow solution is poured into 3 litres of distilled water to give a white flocculent precipitate. This is allowed to settle out, then separated by filtration on a sintered-glass disc of porosity No. 4, washed with distilled water (500 ml) until the washings are colourless and finally dried at 70° C to give an off-white material. The yield is 9 g. A sample twice recrystallised from boiling ethanol (in which it is sparingly soluble) gave a material of m.p. 133° to 133·5° C.

Analysis gave: found, C = 34·1 per cent.; H = 2·4 per cent.; N = 3·16 per cent.; Br = 56·6 per cent.; $C_{12}H_{10}ONBr_3$ requires C = 34·0 per cent.; H = 2·38 per cent.; N = 3·3 per cent.; Br = 56·56 per cent.

SENSITIVITY TESTS—

These were carried out by the method previously described.[4,5,9] Owing to the sparing solubility of the reagents in absolute ethanol, hot solutions of the reagents were used, but the molarity was still 0·02. Precipitation of the reagent caused some difficulty in determining absolute limits for detection of the precipitated complex, so the tubes were observed both when hot and cold. The presence or absence of precipitated complex could usually be observed in the cold tubes by the difference in colour tint obtained, as well as by obvious differences in the nature and quantity of the precipitate.

The results obtained with 7-allyloxine and 7-allyl-5-nitroso-oxine are given in Tables I and II, respectively. The buffers A, B and C employed have been discussed previously.[4,5,9]

RESULTS

It is obvious that the introduction of an allyl group in the oxine structure does not cause any increase in selectivity, and the derivatives closely follow the behaviour of the parent compound. No increase in selectivity is obtained on the introduction of a nitroso group in position 5, although an over-all increase in sensitivity occurs.

It is known that the introduction of a nitroso group in position 5 increases selectivity,[5] and Irving *et al.*[4] have reported increased sensitivity with substituents in position 7, *e.g.*, 7-methyloxine. Increased selectivity is further enhanced in 2-methyl-5-nitroso-oxine,[6] when precipitation of all the Group 3B metals is prevented. Rearrangement of the methyl and nitroso groups to give 5-methyl-7-nitroso-oxine causes a remarkable decrease in selectivity, coupled with some increase in sensitivity.[10] With 7-allyl-5-nitroso-oxine the same type of behaviour exists, and like 5-methyl-7-nitroso-oxine, this derivative is non-selective, but more sensitive than oxine.

REACTIONS WITH ALUMINIUM—

Although the proximity of the allyl chain to the chelating group obviously causes steric hindrance, the ionic radius of the aluminium atom is still sufficiently large to permit a tris complex to be formed, and the precipitation of the chelate occurs in acid and neutral solutions (buffers A and B). Maximum sensitivity is found in buffer B, the reaction in acid solution being somewhat less sensitive, but even so, greater than the sensitivity of oxine (see Table III). No precipitation occurs in buffer C, and it is possible that the tris complex is held up, possibly in the form of complex ions. This behaviour has previously been reported by Irving *et al.*[4], and has been found to occur with 5-fluoro-oxine[9] as well as other derivatives.[11]

A very similar series of reactions occurs with 7-allyl-5-nitroso-oxine. Precipitation of the tris complex occurs in buffers A and B, but no precipitation is obtained in buffer C. In this case, however, a reversal of limiting sensitivities occurs, and whereas 7-allyloxine shows its greatest sensitivity at pH 8·35, 7-allyl-5-nitroso-oxine favours a pH of 5·3.

REACTIONS WITH COPPER—

Precipitation of copper 7-allyloxinate occurs throughout the pH range examined, precipitations occurring under all the test conditions. In this behaviour 7-allyloxine closely follows the behaviour of oxine. With 7-allyl-5-nitroso-oxine, however, although precipitation of the complex occurs in buffers A and B, no precipitation is obtained with buffer C. In this, 7-allyl-5-nitroso-oxine follows the behaviour of 5-nitroso-oxine,[5] which also does not precipitate copper from solutions buffered with buffer C. This behaviour is not shared with other oxine derivatives studied, precipitation in all buffers occurring with oxine, 5-methyl-7-nitroso-oxine[10] and 2-methyl, 5-methyl, 6-methyl and 7-methyloxines.[4] The reactions of the allyl derivatives towards copper show an increase in sensitivity, so that the pL values for 7-allyloxine in buffers A, B and C are all higher than those for copper with oxine (see Table III). With 7-allyl-5-nitroso-oxine, a quite remarkable increase in sensitivity is obtained, and the average pL value reaches its peak of 6·0 in hot solution in buffer A and in cold solution in buffer B.

This increase in sensitivity, of approximately two pL units above the average sensitivity (considered by Irving to be 4·2), is exceptional, particularly as it has been pointed out that individual sensitivities of many oxine derivatives and related compounds do not depart greatly from this average value.

REACTIONS WITH ZINC—

Precipitation of the zinc complex of 7-allyloxine occurs in buffers A, B and C, and in this behaviour the derivative resembles the parent compound. A comparison of the average sensitivities of oxine with both 7-allyloxine and 7-allyl-5-nitroso-oxine shows that the allyl derivatives are somewhat more sensitive, especially 7-allyloxine in hot buffer C.

REACTIONS WITH MAGNESIUM—

No precipitation of the complex magnesium allyloxinate occurs in buffers A or B, but precipitation of the complex occurs in buffer C. This behaviour may be compared with the non-precipitation of magnesium oxinate in buffer A, its partial precipitation in buffer B and its quantitative precipitation in buffer C. With magnesium and 7-allyl-5-nitroso-oxine, however, precipitation occurs in buffers A, B and C. Out of well over 50 oxine derivatives investigated,[11,12] 7-allyl-5-nitroso-oxine and also 5-methyl-7-nitroso-oxine[10] are the only derivatives that will precipitate magnesium from acid solution. As well as precipitating magnesium from acid solution, 7-allyl-5-nitroso-oxine is somewhat more sensitive than oxine, whereas 7-allyloxine, which behaves very similarly to oxine, has very nearly the same pL value (see Table III).

REACTIONS WITH NICKEL—

In its reactions with nickel, 7-allyloxine closely follows the behaviour of oxine, in that precipitation occurs in all three buffers, and the sensitivity remains quite stable through the pH range examined, no decrease in sensitivity occurring in alkaline solution. With 7-allyl-5-nitroso-oxine, some slight degree of selectivity occurs, in that no precipitation takes place in buffer C. Precipitation reactions are obtained in buffers A and B, and are more sensitive than analogous reactions with oxine. The average pL value for the nickel - oxine chelate (4·0) may be compared with that of 6·0 for the allylnitroso chelate.

DISCUSSION OF RESULTS

Many attempts have been made to correlate structure with stability,[13] and stability in its turn with increased sensitivity or selectivity or both, but the difficulties are numerous, and any degree of selectivity cannot be attributed to any one factor. The existence of steric hindrance was offered as a possible explanation for the non-chelation of aluminium with 2-methyloxine,[2] and further investigation showed that other derivatives with substituents in position 2 did not precipitate aluminium under conditions in which precipitation occurred with oxine.[3,4] Steric hindrance was obviously a contributing cause towards this increased selectivity, and possibly could have been the complete explanation in this instance, but further investigation has shown that the phenomenon of selectivity was not connected solely with steric-hindrance factors, when it was demonstrated that precipitation of aluminium and certain other metals did not occur with 5-nitroso-oxine,[5] in which derivative the possibility of steric hindrance would appear to be completely absent.

It has been suggested[5] that a possible explanation for the increased selectivity of 5-nitroso-oxine lies in the fact that the acidity of the chelating function, *i.e.*, the phenolic hydroxyl group and the ring nitrogen, is increased by the presence of the nitroso group in the *para* position to the OH, with a subsequent lowering of the constant pK_2. Lowering of pK_2 is associated with the formation of weaker metallic complexes, and this factor may account for the increased selectivity but decreased sensitivity of 5-nitroso-oxine.

With 7-allyloxine and 7-allyl-5-nitroso-oxine, the possibility of a certain amount of steric hindrance to chelate formation exists, and Irving *et al.*[4] have shown that the presence of a methyl group in position 7 causes some slight increase in sensitivity. It would appear that any lowering of pK_2 by the nitroso group in 7-allyl-5-nitroso-oxine is more than compensated for by the presence of the allyl chain in position 7, but at this stage only very tentative conclusions may be drawn, and any fuller consideration must await determination of ionisation constants of the reagents and stability constants of the metallic chelates.

From the sensitivity limits determined so far some pattern begins to emerge. It is seen from Table III that increased selectivity is generally associated with decreased sensitivity, although anomalies occur from time to time. A complete picture of the behaviour of a series of substituted oxines with metallic ions must await a far greater amount of experimental data.

TABLE III

pL VALUES FOR METALS WITH OXINE DERIVATIVES

Metal	Oxine	5-Nitroso-oxine	5-Methyl-oxine	5-Methyl-7-nitroso-oxine	5-Fluoro-oxine	7-Allyl-oxine	7-Allyl-5-nitroso-oxine	7-Methyl-oxine
Al^{III}	3·3 A, B[g]; 3·3†[c]; 3·5[h]*†	NP[f]	4·0†[c]	5·0A[d]	3·7 A, B[b]	4·65 A[a]	4·45 A[a]	4·3†[c]
Cd^{II}	4·45[h]*†	NP[f]		5·0 A[d]				
Ce^{III}		Indefinite		3·7 A, B[d]				
Ce^{IV}		4·0 C[f]		4·45 A[d]				
Cr^{III}	3·3†[c]	2·7 A[f]	4·0†[c]	4·3 A[d]				4·7†[c]
Co^{II}	4·0[h]*†	5·0 B[f]		5·0 A, B[d]	4·65 A, B[b]			
Cu^{II}	4·5†[c]; 4·3[h]*†	5·0 B[f]	4·5†[c]	3·7 A, B[d]	4·65 A, B[b]	5·0 A, B, C[a]	6·0 B[a]	5·3†[c]
Fe^{II}		3·0 A, B[f]		5·0 A, B[d]	5·0 B[e]			
Fe^{III}	4·0†[c]; 4·3[h]*†	NP A, B, C[f]	4·3†[c]	5·0 A, B[d]	5·0 A, B[e]			4·3†[c]
Ga^{III}	3·24[h]*†; 4·65 B[g]; 4·0†[c]	NP A, B, C[f]	4·0†[c]	5·0 A, B[d]				4·3†[c]
In^{III}	3·65 A, B[g]; 4·3[h]*†	NP A, B, C[g]						
Pb^{II}	4·0[h]*†	NP A, B, C[f]		5·0 A[d]				
Mg^{II}	3·8[h]*†	NP A, B, C[f]		4·3 A[d]	NP A, B[b]	3·7 C[a]	4·3 A, B[a]	
Hg^{I}	4·1[h]*†	3·0 A[f]		4·65 A[d]				
Hg^{II}	3·3[h]*†	3·65 A[f]		5·65 A[d]	4·65 A, B[b]			
Ni^{II}	4·0[h]*†	5·0 B[f]		5·0 A, B, C[d]		5·0 A, B, C[a]	6·0 B[a]	
Tl^{I}		NP A, B, C[f]		4·3 A, B[d]				
Tl^{III}	4·45 A, B[g]	4·3‡ A[g]						
VO_3'	4·2[h]*†	NP A, B, C[f]		5·0 A[d]				
Zn^{II}	4·5†[c]; 4·5[h]*†	4·0 A, B[f]	3·5†[c]	5·45 A[d]		6·0 C[a]	5·0 B[a]	4·2†[c]

* Buffers employed by Berg corresponding approximately to buffers A, B and C. A, B and C refer to the buffers employed (see text for details).

† Given for the medium in which the greatest sensitivity was shown.

‡ Unreliable value.

NP = No precipitate in buffers stated.

[a] Present paper.
[b] Hollingshead.[9]
[c] Irving *et al.*[4]
[d] Hollingshead.[10]
[e] Hollingshead.[14]
[f] Irving, Hollingshead and Harris.[5]
[g] Hollingshead.[15]
[h] Converted from pM values calculated by Irving and Rossotti[11] from values of concentration in μg per ml quoted by Berg.[16]

Tests with 7-dibromoallyl-5-bromo-oxine

Sensitivity tests were carried out with 7-dibromoallyl-5-bromo-oxine by the method previously employed (see above). The same order of precipitation occurs, the reagent being, as expected, not selective. Owing to the very sparing solubility of the derivative in absolute ethanol, it was necessary to add the reagent solution (0·02 *M*) at the boiling point. Even under these conditions some precipitation of the reagent occurred during the addition; therefore the amount of reagent added was not always of identical weight or volume. For this reason, no sensitivity limits are given for the bromo derivative, as these would be indefinite. A tendency to form more stable complexes in acid solution was noted.

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The Separation and Quantitative Determination of Platinum, Palladium, Rhodium and Iridium on Paper Strips

By N. F. Kember and R. A. Wells

Chromatographic methods are described for the separation of platinum, palladium, rhodium and iridium in microgram amounts on paper strips. Three solvent mixtures are recommended, the choice depending on the type of mixture to be analysed. After separation, the metals are extracted from the sections of the strips, and the platinum, palladium and rhodium are determined by colorimetric methods and the iridium by micro-titration. Analyses have been carried out by this technique on mixtures of all four metals containing large amounts of base metals.

The qualitative separation of the members of the platinum-metal group by cellulose chromatography on paper strips has already been reported by a number of workers. Lederer[1] separated silver, copper, palladium, platinum and gold with *n*-butanol saturated with *N* hydrochloric acid by upward diffusion, whilst Burstall, Davies, Linstead and Wells[2,3] reported the separation of rhodium, palladium, platinum and gold with ethyl methyl ketone or methyl *n*-propyl ketone containing concentrated hydrochloric acid. Iridium was shown to be a complicating factor, because of its variation in valency.

No previous attempt has been made to use these separations as the basis of a quantitative analytical method. This would be of particular interest in view of the great difficulty involved in separating microgram amounts of these metals. This paper describes a number of methods for the separation and determination of platinum, palladium, rhodium and iridium on the microgram scale. It has been possible to determine 0·1 per cent. of some components of the mixture in the presence of macro amounts of the other platinum metals or of the base metals. The remaining members of the series, ruthenium and osmium, have not been included in the quantitative analyses, but their general behaviour and possible interference have been studied. These metals can be readily removed by distillation of their volatile oxides.

No single solvent could be used to carry out all the desired separations, which were developed by standard paper-strip techniques with downward diffusion. Three solvents were used finally, the choice of solvent depending on the type of mixture to be analysed. The solvents were—

(*i*) 60 parts of *iso*butyl methyl ketone (hexone), 10 parts of pentanol and 30 parts of hydrochloric acid, sp.gr. 1·18, all parts by volume,
(*ii*) *n*-butanol saturated with 3 *N* hydrochloric acid and
(*iii*) solvent (*ii*) containing hydrogen peroxide.

The first solvent gave three well defined and equally spaced bands of rhodium, palladium and platinum, in that order. Iridium, if present at low concentration, was reduced during the separation to a less mobile form and stayed with the rhodium. This solvent was therefore used in the analysis of rhodium - palladium - platinum or iridium - palladium - platinum mixtures. Analyses were carried out in the presence of a number of common base metals; some metals, however, interfered owing to their position after development of the strip. When these metals were present, the second solvent was used, as they remained immobile. Solvent (*iii*) was used in the separation of iridium from rhodium, since the hydrogen peroxide maintained the iridium in a fully oxidised condition, in which form it moved near the solvent front. By careful choice of solvent and final methods of analysis, sometimes two strips being developed under different conditions, it was possible to analyse mixtures of platinum, palladium, rhodium and iridium in the presence of base metals.

After separation, the strips were cut into sections, each containing the required metal, which was extracted from the paper with dilute acid. Determinations of platinum and rhodium were carried out photometrically with stannous chloride, and palladium was determined similarly with thioglycollic acid or *p*-nitrosodimethylaniline. No satisfactory photometric method was found for the determination of microgram amounts of iridium, but use was made of a micro-titrimetric method developed by Pollard,[4] with a standardised solution of hydroquinone as reducing agent and 3:3′-dichlorobenzidine as indicator.

Experimental

Chromatographic separation—

Most separations described in this paper were carried out on 3-cm wide strips of Whatman's No. 1 filter-paper. Faster (No. 4), slower (No. 20) and thicker (Nos. 3 MM and 15) papers were investigated, but showed no advantage. The apparatus consisted of a tall glass jar, 50 cm high and 7·5 cm in internal diameter, fitted with a stopper from which was suspended a 30-ml solvent container and two strip supports. Downward diffusion of solvent was used, but upward diffusion was shown to give very similar results. A quantity of solvent was placed at the bottom of the jar to aid equilibration of the atmosphere. For all quantitative experiments, separations were carried out at a controlled temperature at 25° C. Chromatograms were left to develop overnight; the strips were then removed from the jar and the excess of solvent was allowed to evaporate from the paper.

Chromatograms were developed in pairs, one of which was used as a control strip, which, when sprayed with a suitable reagent, provided a guide to the position of the bands. This technique also gave an indication of the quantity of metal present, so that a choice may be made of dilution factors in the colorimetric analysis.

Removal of the separated metals from the strip—

With the control strip as a guide, the strip was cut into sections each containing the required metal. Several methods were considered and investigated for the removal of the metals from the paper, but the most practical was shown to be extraction with dilute acid, followed by washing with the colorimetric reagents. This was carried out with the apparatus

shown in Fig. 4 (p. 747), which permitted the extraction to be carried out with a minimum volume of solution.

An attempt was made to carry out the extraction and colour development in one stage in a centrifuge tube by shaking the paper with a measured quantity of reagent, but adsorption of the coloured complexes by the cellulose was found to cause serious error. Extraction in a Soxhlet apparatus was also investigated, but was found to need too large a volume of liquid, so that time was lost during the additional evaporation stage.

METHODS OF FINAL DETERMINATION—

After the metals had been separated and extracted from the strip, the only remaining stage was to determine the microgram amounts of the platinum metals. Since in most cases the metals were now separate from one another, the final methods of determination need not be specific, this being a considerable advantage in platinum-metal analysis. We used photometric methods for platinum, palladium and rhodium, but there is no reason why other methods of analysis of greater convenience to other workers may not be used. No colorimetric method was found to be of sufficient sensitivity or accuracy for the determination of iridium, but a published method involving micro-titration[4] was found to be satisfactory.

Platinum—The recommended method is colorimetric, the reagent being stannous chloride. Sandell's method[5] was first investigated, but it gave erratic results at the lowest limits of determination. By increasing the concentration of the reagents,[6] consistent results were obtained.

Palladium—The recommended methods are colorimetric, the reagent being either thioglycollic acid[7] or *p*-nitrosodimethylaniline.[8] The first reagent was used when possible in view of the wide range of acidity of the final solution that could be accepted. Investigations showed that the interferences caused by other metals with this reagent were more severe than those quoted by the original authors. To gain sensitivity and in the presence of these base metals, the second reagent was used. By using *p*-nitrosodiphenylamine it should be possible to decrease the lower limit of detection further.

Rhodium—The recommended method is colorimetric, the reagent being stannous chloride in 2 *N* hydrochloric acid.[9]

Iridium—The recommended method is a titration, with hydroquinone as reducing agent.[4] A number of colorimetric methods was investigated, but none was found to give the required sensitivity or accuracy.

The majority of published methods fall into one of three groups—

(*a*) Those in which the fully oxidised reddish-brown colour is produced by using reagents such as chlorine water, sodium persulphate or ceric sulphate. An interesting fact was observed as a result of these studies, namely that the spectral curve of the final solution varied considerably, according to the form of the original iridium solution. This may account for disagreement amongst a number of previous authors on colorimetric methods for iridium by this type of reaction.

(*b*) Those in which the purple or blue hydrated oxides are produced by the use of sulphuric - nitric - perchloric acid mixtures. A number of these methods was tested and all gave considerable scatter of results, owing to the critical nature of the acidity and time of heating.

(*c*) Those in which the iridium is first oxidised fully and the excess of reagent is removed, a colour being obtained by means of a redox indicator, *e.g.*, benzidine or *leuco*-malachite green. These methods again gave inconsistent results, probably owing to incomplete oxidation or difficulty in removing excess of reagent without reducing the product.

During the course of these studies, an investigation was made of the reaction of iridium with stannous chloride. Mention has been made[5,9] of the severe interference that iridium causes during the determination of platinum and rhodium, but apparently no study has been made of stannous chloride as a reagent for the determination of iridium. Measurement of the spectral curve showed a sharp rise in the absorption in the ultra-violet region. It was not practicable to use a very low wavelength in view of a similar, although delayed, rise in the spectral curve of the reagents; measurements were therefore carried out at 360 mμ. A surprisingly high sensitivity was obtained, it being possible to detect 0·1 p.p.m. of iridium by this method. Unfortunately, the intensity of colour was found to vary considerably with time and temperature, particularly the latter, since little colour was produced at room

temperature. No method was found of obtaining reproducible results within the limits of accuracy required for colorimetric analysis.

The titrimetric method was finally adopted as being the only method available of sufficient sensitivity for this scale of work. A number of small modifications was made to Pollard's original method. The sample of iridium was oxidised by digestion with perchloric acid to the purple hydrated oxide. The oxidised iridium was then reduced by a standardised solution of hydroquinone, with 3 : 3'-dichlorobenzidine as indicator, a micro-titration technique being used. It was possible to determine amounts of iridium as small as 0·5 μg by this method, although some scatter was experienced at this level, but this might be expected in view of the experimental technique. An attempt was made to determine the intensity of the indicator colorimetrically, but this was found to be impractical owing to its instability.

Separations with hexone

Separations with ethyl methyl ketone and methyl *n*-propyl ketone containing 30 per cent. of hydrochloric acid, sp.gr. 1·18, have already been reported.[2,3] Further investigations with hexone containing 40 per cent. of concentrated hydrochloric acid gave better separations, because of narrower bands, particularly for rhodium. Further, the solvent was considerably more stable on keeping, despite the higher acid concentration. This concentration was necessary to prevent the dividing of the platinum between the wet and dry solvent areas of the strip.

With that hexone solvent a separation was carried out on a 3-cm wide strip of Whatman's No. 1 paper of the chloro acids of platinum, palladium and rhodium in dilute hydrochloric acid solution. The strip was cut into sections and the metals determined as described previously, with the following results—

	Platinum	Palladium	Rhodium
Metal present, μg	40·9	46·0	59·7
Metal found, μg	24·5	45·4	59·7

The results show good agreement for both palladium and rhodium, but a low yield of platinum. Quantitative tests with only platinum present showed that some of the metal occupied a second band in the position normally occupied by palladium.

Prevention of double platinum bands—

It has been observed as a general rule in inorganic chromatography with organic solvents that, if a metal exists in two or more valency states, there is a tendency for the lower valent form to move less rapidly along a strip or column. The double platinum band may therefore be explained by reduction of the platinum, either in the original solution or in the solvent, from $PtCl_6''$ to $PtCl_4''$. Alternatively, the reason may be lack of sodium ions in the mixture, a factor found to be critical in previous work on the separation of gold from the platinum metals.[10]

Solutions were prepared containing 1 mg of platinum per ml in dilute hydrochloric acid and either 1 per cent. of sodium chloride or 1 per cent. of sodium chlorate. These were spotted on strips, dried, developed with the hexone solvent and sprayed with the stannous chloride reagent. The addition of sodium chloride showed no improvement, but the use of sodium chlorate completely prevented the tendency to double-band formation, which confirmed the fact that this had been caused by reduction of the platinum. As a result, sodium chlorate was included in all mixtures analysed; potassium chlorate could not be used, owing to the formation of insoluble platinum compounds.

Effect of temperature and acid concentration

Diffuse bands were occasionally observed during preliminary experiments, and it was suspected that this was due to irregularities in temperature during the lengthy time of development. To investigate this, separations were carried out at controlled temperature over the range 17° to 30° C. At the lower temperatures bands were diffused and at the lowest temperature double bands of platinum were formed, even when sodium chlorate was added to the original solution. Narrowest bands were obtained at 25° C, no improvement being apparent above this temperature, which was therefore adopted for all subsequent experiments.

Separations carried out at 25° C with hexone containing 40 per cent. of hydrochloric acid showed increased movement of rhodium. A series of separations was carried out at

a range of acidity, resulting (see Table I) in the adoption of a solvent containing 30 per cent. of hydrochloric acid, which gave better spaced bands and a solvent more stable to keeping.

TABLE I

EFFECT OF ACID CONCENTRATION OF THE SOLVENT

Concentrated hydrochloric acid in solvent, %	R_F values				Rate of movement, cm per hour
	Rhodium	Palladium	Platinum	Gold	
5	0·00	0·00	0·01	very diffuse	9
10	0·00	0·01	0·04	very diffuse	7
15	0·00	0·14	0·25	very diffuse	5
20	0·06	0·38	0·65	very diffuse	2·4
25	0·10	0·41	0·68	diffuse, 0·9	2·2
30	0·14	0·41	0·68	0·97	1·9
40	0·20	0·41	0·68	0·97	1·8

PREVENTION OF TRAILING OF THE PLATINUM—

By using the best conditions of separation so far achieved, several quantitative analyses were carried out on synthetic mixtures. It was observed that platinum recoveries were still low, particularly when the concentration of the metal on the strip exceeded 200 μg. At the same time the results obtained for rhodium on the same strip were correspondingly high. Qualitative experiments with more than 200 μg of platinum present showed a tendency not revealed with lower concentrations, *viz.*, severe trailing, particularly along the extreme edge of the paper.

It was believed at first that this was due to distortion of the rectangular cross-section of the strip during the machine cutting of the rolls of paper. This was partly confirmed by using strips precisely cut by hand, which gave slightly better results, but did not remedy the effect sufficiently.

The trailing was finally prevented by the addition of small proportions of a higher alcohol to the solvent. The results, shown in Table II, of a series of experiments, each carried out on two strips containing (*a*) 40 μg each of platinum, palladium, rhodium and gold and (*b*) 250 μg of platinum showed that the best solvent mixture was hexone - pentanol - hydrochloric acid, sp.gr. 1·18, in the proportion 60:10:30 by volume (Fig. 1).

The results of a number of analyses with this solvent are given later.

EFFECT AND MOVEMENT OF OTHER IONS—

The effect of a number of anions that might be expected to be present was investigated by adding 50 mg of the acid or of the sodium salt of the ion to 1 ml of a solution containing about 1 mg each of rhodium, palladium, platinum and gold. These were developed with the hexone solvent and sprayed with stannous chloride solution. The following anions were shown to have no effect on the efficiency of the separation: SO_4'', NO_3', Br', I', ClO_4', ClO_3', AsO_4''', SbO_4''', SeO_4'', TeO_4''. The presence of selenium and tellurium imposed other limitations discussed more fully in a later section.

The presence of cyanide in the solution gave very low results owing to the stability of the cyanide complexes. Repeated treatment of the original solution with aqua regia was necessary to break down the complexes to a state suitable for analysis.

Ammonium and potassium salts in the solution also interfered seriously, because of the formation of insoluble chloro salts. It was therefore very important to avoid contamination of the strips with ammonia vapour during the drying of the spots before separation.

The investigations so far have been carried out on rhodium, palladium and platinum. The remaining platinum metals were also investigated with this solvent, although most of the ruthenium and osmium would have been lost during the preparation of the original solution.

Iridium—It has already been reported in studies on the separation of the platinum metals with ketonic solvents[3] that iridium is reduced on the strip to a less mobile form. This has also been shown to be true when the hexone solvent is used. Iridium is found in a band with a diffuse leading edge in the same position as rhodium. The forward diffusion increased with the amount of iridium present. This movement is discussed more fully in a later section.

Ruthenium behaved very similarly to iridium.

Osmium behaved very similarly to platinum.

Base metals—The movement of a large number of metals was recorded by developing strips under standardised conditions, *viz.*, for 18 hours at 25° C on No. 1 paper with hexone - pentanol - hydrochloric acid solvent. The orthodox method of R_F measurement was not used, since it does not take the width of the band into account, a factor in many published

TABLE II

EFFECT OF ALCOHOL ADDITION ON THE EFFICIENCY OF SEPARATION

Number	Solvent mixture: Components	Solvent mixture: Parts by volume	R_F values: Rhodium	R_F values: Palladium	R_F values: Platinum	Solvent travel in 16 hours, cm	Effect on platinum band
1	Hexone Hydrochloric acid	60 40	0·21	0·47	0·68	34·2	Severe trailing, particularly along edges
2	Hexone *n*-Butanol Hydrochloric acid	50 10 40	0·18	0·49	0·71	29·2	Some reduction of trailing. More compact rhodium band
3	Hexone *iso*Butanol Hydrochloric acid	50 10 40	0·20	0·49	0·69	29·0	Further slight improvement
4	Hexone Pentanol Hydrochloric acid	50 10 40	0·19	0·48	0·69	26·5	Only slight tendency for trailing. Rhodium band similar to 2
5	Hexone *n*-Butanol Hydrochloric acid	35 25 40	0·20	0·49	0·69	28·2	Bands tending to become U-shaped, otherwise similar to 2
6	Hexone *n*-Butanol Hydrochloric acid	60 10 30	0·18	0·51	0·70	32·6	Less trailing than in 2, but wider rhodium band
7	Hexone Pentanol Hydrochloric acid	55 5 40	0·17	0·46	0·69	28·0	Similar to 2
8	Hexone Pentanol Hydrochloric acid	50 10 40	0·15	0·46	0·68	28·0	Similar to 4
9	Hexone Pentanol Hydrochloric acid	40 20 40	0·15	0·48	0·67	26·0	Some trailing, although less than in 1. Wider platinum and palladium bands, causing less efficient separation
10	Hexone Pentanol Hydrochloric acid	65 5 30	0·10	0·45	0·72	32·5	Result intermediate between 2 and 4
11	Hexone Pentanol Hydrochloric acid	60 10 30	0·08	0·43	0·70	29·4	Least tendency for trailing
12	Hexone Pentanol Hydrochloric acid	50 20 30	0·09	0·46	0·71	28·0	Similar to 9

tables that does not make clear whether one metal can be separated completely from another. The starting point of measurement was the trailing edge of the original solution band, and measurements were taken to both trailing and leading edges of the developed band as a ratio to the movement of the solvent front. All values were expressed as a percentage to prevent confusion over decimal points.

$$R_T = \frac{\text{movement of trailing edge}}{\text{movement of solvent front}} \times 100$$

$$R_L = \frac{\text{movement of leading edge}}{\text{movement of solvent front}} \times 100.$$

The results are shown in Table III and include the values for the simpler solvent, hexone containing 40 per cent. of concentrated hydrochloric acid. It will be observed that a number of differences occur in the results obtained with the two solvents. With the simpler solvent,

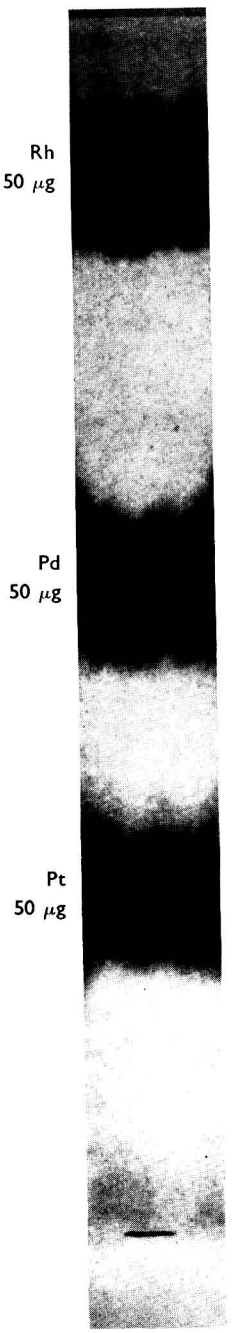

Fig. 1

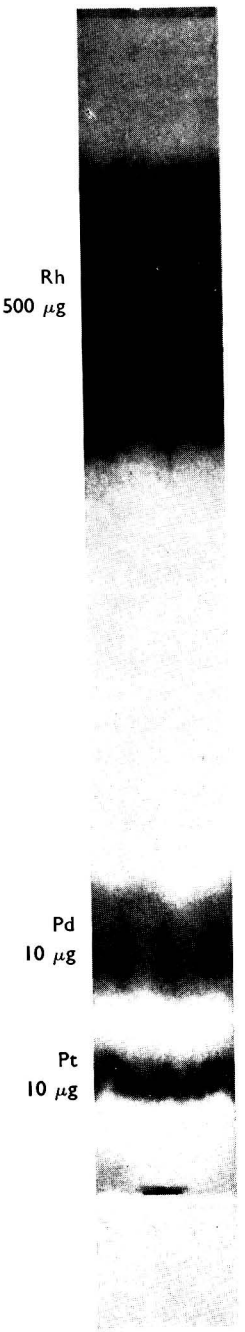

Fig. 2

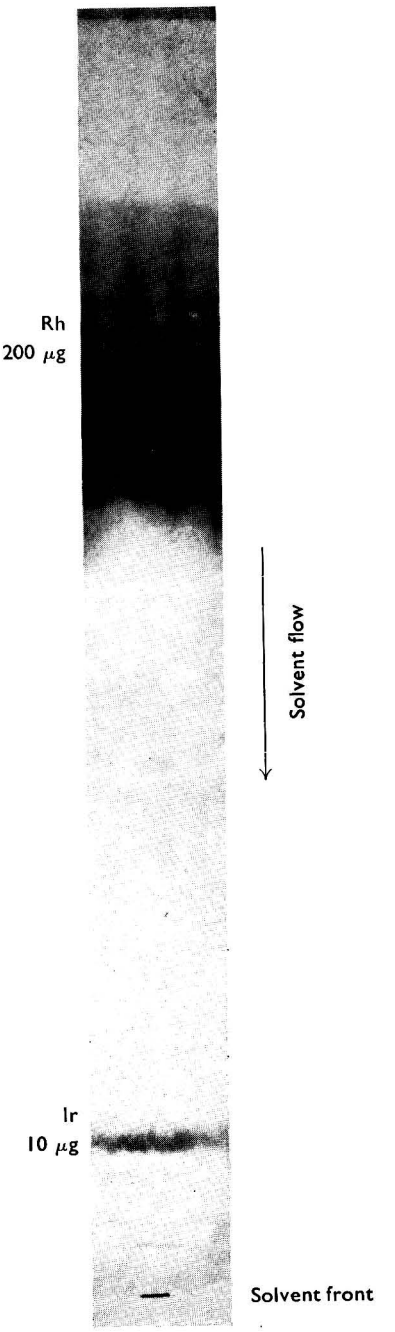

Fig. 3

[To face p. 740

greater differences of movement occur in the less mobile metals and with some metals, *e.g.*, beryllium and manganese, the order of the metals changes. This is due to the considerably different order obtained when an alcohol - hydrochloric acid solvent mixture is used, the results of the more complex ketone - alcohol - acid solvent being intermediate between the two.

The tables indicate a number of other interesting separations. The separations of nickel, cobalt, copper and zinc and of nickel, manganese, cobalt, copper and iron with lower ketone - dilute hydrochloric acid mixtures have already been reported, but the small degree of separation of some of the components does not permit the quantitative separation of macro amounts of one metal and minor proportions of the adjacent metal. Further, the earlier solvents are very dependent on strict control of water and acid concentration for their efficiency. With the hexone solvent, excellent separations of nickel, manganese, cobalt copper and iron have been achieved and they could be made the basis of quantitative analyses.

TABLE III

POSITION OF METALS ON STRIPS DEVELOPED WITH HEXONE - PENTANOL - HYDROCHLORIC ACID SOLVENT SYSTEMS

Metal (about 100 μg)	Hexone - pentanol - hydrochloric acid (60 : 10 : 30)		Hexone - hydrochloric acid (60 : 40)		Metal (about 100 μg)	Hexone - pentanol - hydrochloric acid (60 : 10 : 30)		Hexone - hydrochloric acid (60 : 40)	
	R_T	R_L	R_T	R_L		R_T	R_L	R_T	R_L
Ba	0	9	0	9	Pd	41	49	43	51
Th	1	10	10	19	Cu	55	63	59	67
Sr	2	11	11	20	Co	66	75	71	79
Lanthanons	3	12	19	28	Pt	68	76	62	69
Al	5	14	20	29	Os	68	76	63	70
Rh	6	15	14	28*	As	82	89	80	85
Ru	6	15*	22	29*	Sb	82	89	80	85
Ni	7	16	15	24	Zn	82	90	75	83
Hf	7	16	10	19	Cd	83	90	76	76
Cr	7	16	21	30	Hg	85	91	76	76
Ir	8	20*	20	33*	Mo	85	92	89	96
Ca	8	17	14	23	Se	86	92	89	94
Ti	9	18	19	24	Te	86	92	89	94
Sc	9	18	25	34	Sn	86	92	87	93
Zr	9	18	19	28	U	86	92	83	90
Mg	10	19	25	34	Ga	89	96	83	90
V	17	26	28	37	In	89	96	83	90
Pb	22	31	32	40	Fe	89	96	89	96
Mn	23	30	26	35	Au	95	100	93	100
Be	26	34	44	52					
Bi	38	46	49	57					

* Trail forward.

SEPARATIONS WITH *n*-BUTANOL

The most evenly spaced chromatographic separations were produced with the hexone solvent described in the last section. In the presence of certain base metals this solvent could not be used, owing to the interference of these metals in the chromatographic separation and subsequent colorimetric determinations. A search was carried out for a solvent that would overcome these difficulties.

SELECTION OF THE BEST SOLVENT—

Lederer[1] has reported the use of *n*-butanol saturated with *N* hydrochloric acid, and Burstall[3] and co-workers the use of the same solvent saturated with 2 *N* hydrochloric acid. Investigation showed that there was a tendency in the first solvent to form double bands. This was overcome by the second solvent, which also gave narrower bands for all metals. The strength of the equilibrating acid was therefore increased to 3 *N*, which resulted in further improvement. Further increase in acidity was not possible, since the alcohol was then completely miscible with the acid. With this solvent, platinum was found in a narrow band in the wet solvent front (R_F 0·85), palladium in an equally narrow band a short distance behind and rhodium in a band near the original position (Fig. 2). A large number of other solvents

was tested, but no improvement was found on this result, both from the point of view of narrowness of bands and hold-back of base metals. Water miscible solvent mixtures tended to give diffuse bands. The narrowness of the bands obtained with the *n*-butanol - 3 *N* hydrochloric acid solvent was of particular use in the visual estimation of separated platinum metals, owing to the increase in sensitivity that resulted. The solvent was prepared by shaking *n*-butanol with an equal volume of 3 *N* hydrochloric acid, the solvent phase being used in the solvent container and the aqueous phase in the bottom of the jar. This technique resulted in narrower and better-shaped bands than those obtained when the solvent phase was also used to equilibrate the atmosphere in the jar.

Effect and movement of other ions

Certain anions that might have had an effect on the separation were tested in the same way as that already used with the ketonic solvent; SO_4'', NO_3', Br', I', ClO_3' and ClO_4' had no effect on the efficiency of the separation. The effect of cyanide was the same as that obtained with the other solvent; the presence of potassium and ammonium also had similar effect.

The movement of the other platinum metals was also studied.

Iridium—After development this was found in a narrow brown band coincident with the position of platinum; spraying the strip with sodium hypochlorite solution, however, showed that some of the metal had been reduced and was present both in the original spot and trailing down the strip. This phenomenon and its prevention is more fully reported in a later section.

Osmium—Narrow band coincident with platinum, but with some trailing.

Ruthenium—Similar position to rhodium.

The movement of a large number of metals was recorded and calculated in the same manner as that used previously and the results are shown in Table IV. Also included in the table are the positions of the reduced forms of platinum, iridium and iron referred to later; in these cases no sodium chlorate was present in the original solution.

Table IV

Position of metals on strip developed with *n*-butanol saturated with 3 *N* hydrochloric acid

Metal	R_T	R_L	Metal	R_T	R_L
Ba	4	17	Fe^{III}	46	53
Sr	5	16	Ga	52	59
Ca	5	16	As	67	73
Lanthanons	5	16	Pt^{II}	70	75
Cr	9	19	Pd	71	76
Al	10	23	Mo	71	76
Ni	13	23	Bi	71	76
Mn	13	24	Pt^{IV}	82	85
Co	14	24	Os	82	85
Rh	16	26	Ir^{IV}	82	85
Ti	16	26	Te	85	91
Fe^{II}	17	27	Cd	89	93
V	20	30	Zn	90	95
Cu	24	33	Sb	91	95
Ir^{III}	24	33	Sn	92	96
Ru	27	36	Hg	94	96
U	31	39	Se	96	100
Pb	33	40	Au	96	100
Be	35	42			

Inclusion of iridium in the system

During preliminary work on the separation and determination of the platinum metals it was realised that the presence of iridium in any mixture introduced a number of complications; as a result this metal was left for individual study. The major difficulty was that of converting the metal to and maintaining it in one oxidation state during both the preparation of the original solution and during development of the chromatogram, since the mobility of both oxidation states varied very considerably on the strip. The metal followed the general chromatographic rule that higher valent ions were more mobile. It was considered, and reported previously, that with a higher ketone - hydrochloric acid mixture as solvent, iridium

was reduced,[3] but subsequent investigation with higher loadings and more sensitive methods of detection showed that forward trailing proportional to the quantity of iridium present occurred. Lederer[11] has reported that the partial reduction of iridium with *n*-butanol - dilute hydrochloric acid mixtures may be almost completely prevented by the inclusion of nitric acid in the solvent mixture. Apart from the fact that the oxidation is not absolutely complete, this solvent is unsuitable for quantitative analysis, since rhodium tends to trail forward in the presence of solvents containing nitric acid.

The problem was studied in two ways: (*a*) by attempting to oxidise or reduce the iridium in the original solution to one form that would remain in a single band on the strip, and (*b*) by using solvents with oxidising or reducing properties giving the same result.

OXIDATION AND REDUCTION IN THE ORIGINAL SOLUTION—

The oxidising and reducing properties of a number of reagents were investigated, followed by chromatographic development with both alcoholic and ketonic solvents. It was necessary to study the reaction of the other platinum metals with each treatment in case of secondary effects. This was particularly important with platinum, since it was very readily reduced to a less mobile form, probably $PtCl_4''$, which moved with palladium.

The treatment of platinum-metal solutions with sulphur dioxide to render iridium immobile has already been reported.[3] Although iridium was always completely reduced by this treatment, further investigations showed that platinum was also affected. Chromatographic development of the solutions gave at least two bands, one in the normal position of fully oxidised platinum and the other in the position normally occupied by palladium. The proportion of platinum in each band varied according to the acidity of the original solution, the method of preparation and the quantity of sulphur dioxide added to the solution or solvent. In some cases three or four well defined bands were obtained, possibly owing to the formation of stable sulphito complexes.

A number of other reducing agents, including hydroxylamine, hydrazine and formaldehyde, was investigated, giving similar double-band effects or causing partial or complete precipitation of one of the metals of the group. The effect of oxalic acid in both acid and alkaline solutions was investigated in some detail. Iridium was successfully reduced, but subsequent chromatographic development showed three distinct bands of platinum, one probably due to the formation of a stable oxalato complex.

The reduction of iridium with hydrogen peroxide in alkaline solution showed some promise at first. Solutions were made alkaline with sodium hydroxide, treated with hydrogen peroxide, boiled to remove excess of peroxide and made acid with hydrochloric acid. Chromatographic development and spraying apparently showed complete reduction of iridium and a single platinum band in its normal position separate from palladium. Quantitative experiments, however, gave low results for both these metals, owing to trailing forward of iridium and backward of platinum.

Promising results were also obtained with ferrous sulphate as reducing agent; iridium was completely reduced and, although the platinum was coincident with the palladium on chromatograms, no double band was apparent. Quantitative experiments on platinum - iridium mixtures showed a trace of platinum with the iridium, indicating further reduction of some of the platinum to a completely immobile form, probably finely divided metal.

Complete reduction of iridium to its less mobile form was therefore not achieved with the reagents tested. The secondary effects on the other platinum metals were also too serious for any of these methods to be used. Treatment of the original solution with oxidising agents was also ineffective, since the reducing properties of the chromatographic solvents and cellulose were more powerful.

OXIDISING AND REDUCING SOLVENTS—

Both of the solvents previously used had reducing properties to differing degrees when used in conjunction with cellulose. The reducing powers of the ketonic solvent - cellulose system were investigated in detail, since they affected the quantity of iridium that could be tolerated in a mixture. Several strips were spotted with various quantities of iridium between 50 and 250 μg, as chloro acids in sodium chlorate solution. These were developed with the hexone solvent, and the sections normally occupied by the palladium (centre) and platinum (lower) bands were analysed for iridium. These showed severe trailing forward in the presence of 250 μg of iridium, more than 30 μg being present in the centre section

and 2·0 μg in the lower section. With only 100 μg present, 4·0 μg was found in the centre section and none in the lower section. In the presence of only 50 μg of iridium no forward trailing was detected.

The partial reduction of iridium in the *n*-butanol solvent has already been mentioned. This tendency was prevented by the addition of hydrogen peroxide to the acid used in preparing the *n*-butanol - 3 *N* hydrochloric acid solvent. With this solvent, iridium was completely oxidised and moved in a narrow band with the platinum; none was detected in any other portion of the strip (Fig. 3). It was still found necessary to include sodium chlorate in the original solution. The addition of peroxide to the solvent had no effect on the movement of other metals, even those that may be expected to give more soluble peroxy compounds, *e.g.*, vanadium, titanium, chromium and molybdenum. Addition of hydrogen peroxide to the hexone solvent had little or no effect on the movement of platinum, palladium and rhodium.

Other oxidising techniques investigated included the addition of sodium chlorate to the solvent - acid mixture. This generated chlorine and chlorine dioxide in the solvent, but experiments showed that the reduction of the iridium was only delayed for a short time, causing slightly increased movement of iridium relative to the rhodium, but insufficient for their separation. The use of dry chlorine gas to saturate the solvent gave no change, showing that chlorine dioxide was the active oxidising agent in the previous experiments.

A number of solvents containing nitric acid in addition to and in place of the hydrochloric acid was prepared. These caused increased movement of iridium, but rhodium tended to trail forward, giving diffuse bands overlapping palladium.

Analysis of mixtures containing iridium—

Several methods were investigated to separate all components of platinum, palladium, rhodium and iridium mixtures. After development with ketone - hydrochloric acid solvents, iridium and rhodium were left together at the top of the strip with a reasonable separation from palladium. An attempt was made to develop the strip further after such a separation with the *n*-butanol - hydrochloric acid - hydrogen peroxide solvent to move the iridium away from the rhodium. This proved unsuccessful owing to the extreme fragility of the strip after development with the ketonic solvent and the large amount of acid held by the cellulose having an effect on the separation.

A reversal of this procedure was next investigated. Long strips of paper 65 cm × 3 cm were used in the same jars, the bottom 20 cm being rolled up in a polythene "peg." The strips were developed with the *n*-butanol - hydrochloric acid - hydrogen peroxide solvent overnight, the solvent diffusing about 30 cm down the strip. This left rhodium alone at the top of the strip and platinum, iridium and palladium in two bands about 25 cm down the strip. The strip was now cut at a point 20 cm from the beinning, the extra paper unrolled, the excess of solvent allowed to evaporate and the strip developed with the hexone mixture since the *n*-butanol solvent does not affect the strength of paper. Spraying with the correct reagents showed platinum and palladium well separated and in their correct positions, but iridium was found in a diffuse band all down the strip. This was probably due to the high stability of the peroxy compounds, and an attempt was made to break this down between the two solvent developments by gentle heating or in a jet of steam, but without success.

Later experiments on the micro-titration of iridium showed that the presence of rhodium or platinum caused no interference; this made two-stage separation unnecessary. In its place two separate strips were developed, one in the hexone solvent and the other in the *n*-butanol - peroxide solvent, the first being used for iridium, palladium and platinum analyses and the second for rhodium. This is discussed more fully in the next section.

Selection of the separation and determination procedure

Each method of separation and determination imposed some limitation on the type of solution that could be analysed, but by suitable choice of conditions and by combinations of methods, it was possible to analyse a very wide variety of mixtures of both platinum and base metals. Many of the present limitations could be removed by the use of more sensitive colorimetric tests or the use of other methods of analysis, *e.g.*, polarographic, radiometric and spectrographic. In the analysis of ores a preliminary concentration stage would be desirable; it could be carried out by any of the early stages of standard dry or wet assay methods. For

the analysis of alloys of platinum metals and certain base metals the only limitations would be those of quantity.

LIMITATIONS IMPOSED BY THE SEPARATION METHODS—

An important factor was the maximum quantity of any metal that could be tolerated on the strip before having any effect on the efficiency of the separation. Most tests were carried out on 3-cm wide strips of Whatman's No. 1 paper; it was found that the use of thicker paper offered no advantage, since more diffuse bands resulted. It must be stressed that only one metal was present at its maximum loading.

Platinum—Qualitative tests with the hexone solvent showed that when more than 1·5 mg of platinum was present on the strip severe trailing of the metal occurred, particularly along the extreme edges. With the *n*-butanol solvents less platinum could be tolerated, owing to the closeness of the platinum and palladium bands. Excellent separations were obtained with hexone solvent on a mixture of platinum (1·4 mg of platinum on strip) containing about 0·1 per cent. each of rhodium and palladium (Table VII).

Palladium—The width of the palladium band when the hexone solvent was used increased if the quantity exceeded 200 μg, but unlike the other metals, both leading and trailing edges of the band remained very sharp. A mixture containing palladium (3 mg of palladium on strip) and about 0·1 per cent. each of platinum and rhodium was analysed successfully (Table VII). With the *n*-butanol solvents, the same limitations applied as for platinum.

Rhodium—With all solvents the width of the rhodium band increased with an increase of loading by diffusion towards the palladium band. The upper limits were 3 mg for the hexone solvent and 5 mg for the *n*-butanol solvents. Mixtures of rhodium containing about 0·1 per cent. of platinum, palladium and iridium were successfully analysed (see Table VII).

Iridium—The upper limits for iridium were lower for both solvent mixtures. The limitations of the hexone solvent have already been discussed; with the *n*-butanol solvent the limit was 500 μg, and above this backward trailing caused interference.

Base metals—Analyses have been carried out in the presence of large amounts of commonly occurring base metals that would remain comparatively immobile in one or other of the solvents (see Tables III and IV). Tests have shown that up to 5 mg of some of these metals could be tolerated before distortion of the palladium band occurred. Sodium was only slightly soluble in the solvents and was regarded as an immobile metal.

In the presence of large amounts of metals that move in the centre of the strip, distortion of the platinum-metal bands occurred. This was avoided by a change of solvent, which proved successful in every separation.

Metals that move in or near the solvent front imposed a similar practical limitation. As their quantity increased, the bands occupied more fully the space between the wet and dry solvent fronts, increasing this area above its normal limits. This reduced the space available for the platinum-metal bands, so that not more than 5 mg of base metal could be present on the strip.

LIMITATIONS IMPOSED BY THE DETERMINATION METHODS—

When the analyses were performed at the lowest levels, the final colorimetric solutions were made up to 5 ml; with the reagents recommended, the lower limit of determination with reasonable accuracy when 1-cm cells in a Unicam spectrophotometer were used was 1·0 μg for platinum and rhodium and 0·05 μg for palladium (*p*-nitrosodimethylaniline).

In these procedures some base metals interfered, if present on the strip in similar positions to those metals being determined. This was usually avoided by a change in the choice of developing solvent. The interferences investigated are shown in Table V.

Interferences in the micro-titration method for iridium were also investigated. Up to 100 μg of rhodium or 50 μg of platinum could be present before their colour interfered with the detection of the end-point at the lowest levels of iridium determination. No palladium could be tolerated, since it formed a red precipitate; palladium and iridium were, however, always separate from one another on the strip if the correct conditions were chosen. Osmium did not interfere and up to 20 μg of ruthenium could be tolerated. The lower limit of detection of iridium was 0·5 μg, which gave a positive yellow colour with the indicator, and titrations on known quantities of iridium showed an accuracy of ± 0·5 μg between 1 and 10 μg of iridium and ± 1·0 μg between 10 and 40 μg.

For approximate analyses at very low levels, visual estimation of the colour of the band on the control strip was possible, comparison being made with known standards. This method was surprisingly accurate (± 30 per cent.), probably more so than the spectrophotometric methods at the lowest levels. The lower limit of detection of rhodium, palladium and platinum with stannous chloride reagent was 0·5 μg, and as it was possible to detect, for example, 0·5 μg of platinum in 5 mg of rhodium, *i.e.*, 0·01 per cent. Visual estimation

TABLE V

COLORIMETRIC INTERFERENCES

Method	No significant interference, <1 per cent. relative error	Slight interference, 1 to 5 per cent. relative error	Severe interference >5 per cent. relative error
Platinum by stannous chloride	Fe, Co, Cu	Cr, Ni	Os, Ir, Ru, Rh, Pd, Au, Te, Se
Palladium by thioglycollic acid	Pb, Mn, As, Ir	Rh	Be, Bi, Os, Mo, Pt, Ru, Cu (precipitate)
Palladium by *p*-nitrosodimethylaniline	Fe, Ni, Cu, Ag, Be, Mn, Mo, As	Pt	Au, Bi (precipitate)
Rhodium by stannous chloride	Ni, Mn, V, Ti, Cr, Cu, Co, Fe, U	Ir	Ru

of iridium was also carried out on strips developed with the *n*-butanol - hydrochloric acid - hydrogen peroxide solvent. The sensitivity was surprisingly high, since the iridium formed a very narrow brown band in the wet solvent front; 1 μg of iridium could just be detected by this method. No spraying was necessary, since the solvent maintained the iridium in its fully oxidised and highly coloured form.

TABLE VI

SELECTION OF ANALYTICAL METHOD

Major component of mixture	Upper limit of major component, mg	Analyses required	Solvent* recommended	Notes
Pt (Ir absent)	1·5	Pt, Pd, Rh	Hexone	
Pt (Ir present)	1·5	Pt, Pd, Ir	Hexone	
		Rh	Butanol	
Pd (Ir absent)	3·0	Pt, Pd, Rh	Hexone	
Pd (Ir present)	3·0	Pt, Pd, Ir	Hexone	
		Rh	Butanol	
Rh (Ir absent)	3·0	Pt, Pd, Rh	Hexone	
	5·0	Pt, Pd, Rh	Butanol	
Rh (Ir present)	3·0	Pt, Pd	Hexone	
	5·0	Pd, Ir	Butanol	
	0·5	Rh	Butanol	
Ir	0·2	Pt, Pd	Hexone	
Na	6·0	Pt, Pd, Rh	Hexone	Only minor quantities of platinum metals present
Cu, Co, Ni, Fe	5·0	Pt, Pd, Rh	Butanol	
Zn	5·0	Pt, Pd, Rh	Hexone	

* "Hexone" is *iso*butyl methyl ketone - pentanol - hydrochloric acid, sp.gr. 1·18 (60:10:30), and "butanol" is *n*-butanol saturated with 3 *N* hydrochloric acid. Hydrogen peroxide is included in this solvent if iridium is present.

SELECTION OF ANALYTICAL METHOD—

Taking into account the factors discussed in the last two sections and the position of the bands of some common base metals in the separations, the best methods of analysis were selected for each type of mixture. These are listed in Table VI. For those special mixtures containing base metals not listed in Table VI, the information in Tables III, IV and V gives a guide to the best solvent and determination conditions.

The choice of colorimetric method used for palladium depended on the other metals present near this metal on the strip, on colorimetric interferences of these metals (Table V) and on the sensitivity required.

As regards the presence of osmium and ruthenium, if these have not been removed by distillation or otherwise removed during the preparation of the original solution, not more than 20 μg of ruthenium could be tolerated if iridium was being determined and the hexone solvent used. This could be avoided if the *n*-butanol solvent was used, since the iridium was now removed from the ruthenium. If rhodium was being determined, not more than 2 μg of ruthenium could be tolerated on the strip with either solvent. Osmium interfered seriously with the determination of platinum on account of its position on the strip and its interference in the colorimetric analysis; it would normally be removed by volatisation as the tetroxide on boiling the original solution.

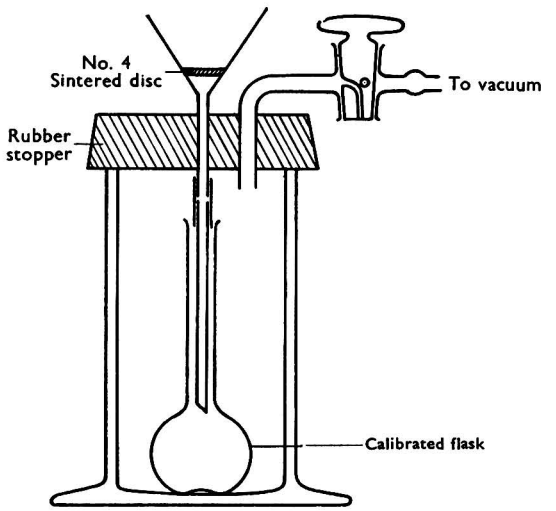

Fig. 4. Extraction apparatus

METHOD

APPARATUS—

Micro-pipettes—Micro-pipettes of 0·05 and 0·01 ml are of sufficient accuracy for most work. When greater accuracy is needed, Agla micrometer syringes should be used.

Chromatographic jars—50 cm tall, 7·5 cm in internal diameter fitted for downward diffusion.[3]

Reagent sprays, glass, of standard design.[3]

Aqueous extraction apparatus—See Fig. 4.

Micro-titration apparatus—See Fig. 5.

REAGENTS—

iso*Butyl methyl ketone* (*hexone*)—Laboratory grade, redistilled, b.p. 116° C.

n-*Butanol*—Laboratory grade, redistilled, b.p. 118° C.

Pentanol (iso*amyl alcohol*)—Analytical-reagent grade.

Hydrogen peroxide, 20 volume—Analytical-reagent grade.

Hydrochloric acid, concentrated—Analytical-reagent grade.

Hydrochloric acid, 5 N, 3 N *and* N—Prepared from the analytical-reagent grade concentrated acid.

Stannous chloride reagent—Dissolve 11·25 g of analytical-reagent grade stannous chloride in 100 ml of 3·5 *N* hydrochloric acid.

Thioglycollic acid—A 2·5 per cent. solution in water.

p-*Nitrosodimethylaniline*—Dissolve 25 mg in 50 ml of ethanol and 50 ml of water.

Sodium acetate solution, M.

Lithium sulphate solution—A saturated solution in sulphuric acid, sp.gr. 1·84.

Hydroquinone solution—Dissolve 0·1424 g of hydroquinone in 500 ml of 1 per cent. v/v sulphuric acid prepared with freshly boiled water.

3 : 3'-*Dichlorobenzidine sulphate indicator*—Warm 0·1 g with 10 ml of 33 per cent. sulphuric acid, and dilute to 100 ml. It is best to recrystallise the reagent from dilute sulphuric acid before use.

TEST SOLUTIONS—

The solutions of chloro acids of the platinum metals used for this investigation were prepared as a series containing about 1 mg of metal per ml; they were prepared from spectrographically pure material.

PROCEDURE FOR SEPARATION—

To the test solution, free from cyanide, potassium and ammonia, in *N* to 5 *N* hydrochloric acid add 1 per cent. w/v of sodium chlorate and make up to a known volume. Transfer accurately 0·05-ml aliquots of the solution to the required number of strips, drawing the tip of the pipette across the strip along a line 7 cm from one end of each 45-cm × 3-cm strip

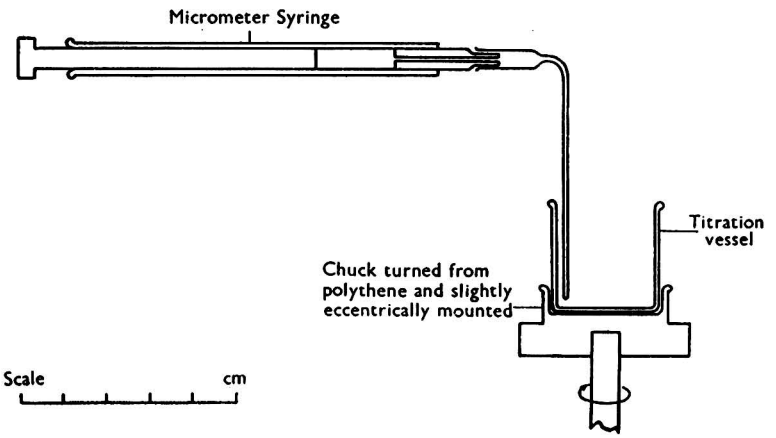

Fig. 5. Micro-titration apparatus

of Whatman's No. 1 filter-paper. The number of strips prepared depends on the analysis required (see Table VI). Two strips are prepared for each analysis, one as a control. Set the strips aside to dry for 1 hour in the air or 30 minutes in a vacuum-desiccator. Desiccator drying is necessary if the atmosphere is humid or if deliquescent salts are present in the mixture in high proportion.

Fold each strip 4 cm from the sample end and place it in the chromatographic jar containing the required solvent for development by downward diffusion. Place the jar in a protected position in which the temperature can be maintained between 22° and 26° C and leave overnight for 14 to 18 hours. By using strips of No. 4 paper this time can be reduced to 6 to 7 hours. This gives slightly wider bands and is not advisable when the quantity of any component of the mixture approaches the upper limit. Remove the strips from the jars and set them aside to dry, preferably by hanging in clips to avoid contamination. The strips are very fragile at this stage if hexone solvent has been used, and they become more brittle as the solvent evaporates. Spray one strip with stannous chloride solution and warm gently to indicate the position of the platinum-metal bands, *viz.*, rhodium, yellow-orange; palladium, brown; and platinum, yellow. If iridium is present and the *n*-butanol - hydrogen peroxide solvent has been used, the position of the metal (brown band) must be marked before spraying, as it will be rendered colourless by the reagent. Lay the other strip or strips on a strip of glass and divide into sections at points between the bands and 1 cm beyond the furthest detected position, using the control strip as a guide. The strip can be divided by placing a small piece of glass across it and lifting the free end with a pair of tweezers, breaking it cleanly along the line. Transfer each section to a 10-ml micro beaker and carry out determinations on these sections as described in the next section.

REMOVAL OF THE METALS FROM THE PAPER—

The aqueous extraction apparatus (Fig. 4) is used to remove the separated metals from each portion of the strip. The same general instructions apply to all metals, the only variation

being the type and order of extraction solutions; these are given in the next section. The volume of the calibrated flask should be chosen to give reasonable light transmission in the final colorimetric determination, 5 or 10-ml flasks normally being used. The extract for the determination of iridium is collected in a tall tube (made from a specimen tube 7·5 cm × 2·5 cm in diameter) provided with a lip.

Add the first extraction solution to the paper, cover the beaker with a watch-glass and warm it on a steam-bath for 10 minutes unless otherwise stated. Break up the paper to a coarse pulp with a small glass rod, which is left in the beaker. Prolonged heating causes the formation of a fine pulp that is difficult to filter off. Allow the pulp to settle and filter the mixture through the funnel of the extraction apparatus directly into a calibrated flask, applying gentle suction; finally transfer the pulp to the funnel, pressing it into a wad with the glass rod. Release the vacuum and add the second extraction solution to the funnel, transferring it via the beaker. Leave to soak for a minute then filter into the flask. Repeat this stage with the other extraction solutions. Remove the flask, make up to the mark with water and measure the transmittance on the spectrophotometer. For iridium analyses remove the tube and wash the filtrate into the titration vessel with the minimum amount of water.

Extraction solutions (volumes for 5-ml flask; alter the volumes in proportion for other sizes of flask)—

Rhodium (upper section)—

1. 1 ml of stannous chloride solution + 1 ml of 5 *N* hydrochloric acid. Heat for 30 minutes.
2. 1 ml of 5 *N* hydrochloric acid.
3. 1 ml of water.

Palladium (*centre section*) *by thioglycollic acid method*—

1. 1 ml of *N* hydrochloric acid + 1 ml of water.
2. 1 ml of thioglycollic acid solution.
3. 1 ml of water.

Palladium (*centre section*) *by* p-*nitrosodimethylaniline method*—

1. 1 ml of sodium acetate solution + 1 ml of water.
2. 0·1 ml of *p*-nitrosodimethylaniline + 0·9 ml of water.
3. 1 ml of water.

Platinum (*lower section*)—

1. 1 ml of 5 *N* hydrochloric acid + 1 ml of water.
2. 1 ml of stannous chloride solution.
3. 1 ml of water.

Iridium (*upper or lower section according to solvent used*)—

1. Add 0·2 ml of liquid bromine to the paper, leave to soak for 5 minutes with the beaker covered, then add 1·8 ml of water.
2. 1 ml of *N* hydrochloric acid.
3. 1 ml of *N* hydrochloric acid.

COLORIMETRIC ANALYSIS—

All final measurements are made on a Unicam spectrophotometer or similar instrument, 1-cm cells (silica for palladium by thioglycollic acid and glass for the others) being used and comparisons being made against reagent blanks. Prepare standard curves, using the same reagents as were used in the aqueous extraction method over the ranges given below. In preparing the standard curve for palladium by the *p*-nitrosodimethylaniline method 0·5 ml of 0·1 *N* hydrochloric acid is added to each 5 ml of solution to give the correct pH. During the actual determinations the residual acid in the paper is sufficient.

The values of wavelength and range are as follows—

	Wavelength	Range in 5 ml of solution, μg
Rhodium	480 mμ	0 to 100
Palladium (thioglycollic acid method)	325 mμ	0 to 50
Palladium (*p*-nitrosodimethylaniline method)	525 mμ	0 to 1·5
Platinum	403 mμ	0 to 100

MICRO-TITRATION OF IRIDIUM—

To the bromine water extract of iridium add 0·4 ml of lithium sulphate solution and heat to fumes of sulphur trioxide, preferably under infra-red lamps in the titration vessel. Some charring will probably occur, because of traces of solvent and decomposed cellulose present. Add 0·2 ml of perchloric acid and heat to fumes again, repeating this until a clear solution is obtained. Add 0·2 ml of perchloric acid, 1 drop of 3 : 3′-dichlorobenzidine indicator and heat to 300° to 320° C. The temperature is fairly critical, and if a lower temperature is used a longer time of heating is necessary; this must be determined by experiment. The solution first turns yellow, then brown. This colour discharges and the final solution is colourless or purple, according to the amount of iridium present. Leave for 10 seconds at this stage, then remove and cool rapidly. Add 2 ml of water and cool again. Transfer the vessel to the titration apparatus and titrate with the hydroquinone solution (diluted 10 times from stock solution, *i.e.*, 0·01 ml of hydroquinone ≡ 1 μg of iridium; the diluted

TABLE VII

ANALYSES OF SYNTHETIC MIXTURES

Metal present					Metal found			
Base metals, μg	Platinum, μg	Palladium, μg	Rhodium, μg	Iridium, μg	Platinum, μg	Palladium, μg	Rhodium, μg	Iridium, μg
0	40·9	46·6	59·7	0	41·0	47·0	59·5	—
0	**253·0**	1·2	3·0	0	251·0	1·4	2·7	—
0	**1400·0**	1·8	1·5	0	—	2·0	1·5	—
0	**1190·0**	0·1	0	0	—	0·1	—	—
0	**545·0**	2·1	2·7	1·8	—	1·9	2·5	1·5
0	**422·0**	3·5	4·2	8·7	—	3·5	4·0	8·0
0	**197·0**	8·2	9·9	18·8	—	8·0	10·2	18·0
0	3·0	**290·0**	6·0	—	3·0	290·0	6·0	—
0	2·0	**3170·0**	3·0	—	2·2	—	3·2	—
0	10·0	**2900·0**	2·4	5·0	9·8	—	2·5	4·0
0	6·1	4·5	269·0	—	6·5	4·8	272·0	—
0	2·0	2·3	**2500·0**	—	2·2	2·5	—	—
0	0	0	**5000·0**	1·0	—	—	—	1·0
0	3·5	3·5	**2500·0**	2·5	4·0	3·0	—	2·0
0	4·1	2·0	2·4	**135·0**	4·2	2·0	2·2	—
Na, 6000	19·0	18·6	23·9	—	18·5	19·0	23·0	—
Zn, 5000	5·7	1·9	2·4	—	5·0	2·3	2·2	—
Cu, 2500	9·5	4·9	4·2	—	9·2	5·0	4·4	—
Ni, 5000 Cu, 250 Co, 250 Fe, 250	4·1	2·5	—	—	4·8	2·5	—	—

A dash indicates that no analysis has been carried out.
* The major component is in bold type.

hydroquinone solution is standardised by a series of titrations against known quantities of iridium) first to discharge the purple colour, and then add one drop of indicator to produce a yellow colour; add further hydroquinone solution until this colour is just discharged.

The titration may be repeated after evaporating the solution again to fumes of sulphur trioxide and repeating all stages.

RESULTS OF ANALYSES OF SYNTHETIC MIXTURES

With the procedures described in the previous section, a number of synthetic mixtures containing noble and base metals was analysed. Mixtures containing each metal as a major component were included, the other platinum metals being present only in trace amounts. The results in Table VII show good agreement with the known quantities present.

CONCLUSIONS

This investigation has shown that it is possible to analyse complex mixtures of noble and base metals for platinum, palladium, rhodium and iridium with a high order of accuracy in view of the quantities used. The use of control strips on which approximate visual confirmation analyses may be carried out adds a further advantage to the method.

Many other points of interest have emerged during the investigation, particularly with a view to developing rapid methods of trace analysis of the platinum metals. One of these has been the use of slotted strips, first developed in this laboratory for geochemical analysis,[12] which have been used for the detection of traces of iridium in rhodium with the *n*-butanol – hydrogen peroxide solvent. Rapid separations of iridium from rhodium with simple solvents, such as acetone containing hydrochloric acid, in an atmosphere of bromine also show promise by this method.

Investigations on the larger-scale separation of the platinum metals on cellulose columns have also been made.

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The Determination of Sulphur in Plain Carbon Steel

BY R. BELCHER, D. GIBBONS AND T. S. WEST

A method is described for the determination of sulphur in steel, in which the sulphur is precipitated as barium sulphate after most of the ferric iron has been extracted from hydrochloric acid solution with *iso*amyl acetate. The barium sulphate is filtered off and dissolved in an excess of ammoniacal ethylenediaminetetra-acetic acid; the unused reagent is titrated with standard magnesium chloride solution, Solochrome black being used as indicator.

THERE are three methods available for the determination of sulphur in steel, but the only one that is non-empirical is the gravimetric method, in which the sulphur is finally determined as barium sulphate. Unfortunately, this method is lengthy and is subject to a number of errors, because of the unfavourable conditions under which the barium sulphate must be precipitated.

In this method, a long standing time is necessary for quantitative precipitation of the barium sulphate, because of the high concentration of ferric iron present and the high acidity necessary to prevent its hydrolysis. Co-precipitation of the iron could be avoided by addition of a suitable complexing agent, such as ethylenediaminetetra-acetic acid (EDTA); precipitation could then be effected at a lower acidity, and only the high electrolyte concentration would influence the standing time. The latter effect could possibly be overcome by using an even greater excess of the precipitant. However, the excessive amount of EDTA necessary to form a complex with the large amount of ferric iron present in the usual 5-g sample of steel makes this procedure impracticable.

An alternative procedure would be to effect the precipitation at the minimum acidity possible without hydrolysis of the iron (pH 2 to 3), and then to dissolve the precipitate, together with the co-precipitated iron, in ammoniacal EDTA and re-precipitate the pure

barium sulphate by acidification, according to the method of Přibil and Maricova.[1] When we examined this procedure, however, it was found to be unsuitable, for although quantitative recoveries were obtained, the time occupied was found to be as great as in the original method.

It was also found that in the gravimetric method, even though the conditions recommended in the British Standard method[2] were used, co-precipitated iron was trapped in the barium sulphate. Although this did not have a significant effect on the gravimetric figures, when a titrimetric finish was applied slight interference was noted. Moreover, the standing time can be reduced considerably when excessive quantities of ferric iron are absent, since the precipitation can be effected at a lower acidity.[3] Accordingly, it was decided to attempt removal of the iron before the precipitation. Because it is now possible to evaluate barium sulphate precipitates titrimetrically,[4] we have also sought to apply this method as an alternative to the conventional finish.

Experimental

Removal of ferric iron before precipitation—

Several organic solvents were used to extract ferric iron in continuous liquid - liquid extractors, but all proved to be unsatisfactory for one reason or another. Wells and Hunter[5]

Table I

Recoveries of sulphur from pure solution

5 g of electrolytic iron were added in each case and the solution was extracted with *iso*amyl acetate

Sulphur added, mg	Sulphur found (gravimetric), mg	Sulphur found (titrimetric), mg
1·5	1·48	1·48
2·0	1·98	1·99
2·5	2·49	2·51
2·0	3·01	3·01
3·5	3·49	3·50
4·0	3·98	3·99
4·5	4·51	4·51
5·0	5·02	5·00

have shown, however, that 99·95 per cent. of the ferric iron can be removed from a concentrated hydrochloric acid solution in the presence of sulphuric acid by a single cold extraction in a separating funnel with *iso*amyl acetate. Without the addition of sulphuric acid 99·6 per cent. extraction is attained.

A pure sulphate solution was evaporated to dryness and the residue was dissolved in concentrated hydrochloric acid. The solution was extracted with *iso*amyl acetate, and, after

Table II

Determination of sulphur in steels

Steel sample	Sulphur present, %	Sulphur found, %		
Bureau of Analysed Samples No. 0, mild-carbon steel ..	0·029	0·028	0·029	0·029
Bureau of Analysed Samples No. 1C, mild-carbon steel ..	0·048	0·049	0·048	0·049
Bureau of Analysed Samples No. 2C, medium-carbon steel	0·044	0·043	0·044	0·044
Bureau of Analysed Samples No. 3B, high-carbon steel ..	0·028	0·028	0·027	0·029
Bureau of Analysed Samples No. 21, high-carbon steel ..	0·050	0·050	0·049	0·049
Bureau of Analysed Samples No. 21A, high-carbon steel ..	0·054	0·054	0·053	0·054
B.C.S. 228, free-cutting steel	0·075	0·076	0·076	0·074
B.C.S. 240, medium-carbon steel	0·041	0·040	0·041	0·041
B.C.S. 260, iron	0·006	0·006	0·005	0·005

a further evaporation to dryness, the residue was dissolved in water and the sulphate was determined by the conventional procedure, the mixture being set aside overnight. There was no loss of sulphate ion. This procedure was repeated in the presence of ferric iron, and the sulphate recoveries again corresponded to the amounts of sulphate originally taken.

As several workers have suggested that barium sulphate can be precipitated quantitatively, with a fairly short standing time, provided that the electrolyte concentration is not abnormally high, and that the usual acidity at which barium sulphate is precipitated (0·05 *N*) is maintained, an attempt was made to shorten the standing time employed in the above procedure. It was shown that, even with the small amounts of sulphur encountered in steel analysis, a standing time of 1 to 2 hours gave perfectly satisfactory results under the conditions described.

TITRIMETRIC PROCEDURE—

It has already been shown[4] that barium sulphate precipitates may be evaluated titrimetrically, by dissolving the barium sulphate in a known amount of a standard solution of EDTA and titrating the unused EDTA with a solution of magnesium chloride, with Solochrome black as indicator.

The titrimetric procedure was therefore applied to the determination of sulphur in synthetic sulphate - iron samples, and the results, shown in Table I, were satisfactory.

In the gravimetric sulphur method, silicates interfere by forming insoluble barium silicate. This is usually overcome by removing the silicate as insoluble silica. In the titrimetric method the silica is also rendered insoluble. But it is not necessary to filter it off, for it is insoluble in ammoniacal EDTA. It is essential, however, to convert it completely to insoluble silica, otherwise high results are obtained, owing to the formation of barium silicate, which is soluble in ammoniacal EDTA.

The method was applied to the determination of sulphur in a variety of plain steels. Satisfactory results were obtained, as shown in Table II.

It should be noted that, when moderate amounts of certain interfering ions, in particular chromium, are present, their effect may be overcome by carrying out the precipitation of the barium sulphate in the presence of EDTA.[1] When excessive amounts are present, the contaminated precipitate must be redissolved in ammoniacal EDTA and a pure precipitate obtained by acidification. The method might be applied to highly alloyed steels by employing this modification.

METHOD

REAGENTS—

Nitric acid, concentrated.
Hydrochloric acid, concentrated.
iso*Amyl acetate.*
Hydrochloric acid, 2N.
Barium chloride solution—A 20 per cent. solution of the analytical-reagent grade salt.
Ammonia, 9 M.
Ethylenediaminetetra-acetic acid (*disodium salt*), 0·02 M.
Magnesium chloride, 0·01 M—A solution of the analytical-reagent grade salt, standardised against recrystallised EDTA.
Solochrome black indicator—A 0·5 per cent. solution in ethanol, which should be freshly prepared every 3 days.
Potassium nitrate—Analytical-reagent grade.

PROCEDURE—

Weigh 5 g of steel into a 600-ml beaker containing 0·1 g of potassium nitrate, and dissolve it in a mixture of 35 ml of concentrated nitric acid and 25 ml of concentrated hydrochloric acid. Digest, and evaporate the solution as in the British Standard method.[2] Bake the residue for 30 minutes at 300° C until no more acid fumes are evolved.

Dissolve the residue in 40 ml of concentrated hydrochloric acid (the mixture must not be warmed excessively or the acidity will be reduced; fuming hydrochloric acid should preferably be used or, alternatively, the solution should be re-saturated with hydrochloric acid gas before the extraction) and transfer to a separating funnel with the aid of 10 ml of concentrated hydrochloric acid. Shake the solution with 200 ml of *iso*amyl acetate, and finally wash the organic layer with 20 ml of concentrated hydrochloric acid. Combine the two acid layers.

Evaporate the acid solution to dryness in a 100-ml conical beaker, and bake for a further 10 minutes to ensure that all the silica is rendered insoluble. Dissolve the residue in 50 ml of water.

Acidify the solution with 1 to 2 ml of 8 *N* hydrochloric acid, heat it to boiling, treat it with 2 ml of 20 per cent. barium chloride solution and set it aside for 2 hours. Filter off the precipitated barium sulphate on a paper-pulp pad; wash the filter with water to remove excess of barium salts, and suck the precipitate dry under slightly reduced pressure from a water-pump.

Transfer the pad and precipitate back to the original conical beaker and add a minimum twofold excess of EDTA solution and then 5 ml of 9 *M* ammonia solution. Boil the mixture for 5 to 10 minutes. Cool the beaker in running water and, if necessary, make the solution ammoniacal. Add 10 to 12 drops of Solochrome black indicator and titrate with magnesium chloride solution to a clear red colour.

Good results were also obtained by using glass filter-mats (supplied by Hurlbut Paper Co., U.S.A.) supported on an ordinary Gooch crucible. These had the advantage of allowing the end-point to be seen more clearly than when the paper pulp is present, although the latter has never given undue trouble by obscuring the end-point. As far as is known, these filter-mats are not available in this country, and so the paper-pulp method, which has been used throughout most of the work described in this paper, is recommended.

1 ml of 0·02 *M* EDTA solution $\equiv$ 0·6412 mg of sulphur.

A blank determination must be carried out on the reagents used.

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The Absorptiometric Determination of Polyethyleneglycol Mono-oleate

By E. G. BROWN and T. J. HAYES

The absorptiometric determination of polyethyleneglycol mono-oleate in aqueous solution is described. The procedure depends on the formation under controlled conditions of a blue complex between the compound and ammonium cobaltothiocyanate, this complex being extracted into chloroform. Measurement of the optical density of the blue chloroform solution at either 318·5 or 620 mμ then gives the concentration of the polyethyleneglycol mono-oleate, and the procedure is applicable at concentrations from 0 up to 1 g per litre. The method is applicable in the presence of low molecular weight polyethyleneglycol monoesters, triethyleneglycol monostearate having been studied in detail in this connection. If necessary, the method can be adapted to a Spekker absorptiometer, when the complete determination takes about 45 minutes. The qualitative application of the procedure to other compounds is briefly considered.

An aqueous solution of polyethyleneglycol mono-oleate has been used in these laboratories for the experimental softening of viscose rayon yarn, and in this connection a speedy and accurate method for its determination in solution was required. Published methods for the determination of polyethyleneglycols and related compounds may be classified under the three main headings gravimetric, colorimetric and volumetric.

Shaffer and Critchfield[1] observed that high molecular weight polyethyleneglycols (Carbowax compounds, Carbide and Carbon Chemicals Company, U.S.A.) gave a precipitate with silicotungstic acid in the presence of barium chloride and hydrochloric acid and they elaborated a gravimetric procedure based on this principle. The gravimetric method of Oliver and Preston[2] is based on precipitation with phosphomolybdic acid in a barium chloride - hydrochloric acid medium. Haakh, von Candié and Möbus precipitated ethylene oxide products with a resorcinol - glucose condensation compound and determined the resultant complex gravimetrically.[3]

Colorimetric procedures include that of Shaffer and Critchfield,[1] who precipitated the glycol with phosphomolybdic acid and determined the molybdenum content of the precipitate. A similar procedure is due to Stevenson,[4] who also described a second colorimetric method; both his methods are used only for the non-ionic detergent Lissapol N (I.C.I. Ltd.). Coppini and Cameroni[5] determined Carbowaxes 1500 and 4000 by precipitation with potassium ferrocyanide and determination of the excess of ferrocyanide with ferric chloride. MacAllister and Lisk[6] determined polyoxyethylene stearate in dilute solution by the formation of a complex with the amylose fraction of potato starch, the amylose not involved in the complex then being free to form an amylose - iodine complex, which was measured colorimetrically.

A routine volumetric method has been published by Schönfeldt,[7,8] who precipitated ethylene oxide adducts with excess of potassium ferrocyanide and titrated the unused reagent with zinc sulphate solution. Coppini and Grassi[9] determined certain Carbowax compounds iodimetrically.

The principle disadvantages of published methods are that they are slow, are often not capable of high accuracy and do not differentiate between mixtures of polyethyleneglycol products.

We have investigated a procedure for the rapid determination of polyethyleneglycol mono-oleate based on the qualitative test of van der Hoeve[10,11] with ammonium cobaltothiocyanate. It was observed by I. P. Forshaw of this Department that polyethyleneglycol mono-oleate reacted with ammonium cobaltothiocyanate to yield a blue precipitate, which could then be extracted into chloroform to give a blue solution, the reagent itself not being extracted. Further work led to a precise and accurate absorptiometric method for the determination of polyethyleneglycol mono-oleate. This method was shown to be applicable in the presence of low molecular weight polyethyleneglycol monoesters, which did not react with the reagent. Triethyleneglycol mono-stearate was studied in detail in this connection.

This observation is of importance, because there are no references in the literature to the analysis of mixtures of polyethyleneglycol products.

THE AMMONIUM COBALTOTHIOCYANATE TEST—

Gnamm[12] first described the ammonium cobaltothiocyanate reaction as a test for alcohols, ketones and esters. The test was adapted by van der Hoeve[10,11] to polyethyleneglycols and described also by Wurzschmitt.[13] The reaction is stated[14] to be available as a quantitative method, but it has not proved possible to trace the source of this suggestion. There is a wide variation in the composition of the reagents proposed by the various workers. These are summarised in Table I.

TABLE I

PROPOSED AMMONIUM COBALTOTHIOCYANATE REAGENTS

Author	Ammonium thiocyanate, g per litre	Cobalt nitrate hexahydrate, g per litre	Molecular ratio of ammonium thiocyanate to cobalt nitrate hexahydrate
Gnamm[12]	347	1·4	946 to 1
Van der Hoeve[10,11]	174	2·8	236 to 1
Wurzschmitt[13] ..	174	28	23·6 to 1

There is some discrepancy in the literature regarding the test. Van der Hoeve[10,11] states that the test solution is shaken vigorously with the reagent and that after 2 hours the

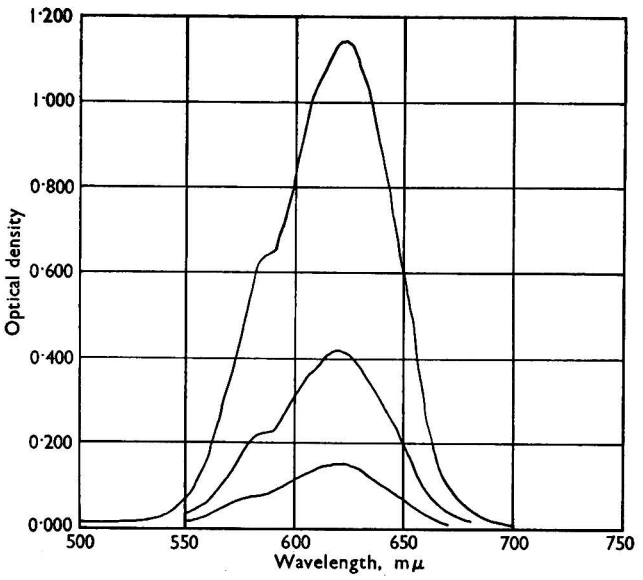


Fig. 1. Absorption spectra of polyethyleneglycol monooleate - cobaltothiocyanate complex in chloroform. Absorption maximum at 620 mμ

colour of the remaining liquid should be blue, and not red-violet, if a polyethyleneglycol product is present. A blue precipitate indicates the presence of cation-active compounds. Wurzschmitt,[13] however, considers the production of a sky-blue precipitate as evidence of polyethyleneglycols and similar compounds. Van der Hoeve[10] showed that the higher the degree of polymerisation, the less was the amount of polyglycol required to give a positive test. In his subsequent paper,[11] this author states that the importance of the test for single substances has greatly decreased.

EXPERIMENTAL

PRELIMINARY INVESTIGATIONS ON THE AMMONIUM COBALTOTHIOCYANATE PROCEDURE—

Preliminary work on the proposed procedure with van der Hoeve's reagent gave unsatisfactory results; the reagent due to Wurzschmitt proved to be more suitable. Experiments showed the following factors to be of importance in obtaining reproducible results: amount

of reagent, ratio of reagent volume to test volume, volume of extracting solvent and method of removal of water from chloroform solutions of the complex. Later it was found that the temperature of the solution before the extraction with chloroform was important. The optimum reagent composition was also studied.

All the work described in this paper was done on a polyethyleneglycol mono-oleate corresponding to a nominal molecular weight of 1000 (based on the original glycol) supplied by the Gemec Chemicals Company. All the optical measurements were made with the Unicam SP500 spectrophotometer.

Absorption spectrum of the polyethyleneglycol mono-oleate - cobaltothiocyanate complex—

The blue chloroform solution of the polyethyleneglycol mono-oleate - cobaltothiocyanate complex shows an absorption maximum at 620 mμ. Fig. 1 shows absorption spectra of the complex at various levels of optical density. In addition, there is an absorption peak in the ultra-violet region at 318·5 mμ, which corresponds to an optical density about six times as great as at 620 mμ (see Fig. 2). Hence, the possibility exists of determination both at 318·5 mμ and at 620 mμ, with much greater sensitivity in the ultra-violet region.

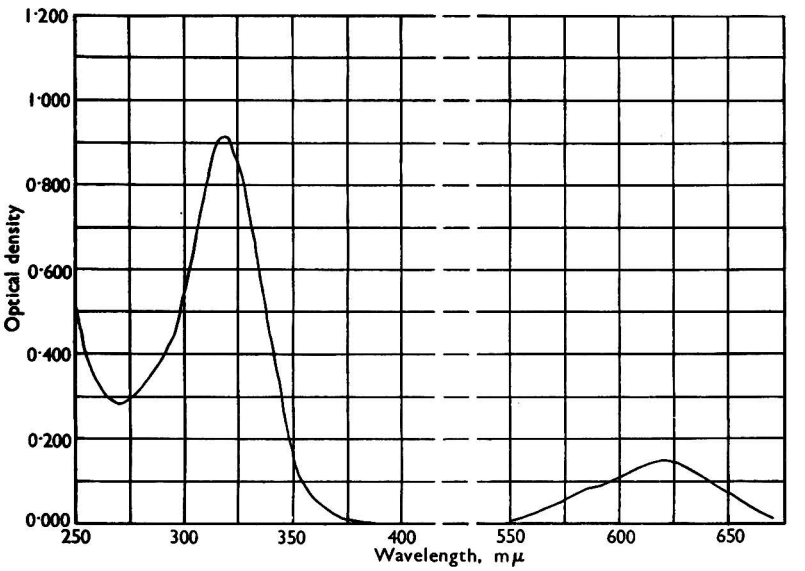


Fig. 2. Absorption spectrum of polyethyleneglycol mono-oleate - cobaltothiocyanate complex in chloroform. Absorption maxima at 318·5 and 620 mμ

Removal of water from the chloroform extract—

The chloroform extract of the polyethyleneglycol mono-oleate - cobaltothiocyanate complex is usually turbid, owing to suspended water droplets. This leads to poor reproducibility in the measured optical density. Any attempt to remove this water by filtration through glass-wool or filter-paper or by drying with anhydrous sodium sulphate resulted in a large decrease in the optical density, as compared with the original solution, results not being reproducible (see Table II).

It was concluded that substantial amounts of the blue complex were lost by adsorption on the large surface areas presented by the various media used, which pointed to the fact that the compound is itself probably surface active.

However, removal of water by centrifuging proved satisfactory, provided that the centrifuge tube was stoppered to prevent chloroform evaporation. Uncentrifuged solutions gave somewhat high and variable results, but the precision of optical-density reading on centrifuged solutions was excellent. Centrifugation of different portions of the same solution gave results in good agreement. Typical results are shown in Tables III and IV. The centrifuging technique was adopted as a standard method of clearing the solution.

TABLE II

EFFECT OF METHODS OF WATER REMOVAL ON OPTICAL DENSITY OF POLYETHYLENEGLYCOL MONO-OLEATE - COBALTOTHIOCYANATE COMPLEX IN CHLOROFORM

Solution	Method of removal of water droplets	Optical density
A	a. Original solution	0·208
	b. Filtered through filter-paper containing anhydrous sodium sulphate	0·129
	c. Shaken with anhydrous sodium sulphate	0·193
B	a. Original solution	0·303
	b. Filtered through glass-wool	0·241
	c. Repeat filtration through glass-wool	0·265
C	a. Original solution	0·348
	b. Filtered through filter-paper	0·239
	c. Centrifuged (unstoppered)	0·351

TABLE III

EFFECT OF CENTRIFUGING ON OPTICAL DENSITY OF POLYETHYLENEGLYCOL MONO-OLEATE - COBALTOTHIOCYANATE COMPLEX IN CHLOROFORM

Solution	Condition	Optical density
A	"Wet"	0·304, 0·308, 0·310, 0·313, 0·312
	Centrifuged	0·248, 0·251, 0·250, 0·249
B	"Wet"	0·256, 0·260, 0·267
	Centrifuged	0·253, 0·251, 0·251

TABLE IV

REPRODUCIBILITY OF MEASUREMENT OF OPTICAL DENSITY OF CENTRIFUGED SOLUTIONS OF POLYETHYLENEGLYCOL MONO-OLEATE - COBALTOTHIOCYANATE COMPLEX IN CHLOROFORM

Solution		Optical density
A	Aliquot 1	0·203
	Aliquot 2	0·204
B	Aliquot 1	0·201
	Aliquot 2	0·203
C	Aliquot 1	0·199
	Aliquot 2	0·202

STABILITY OF POLYETHYLENEGLYCOL MONO-OLEATE - COBALTOTHIOCYANATE COMPLEX—

The blue complex in chloroform shows a slight increase in optical density with time. For example, a solution of initial optical density 0·450 showed a 2·4 per cent. increase in 3¼ hours; one of optical density 0·269 showed a 1·5 per cent. increase in 3 hours. This may be due to slight evaporation of the chloroform solvent, even though the solutions were kept in stoppered flasks. Hence it is advisable to measure the optical density of the solution immediately after preparation of the complex.

STUDY OF REACTION VARIABLES—

After the establishment of the above basic results, the effect of the following variables on the procedure was studied: volume of test solution, volume of reagent, time of reaction and volume of chloroform used for extraction. The following conclusions were reached, Wurzschmitt's reagent being used in the tests.

Volume of test solution—The volume of solution taken for analysis should be constant; 5 ml was chosen as standard.

Volume of reagent—Fig. 3 shows the increase in optical density (at 620 mμ) for 5ml of a 1·01 g per litre polyethyleneglycol mono-oleate solution, treated with various volumes of Wurzschmitt's reagent for 1 minute and set aside for 5 minutes, extracted with chloroform and made up to 25 ml. Above 10 ml of reagent there is a small constant increase in optical density up to 25 ml. As there is no blank on the reagent, the reason for this is not clear. The amount of reagent used was standardised at 20 ml.

Time of reaction—The reaction of the glycol with ammonium cobaltothiocyanate may be considered complete after shaking for 1 minute with 20 ml of the reagent and then setting aside for 5 minutes. The optical density after 1 hour is 2·8 per cent. greater than that after 5 minutes and there is no further change after 24 hours. The reaction time was accordingly standardised at a total of 6 minutes. The effect of setting the mixture aside for various times was as follows—

Time of standing ..	.. 5 minutes	1 hour	24 hours
Optical density ..	.. 0·250	0·257	0·256

Volume of solvent—At the 1 g per litre level, it was found necessary to use 4 separate 5-ml volumes of chloroform for complete extraction of the complex. These combined extracts were then made up to 25 ml in a calibrated flask. Usually a slight blue scum was formed in the solution after the initial chloroform extraction. This scum was completely insoluble in chloroform; its cause is unknown.

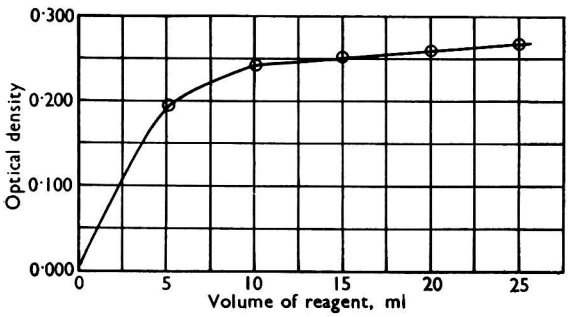


Fig. 3. Effect of volume of reagent on optical density (5 ml of a solution of polyethyleneglycol mono-oleate containing 1·01 g per litre treated with Wurzschmitt's reagent and extracted into 25 ml of chloroform)

One extraction with a single volume of chloroform would be quicker for routine work and about 85 per cent. of the complex is so extracted. It would require careful temperature control and so this modification was not pursued further.

OPTIMUM REAGENT COMPOSITION—

All the above results were obtained with Wurzschmitt's reagent; the optimum reagent composition was then studied.

Determinations were carried out on a 1 g per litre polyethyleneglycol mono-oleate solution, three series of reagents being used, in one of which the cobalt nitrate concentration was constant and the ammonium thiocyanate varied. In the second series of two reagents, the reverse plan was adopted. From a study of the results (obtained at 620 mμ), plotted in the form of two-dimensional graphs relating the two variables to optical density, a series of reagents was prepared in which both cobalt nitrate and ammonium thiocyanate were simultaneously varied. A contour map (Fig. 4) was then drawn, relating concentrations of the two compounds to optical density.

Horizontal cross-sections taken from Fig. 4 show that with increasing concentrations of ammonium thiocyanate, the optical density rises and then becomes constant; a vertical cross-section shows the same effect for cobalt nitrate. The plateau represents conditions in which the optical density is a maximum and least affected by changes in the ammonium thiocyanate or cobalt nitrate concentrations. Optimum proportions of the reagents are such as to give an optical density reading on the plateau, and it is seen that Wurzschmitt's reagent fulfills these conditions. The van der Hoeve reagent is entirely unsatisfactory for quantitative work; the reagent due to Gnamm does not appear on the present map. We chose a reagent consisting of 30 g of cobalt nitrate hexahydrate and 200 g of ammonium thiocyanate made up to 1 litre as the optimum reagent, somewhat further removed from the edge of the plateau than Wurzschmitt proposed. Reagents containing very high concentrations of the two components were considered unsatisfactory in that complete extraction of the blue complex by chloroform then proved difficult. None of the reagents shows a blank value when extracted with chloroform. However, attempts to incorporate acetone or ethanol in the

reagent, thereby increasing the available ammonium cobaltothiocyanate concentration, failed, as then an appreciable blank was given on extraction with chloroform.

CHOICE OF SOLVENT—

The disadvantage of chloroform as a solvent is its volatility. Several other solvents were tested, the results being summarised in Table V for positive or negative extraction.

TABLE V

USE OF VARIOUS SOLVENTS FOR EXTRACTION OF THE POLYETHYLENEGLYCOL MONO-OLEATE - COBALTOTHIOCYANATE COMPLEX

Solvent	Results
o-Dichlorobenzene	positive
Bromoform	positive
Trichloroethylene	positive
Methylene chloride	positive
Benzotrichloride	yellow green extract
Benzyl chloride	positive, but extract slightly yellow green
Carbon tetrachloride ..	negative

o-Dichlorobenzene (b.p. 170° C) was the most promising alternative to chloroform, being relatively non-volatile. The absorption maximum of the complex is shifted to 625 mμ

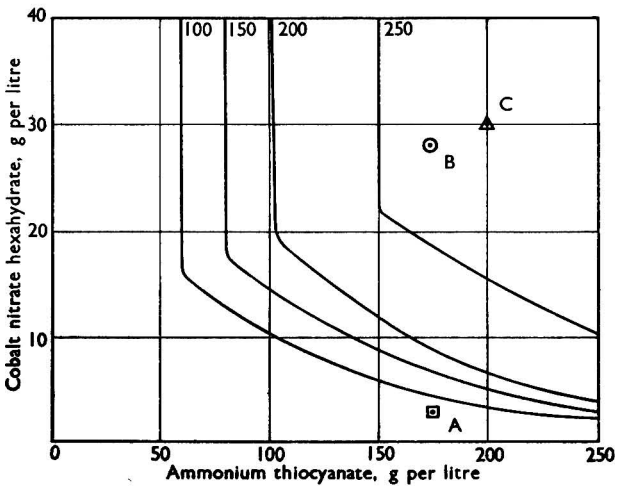


Fig. 4. Contour map showing optimum ammonium cobaltothiocyanate reagent. Contours are optical density × 1000: A, van der Hoeve reagent; B, Wurzschmitt reagent; C, Brown and Hayes reagent

in this solvent. Unfortunately, extraction is less clean with this compound and it is unpleasant to work with. No other solvents offered any advantage over chloroform.

EFFECT OF pH OF SOLUTION ON THE REACTION—

Our sample of aqueous polyethyleneglycol mono-oleate had a pH of 5, that of Wurzschmitt's ammonium cobaltothiocyanate reagent being 3·7. On addition of the reagent to the glycol solution, the pH of the resulting mixture is about 3·9. It was considered that the reagent is so strongly buffered as to render a study of pH effect superfluous. The optimum reagent recommended above had a pH of 3·0.

EFFECT OF TEMPERATURE DURING REACTION AND EXTRACTION—

During the preparation of calibration graphs by the standardised procedure, an occasional anomalous result was obtained. Such points were invariably low. These figures were always obtained during a period of high room temperature (25° C or above). Results are summarised in Table VI, together with some results at low and normal temperatures.

Conclusions are that low temperatures down to 10° C have little effect on the accuracy of the determination, but temperatures over 26° C cause low results, possibly because the complex decomposes.

In the above experiments, owing to local conditions, it was not possible to distinguish between the possible effects of high temperatures and of exposure to sunlight. It is thus

TABLE VI

EFFECT OF TEMPERATURE ON THE REACTION

Polyethyleneglycol mono-oleate present, g per litre	Polyethyleneglycol mono-oleate found, g per litre	Temperature, ° C	Relative error, %
0·946	0·936	20	−1·1
0·946	0·946	20	—
0·946	0·859	28	−9·2
0·824	0·738	27	−10·0
0·754	0·673	26	−10·7
0·733	0·731	23	−0·3
0·733	0·732	21	−0·1
0·522	0·515	15	−1·3
0·522	0·510	10	−2·3

recommended that for accurate results the determination should be carried out with solutions and room temperature as near as possible to 20° C and in the absence of direct sunlight.

METHOD

REAGENTS—

Ammonium cobaltothiocyanate solution—Dissolve 15 g of AnalaR cobalt nitrate hexahydrate and 100 g of AnalaR ammonium thiocyanate in water and adjust the temperature to 20° C. Make up to 500 ml with distilled water.

Chloroform—AnalaR, suitable for ultra-violet spectrophotometry.

PROCEDURE—

In a 60-ml separating funnel place by pipette 20 ml of ammonium cobaltothiocyanate reagent at 20° C and then by pipette 5 ml of polyethyleneglycol mono-oleate solution at 20° C. Shake the whole solution vigorously for 1 minute, timed by a stop-watch, and set aside for a further 5 minutes at 20° C. Add 4 ml of chloroform, shake vigorously for 1 minute and set aside for the two layers to separate, swirling to facilitate this. Run off the chloroform layer into a 25-ml calibrated flask. Repeat this operation with a further 3 or 4 separate 4-ml portions of chloroform. Rinse the stem of the funnel with a small volume of chloroform, adding the rinsings to the flask, and stand the flask in a water-bath at 20° C for 5 minutes. Finally make up to the mark with chloroform. Shake well and transfer a portion of the blue solution to a stoppered 5-ml centrifuge tube. Centrifuge the solution free of water droplets (which takes 1 to 2 minutes) at a minimum of 100 ***g***. Measure the optical density of the resultant solution at 318·5 and 620 mμ on the Unicam SP500 spectrophotometer or other suitable instrument, using 1-cm quartz and glass cells, respectively, and chloroform as the reference solution.

From the appropriate calibration graph read off the amount of polyethyleneglycol mono-oleate in mg corresponding to the measured optical density, and calculate the glycol concentration in g per litre.

PREPARATION OF CALIBRATION GRAPHS—

From a given batch of polyethyleneglycol mono-oleate weigh out the appropriate quantity for the required concentration into a 250-ml beaker. (As the polyethyleneglycol mono-oleate of the molecular weight used in our work is a waxy solid, weighing is best done by difference from a weighing bottle, a thin glass rod being used, the weight taken being as close as possible to the weight required.) Add about 50 ml of water and warm to 40° C on a steam-bath, stirring until all solid is dissolved. Cool and make up to 500 ml in a calibrated flask. Take 5 ml of this solution to carry through the given procedure. Repeat with separate weighed portions of polyethyleneglycol mono-oleate and plot all results as calibration graphs relating optical density to mg and also g per litre of the compound. Prepare a graph up to 5 mg in a

5-ml aliquot, *i.e.*, 1 g per litre, for the 620-mμ measurement and up to 3 mg in a 5-ml aliquot, *i.e.*, 0·6 g per litre, for the 318·5-mμ measurement.

Results

Calibration graphs prepared from a purified sample of polyethyleneglycol mono-oleate are shown in Figs. 5 and 6. Statistical analysis of the data of the 318·5-mμ curve up to 3 mg gives a standard deviation of 0·035 mg about the least-squares regression line, *i.e.*, the equivalent of 0·0069 g per litre; statistical analysis of the 620-mμ curve up to 5 mg gives a standard deviation of 0·047 mg about the least-squares regression line, *i.e.*, the equivalent of 0·0094 g per litre.

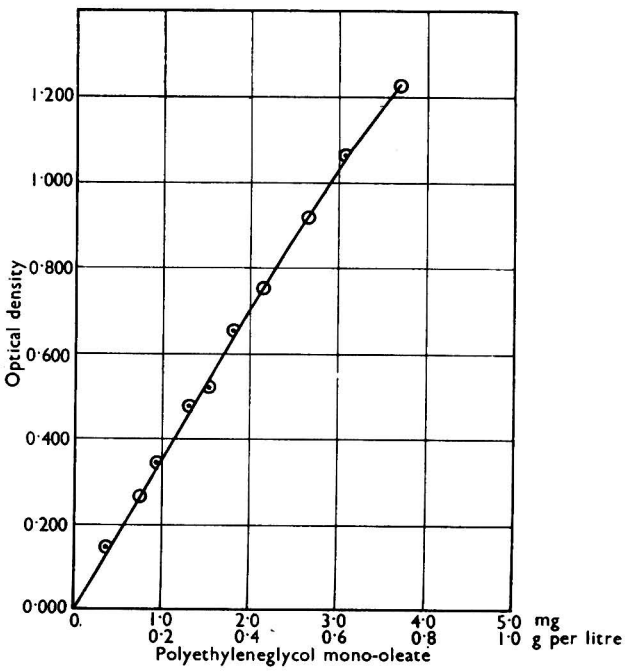


Fig. 5. Calibration graph for polyethyleneglycol mono-oleate - cobaltothiocyanate complex at 318·5 mμ

The 620-mμ graph obeys Beer's law up to about 5 mg in a 5-ml aliquot, *i.e.*, 1 g per litre, the 318·5-mμ graph up to about 3 mg in a 5-ml aliquot, *i.e.*, 0·6 g per litre.

The average $E^{1\%}_{1\,cm}$ value of the compound at 620 mμ, calculated from the calibration data, is 2·736 $\pm$ 0·032; that at 318·5 mμ is 17·60 $\pm$ 0·22. This gives a ratio of E values of 6·43 to 1 in favour of the ultra-violet measurement.

Discussion

Purification of polyethyleneglycol mono-oleate—

Our calibration values were obtained with a sample of polyethyleneglycol mono-oleate that had been purified by recrystallisation from diethyl ether and dried over phosphorus pentoxide under reduced pressure. This sample was examined for the number of ethylene oxide groups (n) present by hydrolysis with an excess of standard alcoholic potassium hydroxide solution and titration of the unused alkali with standard acid; the molecular weight was calculated and then the number of $-CH_2-O-CH_2-$ groups present was computed, assuming that the compound was present solely as the monoester. The material appeared to be $HO-CH_2-(CH_2-O-CH_2)_{15\cdot0}-CH_2-O-CO-C_{17}H_{33}$, molecular weight 988. (In actual fact the number of $-CH_2-O-CH_2-$ groups (n) should be of the order of 20 to 25, according to the manufacturers.) Storage of the sample resulted in water absorption; it is essential to dry any sample of polyethyleneglycol mono-oleate before preparation of calibration data.

A further method of purification is to dissolve the compound in diethyl ether, filter and pass the resultant yellow solution down a column of activated charcoal. The resultant solution is very pale yellow and yields a practically colourless wax on evaporation of the solvent. The wax is then dried overnight over phosphorus pentoxide under reduced pressure.

VARIATION IN COMPOSITION OF POLYETHYLENEGLYCOL MONO-OLEATE—

Polyethyleneglycol mono-oleate is a compound of varying composition, the average molecular weight depending on the weight proportion of each molecular size present. In practice, the composition depends on the proportions of the various polyethyleneglycols present in the glycol used by the manufacturer in the preparation of the monoester. Two further samples, each representing a given production batch of material, were examined for the number of ethylene oxide groups present.

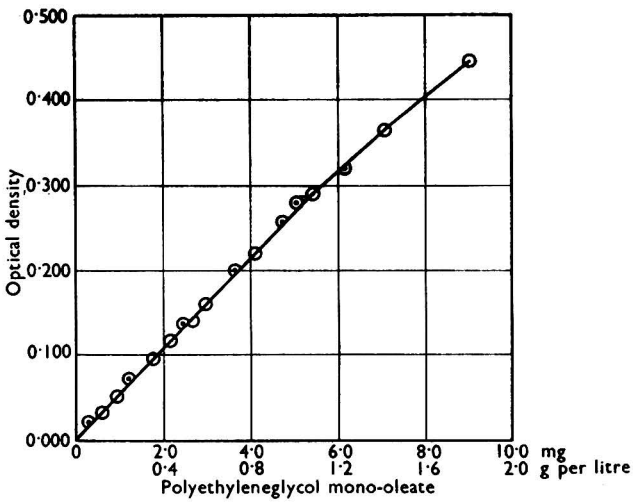


Fig. 6. Calibration graph for polyethyleneglycol mono-oleate - cobaltothiocyanate complex at 620 mμ

One sample gave $n = 19{\cdot}7$ (molecular weight 1193) and the other 20·4 (molecular weight 1230). After purification of this latter sample, n was 19·7 (molecular weight 1193). These figures show a somewhat wide divergence from those of the calibration sample. Accordingly, a solution of the purified sample of known concentration was examined by the standard procedure, the initial calibration graph for determining the amount of polyethyleneglycol mono-oleate being used. This result, and also a similar test on the unpurified compound mentioned above, which show reasonable accuracy, were as follows—

Polyethyleneglycol mono-oleate present, g per litre	0·827	0·689
Polyethyleneglycol mono-oleate found at 318·5 mμ, g per litre	0·821	0·695
Polyethyleneglycol mono-oleate found at 620 mμ, g per litre	0·844	0·716

However, it is considered desirable to prepare a fresh calibration graph for each sample of polyethyleneglycol mono-oleate, each sample to be representative of a given batch of material as prepared by the manufacturerer. Over a period of time this enables information about the batch-to-batch variation of the material to be accumulated.

STABILITY OF POLYETHYLENEGLYCOL MONO-OLEATE SOLUTIONS—

Aqueous polyethyleneglycol mono-oleate solutions at concentrations of 1 and 10 g per litre gradually become turbid on standing. A similar effect is noticed more slowly with less concentrated solutions. However, a solution of 0·73 g per litre concentration proved stable over at least 28 days when examined by the proposed procedure.

Solutions of the glycol for calibration purposes should not be prepared by the dilution of concentrated solutions.

NON-INTERFERENCE OF COMPOUNDS PRESENT IN RAYON-SOFTENING SOLUTIONS—

In the experimental use of a polyethyleneglycol mono-oleate solution for the softening of rayon yarn, the only substance present in addition to the glycol was a tinting dye used to identify the yarn. This colour, a mixture of tartrazine and azogeranine, did not interfere with the procedure, as it was not extracted by chloroform and as was also proved by quantitative recovery of an added amount of the glycol from a solution containing the tinting agent. The use of other dyes would require a separate check to establish their non-interference.

Dissolved cellulosic impurities, which tend to accumulate in the solution during the processing of rayon, also did not interfere, as was shown by similar recovery experiments.

THEORETICAL ASPECTS OF THE REACTION—

Affsprung, Barnes and Potratz[15] determined cobalt as tetraphenylarsonium cobaltothiocyanate and obtained an absorption spectrum in chloroform between 500 and 700 mμ identical with that shown in Fig. 1. According to these and other authors, the blue colour of non-aqueous cobaltothiocyanate solutions is attributable to the $[Co(CNS)_4]''$ anion. It is probable that in our method ammonium cobaltothiocyanate behaves as a dual reagent towards polyethyleneglycol mono-oleate, first forming a polyoxonium compound by means of the ammonium cation and then acting as a precipitant by means of the cobaltothiocyanate anion, in accordance with the view of Wurzschmitt.[13] According to this author, only a proportion of the ethylene oxide groups form oxonium compounds for a given starting compound and reaction. Van der Hoeve,[11] however, appears to dispute the conception of polyethyleneglycol derivatives as oxonium compounds.

Affsprung *et al.*[15] calculated the specific extinction coefficient for the tetraphenylarsonium cobaltothiocyanate complex as 30·1 for a 1 g per litre cobalt solution. On this basis, the empirical formula for the polyethyleneglycol mono-oleate - cobaltothiocyanate complex was calculated as $1{\cdot}3x.[Co(CNS)_4]$ for a molecular weight of 988, dropping to $1{\cdot}1x.[Co(CNS)_4]$ for a molecular weight of 1193, where x represents the polyethyleneglycol mono-oleate grouping.

The absorption maximum at 318·5 mμ is also to be ascribed to the $[Co(CNS)_4]''$ anion; an absorption spectrum of ammonium cobaltothiocyanate in ethanol showed a well defined maximum at 313 mμ and an identical spectrum form to that obtained with the polyethyleneglycol mono-oleate - cobaltothiocyanate complex. Polyethyleneglycol mono-oleate itself only shows an absorption maximum in aqueous solution at about 225 to 230 mμ. The existence of absorption maxima at 313 and 620 mμ for the cobaltothiocyanate anion (in ethanol) was confirmed from the literature.[16]

The claim of Ellis and Gibson[17] that the cobaltothiocyanate anion shows an absorption maximum at 627 mμ independent of cation or solvent could not be substantiated; the maximum is 620 mμ in chloroform, 625 mμ in *o*-dichlorobenzene.

APPLICATION OF THE METHOD TO POLYETHYLENEGLYCOL MONO-OLEATE - TRIETHYLENEGLYCOL MONOSTEARATE MIXTURES

Triethyleneglycol monostearate was chosen as an example to illustrate the application of the procedure in the presence of low molecular weight polyethyleneglycol monoesters.

As noted above, ammonium cobaltothiocyanate either forms a compound with triethyleneglycol monostearate that is not extractable by chloroform or does not form a compound at all. With 1 ml of a 4 g per litre triethyleneglycol monostearate solution and 5 ml of Wurzschmitt's reagent a blue precipitate is formed. This precipitate is not extractable by chloroform. With a very concentrated reagent (40 g per litre of cobalt nitrate hexahydrate and 400 g per litre of ammonium thiocyanate) a very deep blue precipitate is formed, whilst with a weak reagent (40 g per litre of cobalt nitrate hexahydrate and 50 g per litre of ammonium thiocyanate) a pale pink precipitate is given. Neither precipitate is soluble in chloroform. These results indicate a salting-out effect of the reagent on the triethyleneglycol monostearate solution and subsequent adsorption on the precipitate, with no formation of an actual complex between ammonium cobaltothiocyanate and triethyleneglycol monostearate.

EXAMINATION OF SYNTHETIC TRIETHYLENEGLYCOL MONOSTEARATE - POLYETHYLENEGLYCOL MONO-OLEATE SOLUTIONS—

Various synthetic aqueous emulsions of triethyleneglycol monostearate and polyethyleneglycol mono-oleate were examined by the proposed procedure, with the results shown in Table VII.

TABLE VII

ANALYSIS OF SYNTHETIC TRIETHYLENEGLYCOL MONOSTEARATE - POLYETHYLENEGLYCOL MONO-OLEATE SOLUTIONS

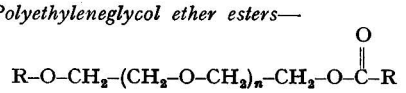
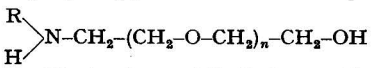
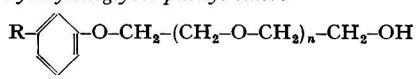
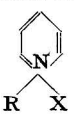
Triethyleneglycol monostearate present, g per litre	Polyethyleneglycol mono-oleate present, g per litre	Polyethyleneglycol mono-oleate found: at 318·5 mμ, g per litre	Polyethyleneglycol mono-oleate found: at 620 mμ, g per litre	Relative error: at 318·5 mμ, %	Relative error: at 620 mμ, %
8·0	0·211	0·231	0·230	+ 9·0	+ 9·0
8·0	0·254	0·258	0·252	+ 1·6	+ 0·8
8·0	0·301	0·321	0·300	+ 6·6	+ 0·3
4·0	0·381	0·387	0·380	+ 1·6	+ 0·3
6·0	0·423	0·436	0·431	+ 3·1	+ 1·9
2·0	0·508	0·493	0·493	− 3·0	− 3·0
6·0	0·601	0·587	0·585	− 2·3	− 2·7
4·0	0·634	0·637	0·650	+ 0·5	+ 2·5
2·0	0·854	—	0·829	—	− 1·9
4·0	0·902	—	0·845	—	− 6·3

TABLE VIII

THE AMMONIUM COBALTOTHIOCYANATE - CHLOROFORM EXTRACTION TEST APPLIED TO VARIOUS COMPOUNDS

Chemical name and general formula	Product	Source	Reaction
Polyethyleneglycols—			
$HO–CH_2–(CH_2–O–CH_2)_n–CH_2–OH$			
Triethyleneglycol	—	A	negative
PEG* 200	—	B	negative
PEG 300	—	B	negative
PEG 400	—	B	slightly positive
PEG 600	—	B	positive
PEG 1000	—	B	positive
PEG 1500 (mixed compound)	—	B	slightly positive
PEG 4000	—	B	negative
Polyethyleneglycol monoesters—			
$R–C(=O)–O–CH_2–(CH_2–O–CH_2)_n–CH_2–OH$			
DEG† monostearate	Nonex 411	B	negative
DEG monolaurate	Nonex 413	B	negative
DEG mono-oleate	Nonex 409	B	negative
PEG 200 monostearate	Nonex 54	B	negative
PEG 200 monolaurate	Nonex 31	B	negative
PEG 300 monostearate	Nonex 28	B	slightly positive
PEG 300 monolaurate	Nonex 27	B	slightly positive
PEG 400 monostearate	Nonex 29	B	positive
PEG 400 mono-oleate	Nonex 30	B	positive
PEG 600 monostearate	Nonex 53	B	positive
PEG 600 mono-oleate	Nonex 54	B	positive
PEG 1000 monostearate	Nonex 63	B	positive
PEG 1000 monolaurate	Nonex 99	B	positive
PEG 1000 mono-oleate	Nonex 64	B	positive
Polyethyleneglycol diesters—			
$R–C(=O)–O–CH_2–(CH_2–O–CH_2)_n–CH_2–O–C(=O)–R$			
PEG 400 distearate	Nonex 76	B	negative
Polyethyleneglycol monoethers—			
$R–O–CH_2–(CH_2–O–CH_2)_n–CH_2–OH$			
Ethyleneglycol monomethyl ether	—	A	negative
PEG 350 monomethyl ether	—	B	positive
PEG 550 monomethyl ether	—	B	positive
PEG 750 monomethyl ether	—	B	positive

TABLE VIII—*continued*

Chemical name and general formula	Product	Source	Reaction
Polyethyleneglycol ether esters—			
			
PEG 200 ethyl ether stearate ester	—	B	negative
PEG 600 ethyl ether stearate ester	—	B	positive
Polyethyleneglycols condensed with a primary aliphatic amine—			
			
Oleylamine + 6·5 ethylene oxide groups	—	C	positive
Oleylamine + 10 ethylene oxide groups	—	C	positive
Oleylamine + 15 ethylene oxide groups	—	C	positive
Oleylamine + 30 ethylene oxide groups	—	C	positive
Dodecylamine + 6 ethylene oxide groups ..	—	C	positive
Dodecylamine + 10 ethylene oxide groups ..	—	C	positive
Dodecylamine + 15 ethylene oxide groups ..	—	C	positive
Polyethyleneglycol phenyl ethers—			
	Lissapol N	D	slightly positive
Quaternary ammonium compounds—			
$(R_4N)^+X^-$ or 			
Phenyltrimethylammonium iodide	—	A	negative
Tetramethylammonium bromide	—	E	negative
Cetyltrimethylammonium bromide	Lissolamine A	D	positive
Lauryldimethylallylammonium chloride	—	F	positive
Lauryldimethylbenzylammonium chloride	—	F	positive
Laurylpyridinium bromide	Fixanol D	D	positive
Cetylpyridinium chloride	—	F	positive

* PEG = polyethyleneglycol.
† DEG = diethyleneglycol.

All numbers in the above table refer to molecular weights based on the original polyethyleneglycol.

Suppliers: A, The British Drug Houses Limited.
B, Gemec Chemicals Company.
C, Aktiebolaget Berol, Sweden.
D, Imperial Chemical Industries Limited.
E, Hopkin and Williams Limited
F, Leda Chemicals.

Statistical analysis of the data in Table VII shows that for 318·5 mμ the over-all standard deviation about the least-squares regression line is 0·011 g per litre. For 620 mμ the overall standard deviation about the least-squares regression line is 0·017 g per litre. These data show satisfactory accuracy for the determination of polyethyleneglycol mono-stearate at concentrations up to about 1 g per litre in the presence of concentrations of triethyleneglycol monostearate up to 8 g per litre.

Triethyleneglycol monostearate forms a poorly dispersed emulsion in water, and evidence is available that there is considerable variation in particle size in such an emulsion. It is our opinion that this non-homogeneity may lead to local variations in the polyethyleneglycol mono-oleate concentration in such a dispersion, owing to adsorption of the mono-oleate on the monostearate particles. This may account for the relatively large errors shown in some determinations in Table VII.

APPLICATION OF THE METHOD TO THE SPEKKER ABSORPTIOMETER—

If necessary, the method may be applied in the visible region, when a Spekker absorptiometer, together with 4-cm cells and Ilford Spectrum No. 607 orange filters are used. A calibration graph prepared in this way obeyed Beer's law at concentrations up to about 3 mg

of polyethyleneglycol mono-oleate, *i.e.*, the equivalent of 0·6 g per litre. In practice the graph may be applied at concentrations up to 5 mg, *i.e.*, the equivalent of 1·0 g per litre.

Extension of the Method

It was expected that other polyethyleneglycols and their derivatives would react with ammonium cobaltothiocyanate and then be extractable with chloroform, according to the number of ethylene oxide groups present in the compound. Some quaternary ammonium compounds would also react. Qualitative tests were performed on a series of available compounds in the following manner.

To 5 ml of a 1 g per litre solution or emulsion of the compound, add 20 ml of ammonium cobaltothiocyanate reagent, shake for 1 minute and set aside for 5 minutes. Extract with 5 ml of chloroform and run off into a dry test tube. Observe the colour of the chloroform extract.

Results are summarised in Table VIII; positive means that the chloroform extract is coloured blue and negative that the chloroform is colourless.

The results in Table VIII show that polyethyleneglycols of molecular weight from 400 up to 1000 give a positive reaction and the compound of molecular weight 4000 gives a negative reaction. For polyethyleneglycol monoesters, only compounds prepared from original polyethyleneglycols of average molecular weight more than 300 give the reaction. The effect of the terminal grouping of substituted polyethylene glycols may be important; polyethyleneglycol 400 monostearate gives a positive reaction, whereas polyethyleneglycol 400 distearate does not react. In general, increasing reactivity of the compounds is parallelled by increasing solubility in water.

All the quaternary ammonium compounds giving a positive reaction contain a long-chain alkyl group.

The Table shows the possibility of elaborating quantitative procedures for both single substances and various combinations of compounds; it is not intended to be exhaustive in its treatment of various classes of compounds, but is an indication of the scope of the present procedure.

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The Micro-determination of Potassium with Dipicrylamine

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An analytical procedure has been developed for the micro-determination of potassium in biological samples. After the sample has been dry-ashed, the potassium is precipitated as its dipicrylaminate salt, and the amount of the precipitate found by colorimetric determination of the intensely coloured dipicrylaminate ion. Interference from elements likely to be present in biological material is very small, and the standard error of a single analysis is less than $\pm$ 2 per cent. for amounts down to 20 μg of potassium. Radioactive potassium was used in the developing and testing of the analytical procedure. These tracer experiments emphasised the undesirability of washing the precipitate with a saturated solution of itself, a practice that is all too common in micro-techniques used for the analysis of biological material.

Potassium is a major inorganic constituent of many tissues, but most conventional methods are unsuitable for its accurate determination in small biological samples. Potassium perchlorate and platinichloride are too soluble; sodium cobaltinitrite gives a precipitate of variable composition, and silver cobaltinitrite cannot be used in the presence of chloride. The use of dipicrylaminate (the anion of hexanitrodiphenylamine) as the precipitating ion suffers from none of these disadvantages: its potassium salt crystallises easily, is relatively insoluble at 0° C, has a constant composition and is easily determined without further chemical treatment, since the dipicrylaminate ion is very highly coloured. A micro-method was therefore developed in this laboratory for the routine determination of potassium as its dipicrylaminate, primarily for the analysis of nerve and muscle. Most of the results obtained by this method have already been published,[1,2] but not a full account of the method itself. Although primarily developed for the determination of potassium in small samples of biological material, it could be equally well used for the analysis of other types of material.

The micro-determination of potassium as its dipicrylaminate had been studied by Kolthoff and Bendix[3] and later, in more detail, by Harington,[4] but neither had obtained very accurate results in the important range 20 to 100 μg of potassium. There was no obvious theoretical reason why the method should not be accurate over this range, so a careful study was made of possible errors in the technique, largely with radioactive potassium-42 as a tracer. Several possible sources of error were discovered, and an analytical procedure was developed to reduce these to a minimum. This procedure was simple and gave reproducible results with amounts of potassium down to 20 μg. If necessary, the sensitivity could have been increased—but only by scaling down the whole procedure. Similar methods for the determination of potassium in serum have been described,[5,6] but these are not so accurate in the range considered in this paper.

Experimental

Procedure for 20 to 100 μg of potassium—

The samples, contained in 5-ml platinum crucibles with loose-fitting lids, were dried at 105° to 110° C and then incinerated in an electric furnace until reduced to a greyish-white ash; small specks of unburnt carbon in no way interfered with the subsequent analysis. The temperature was never allowed to exceed 550° C. Silica crucibles are unsuitable for these small amounts of potassium, since the alkali metals attack the surface, but incineration on a small piece of platinum foil in a silica crucible proved quite satisfactory, and the foil could be re-used almost indefinitely. The precipitation was carried out in the crucible used for incineration. If the sample was incinerated on platinum foil, the ash was first washed into the silica crucible with about 0·5 ml of water and evaporated to dryness.

The ash was dissolved in 2 or 3 drops of water and one drop of reagent added, followed by another drop 10 minutes later. During this 10-minute interval, the outside of the crucible was tapped once or twice to speed up crystallisation. The precipitate should consist solely of small deep-red crystals; if any pale orange precipitate is visible, the crucible should

be warmed in the hand and the fluid gently swirled round until this precipitate has disappeared; this process should be repeated, if necessary, after addition of the second drop. About 10 minutes later a third drop was added, followed by sufficient extra reagent to give about a twofold excess of the dipicrylaminate ion. It was convenient to use dropping tubes with a drop volume of about 30 μl for water and reagent, the optimum numbers of drops then being as follows—

Amount of potassium, μg	20 to 40	40 to 60	60 to 100
Number of drops of water	2	3	3
Number of drops of reagent	3	4	6

The crucibles were then placed in a shallow bath containing water at room temperature, and some 15 to 20 minutes after the final addition of reagent this bath was transferred to a refrigerator with an air temperature of 1° to 5° C. After 2 hours, crushed ice can be added to the water-bath and the filtrations carried out 1 hour later; but it was often more convenient to leave the crucibles in the refrigerator overnight before adding the ice. For all the filtrations the crucibles were kept in the ice-bath and a small sintered-glass filter-stick used, with an asbestos pad similar to that described by Cunningham, Kirk and Brooks[7]; the methods of making and mounting the filter-stick are described later.

All the supernatant liquid was removed by suction and the precipitate washed with four separate drops of distilled water cooled in crushed ice. A small glass rod about 1 mm in diameter was used to ensure that the sides of the crucibles were adequately washed. When washing was complete, the asbestos pad was detached from the filter-stick with a pin and allowed to fall into the crucible. The most convenient way of dissolving the precipitate was to add hot water to the crucible from a wash-bottle and draw the solution through a commercial grade-3 filter-stick directly into a calibrated flask by the syphon arrangement described below. This washing was continued until all the coloured potassium dipicrylaminate had been transferred to the flask. For the colorimetric measurement it was convenient to dilute the solution of potassium dipicrylaminate until it contained the equivalent of 30 to 70 μg of potassium per 100 ml and then measure its extinction in a 1-cm cell with a blue filter (Ilford Nos. 601, 602, 621 or 622). With any of these filters the calibration curve is linear except for some slight deviation at the upper end of the quoted concentration range. Before making up the solution to the desired volume, sufficient 0·1 N sodium hydroxide was added to make the final concentration of alkali about 0·001 N.

This procedure can be used for the determination of larger amounts of potassium, up to about 250 μg. A smaller excess of reagent is adequate and it is an advantage to use reagent of double the normal concentration. It is usually necessary: (*a*) to warm the crucible to obtain a crystalline precipitate, and (*b*) to wash with 5 or 6 drops of ice-cold water to remove excess of reagent. Analysis for these larger amounts of potassium is more tedious and the accuracy obtainable only slightly greater.

Syphoning apparatus—Two types of syphoning apparatus were designed to facilitate the handling of the precipitate; one for washing it and the other for dissolving it. That for washing, shown in Fig. 1, is conveniently held in the hand and suction can be applied to the filter as required by covering the air inlet, A, with the top of the forefinger. The apparatus used for dissolving the precipitate and transferring it to a calibrated flask is shown in Fig. 2. It is convenient to have several connectors, C, of different sizes made from rubber or polythene tubing; these can be made interchangeable to allow flasks of various sizes to be used. (This apparatus is also suitable for the handling of highly radioactive precipitates and is used in the preparation of ^{42}KCl solutions in this laboratory.[1])

Manufacture of the sintered-glass filter-sticks—A modification of the technique described by Kirk[8] was used. The powdered glass was prepared by grinding up globules produced by dropping molten soft glass into a mortar containing cold water. A 4-inch length of 4-mm diameter Pyrex-glass tube, sealed at one end, was about one-quarter filled with fine sand. This sand was covered with sufficient powdered glass to form a disc about 2 mm thick, and the tube was nearly filled with more sand. The tube was held vertical during filling and frequently tapped to pack the contents down. A short plunger, *e.g.*, a 2-inch wire nail, was inserted into the open end. The lower part of the tube was cautiously heated in a flame until a dull red glow from the powdered glass was just visible. After a few seconds at this temperature, the tube was allowed to cool very gradually by slowly moving to progressively cooler parts of the flame. Throughout this sintering process the tube was rotated in the fingers and gentle pressure was maintained on the plunger. When the tube was cold, the

sealed end was broken off as close as possible to the sintered disc and any excess of tubing ground off on a fine grindstone. When not in use, the filter-sticks are best stored in dilute hydrochloric acid; but they must be well washed with distilled water each time to remove all trace of acid.

Preparation of the reagent—Commercial dipicrylamine was recrystallised from glacial acetic acid (this recrystallisation was found to be essential for accurate work). A solution of 0·3 g of Specpure lithium carbonate dissolved in 100 ml of water was warmed with 1 g of dipicrylamine and boiled gently until all the dipicrylamine had dissolved. The dipicrylaminate in 1 μl of this solution is equivalent to about 1 μg of potassium.

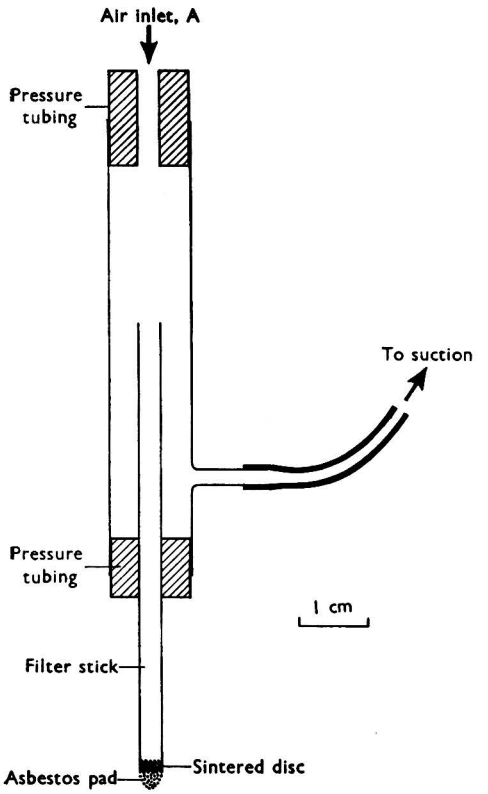

Fig. 1. Syphon apparatus for filtering off and washing the precipitate

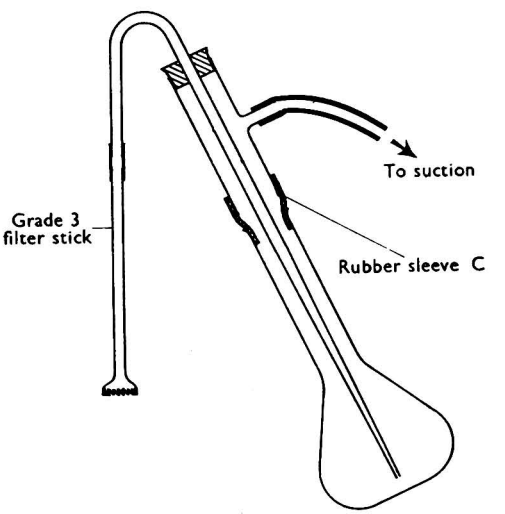

Fig. 2. Syphon apparatus for transferring the dipicrylamine solution to a calibrated flask

ANALYSIS WITHOUT FILTRATION—

An alternative technique suitable for the analysis of some samples was originally suggested by Amdur[9]; if an accurately known amount of dipicrylaminate is used, the potassium precipitated can be calculated from the amount of dipicrylaminate remaining in the supernatant liquid. The following procedure has been found to be very satisfactory for the determination of 40 to 50 μg of potassium. Other amounts can be similarly determined by appropriate alteration of the volumes used.

A 120-μl portion of a solution containing about 10 millimoles per litre of potassium was placed by pipette in a 1-ml micro-beaker, followed by 50 μl of the lithium dipicrylaminate reagent. A further 50 μl of reagent was added about 10 minutes later and a rubber cap fitted over the beaker to prevent evaporation. Half an hour later the beaker was transferred to a refrigerator at a temperature of 0° to 4° C for a minimum of 3 hours (and overnight if convenient). A 50-μl portion of the supernatant liquid was then withdrawn, diluted to 25 ml and the extinction of this solution measured. Samples of a standard solution of 10 millimolar potassium chloride and of distilled water were similarly treated, the supernatant liquid sample from the latter being diluted to 50 ml. If E_v, E_s and E_w represent the extinction

obtained with unknown, standard and water, respectively, the millimolar concentration of potassium in the unknown equals—

$$10 \times \frac{2E_w - E_v}{2E_w - E_s}.$$

The precise volumes of solution, reagent and supernatant liquid do not come into the calculation, provided the same volumes are used in each determination; so it is convenient to use micro-pipettes of a type that will deliver a reproducible volume.

A STUDY OF POSSIBLE ERRORS

The use of radioactive potassium-42 and the intense colour of the dipicrylaminate ion made possible the accurate determination of the small losses (or gains) of these ions occurring at each stage of the analytical procedure. The radioactive techniques used were essentially those described by Keynes and Lewis[1,10] For convenient consideration the procedure is divided into 3 stages: preliminary treatment of the samples; precipitation; and washing and determination of the precipitate. The experimental findings that influenced the final choice of the detailed procedure in each stage are mentioned below. Estimates of the over-all accuracy and reproducibility of the ultimate procedure are also given.

ERRORS IN THE PRELIMINARY TREATMENT—

In order to study these errors some leg nerves from *Carcinus maenas* were soaked in Ringer's solution containing a high concentration of radioactive potassium[1] and others were irradiated in the Harwell pile.[10] It was found that appreciable amounts of potassium (and to a lesser extent sodium) were lost by volatilisation at temperatures above 600° C. In silica crucibles appreciable combination of the alkali metals with the crucible surface occurred even at a temperature of 500° C. (This probably explains the 4 per cent. loss reported by Linderstrøm-Lang[11] when attempting the micro-determination of sodium plus potassium.) During incineration, therefore, the tissue samples were never allowed to come in contact with anything except platinum, and the temperature was not allowed to rise much above 500° C, with 550° C as an upper limit. All the potassium in the incinerated ash was immediately soluble in cold water, and under the conditions chosen the total loss of potassium during the incineration procedure was well below $\frac{1}{2}$ per cent.

ERRORS IN THE PRECIPITATION STAGE—

There are two main sources of error in this stage: incomplete precipitation of potassium dipicrylaminate, largely owing to its appreciable solubility, and co-precipitation of other sparingly soluble dipicrylaminate salts. The equilibrium solubility of pure recrystallised potassium dipicrylaminate at 0° C was found to be equivalent to about 25 μg of potassium per ml. The rate at which this equilibrium solubility is approached in an analysis was found by determining the radioactive potassium remaining in the supernatant liquid at intervals after addition of the reagent. In the absence of any stirring, the rate of precipitation increased as the total precipitation volume was decreased and was greatest in a shallow layer of liquid; so it is an advantage to keep this volume to a minimum. Under the chosen precipitation conditions the loss of potassium was between 1·0 and 0·5 per cent. for the range 20 to 100 μg of potassium; this loss was reproducible and could not conveniently be further reduced.

The most potentially serious error is co-precipitation of the not very soluble sodium salt. The use of a sodium dipicrylaminate solution as the precipitating reagent was found to give slightly high results, and experiments with sodium-24 confirmed the occurrence of significant co-precipitation. Lithium dipicrylaminate caused much less co-precipitation of sodium, and tracer experiments indicated how this could be further reduced by the use of a reagent solution as dilute as possible added in only moderate excess. It was also desirable to produce a coarse crystalline precipitate, not only to reduce co-precipitation errors, but also to minimise errors in the washing procedure discussed below. (A fine amorphous precipitate is more soluble in washing fluids than a crystalline one and retains excess of reagent more strongly.) The control of precipitation conditions in inorganic analyses has been fully discussed by Smith.[12] To obtain satisfactory crystallisation the precipitating ion was added as a not too concentrated solution in several portions at room temperature (or slightly higher when necessary), with adequate time for crystallisation allowed between separate additions of reagent and before transfer to the refrigerator. A reagent solution

containing some carbonate was used to prevent precipitation of free dipicrylamine by any traces of acid.

Negligible interference is to be expected from other elements likely to be present in biological materials: known amounts of potassium were determined in the presence of one or more of these elements at a time, with results which are summarised in Table I. Most of

TABLE I

MAXIMUM INTERFERENCE PRODUCED BY LIKELY CONSTITUENTS OF BIOLOGICAL MATERIALS

	Concentration relative to potassium, *M*	Error, %
Na^{+}	1	<0·1*
	5–10	0·1 to 0·2*
	30	<1
	110	2 to 5
HPO_4''	16	<1
Ca^{++}	110	2
Mg^{++}	110	2 to 4
Fe^{+++}	1	<3†
Cu^{++}	2	2
Zn^{++}	5	3

* Values obtained with sodium-24.

† Value obtained by the analysis of washed red cells.[13] Other values obtained by the routine analytical procedures.

the elements were tested at greater concentrations than those likely to be encountered. These results show that with the precipitation conditions chosen the error to be expected in the analysis of body fluids with a sodium to potassium ratio as high as 50 to 1 should not be greater than about 2 per cent. Many heavy metals, *e.g.*, lead, form sparingly soluble dipicrylaminates. Fortunately, most of these will be converted during incineration into compounds insoluble in water, *e.g.*, phosphates, carbonates or oxides, and so will not interfere. In fluids that are examined without incineration these elements should be removed, preferably by the prior addition of 1 drop of lithium carbonate solution.

ERRORS IN WASHING AND DETERMINING THE PRECIPITATE—

Kolthoff and Bendix[3] recommended washing the precipitate with 2 drops of water, 10 drops of a saturated solution of potassium dipicrylaminate and a final drop of water, all at 0° C. Only moderately consistent results could be obtained with this procedure; and standardisation against recrystallised potassium dipicrylaminate showed that more than 100 per cent. recovery of potassium from potassium chloride solutions was sometimes obtained, the excess presumably coming from the saturated solution used for washing. Radioactive potassium was therefore used to study the whole washing procedure. In one series of analyses the precipitate was washed with a solution of radioactive potassium dipicrylaminate; in a second series the precipitate was active, but the washing solution was inactive; in a third, both were active; and in a fourth, radioactive precipitates were washed only with successive drops of ice-cold water. The concentrations of potassium-42 and of dipicrylaminate in each drop of washing fluid were determined after it had been filtered off. From these measurements it was possible to calculate the amount of excess of reagent removed, the amount of precipitate dissolved and the extra precipitation (if any) caused by each successive drop of washing fluid. These calculated quantities showed that 4 separate drops of washing fluid were sufficient to remove all but traces of excess of the reagent. The use of a saturated solution of dipicrylaminate appeared to be undesirable; solution of the precipitate was only slightly reduced and differing amounts of extra precipitate were thrown down by reagent not removed by the first two drops of water.

Washing with 4 separate drops of ice-cold water caused only a small loss of precipitate, ranging from 2 to 2·5 per cent. for 20 μg of potassium to just under 1 per cent for 100 μg. The positive error due to unremoved reagent did not exceed 1 per cent.; so this washing procedure was adopted for routine analyses.

In some early analyses[1] commercial grade-4 filter-sticks with a 10-cm diameter sintered disc were used without any asbestos. As many as ten drops of washing fluid were required to remove the excess of reagent from the dead-space above the disc; so, as a compromise,

eight drops of an approximately half-saturated solution of potassium dipicrylaminate (containing 0·15 mg per ml) were used for washing (preceded by an initial wash with two drops of water and followed by a final wash with another drop). The use of a filter-stick with an asbestos pad is to be preferred, however, whenever possible.

The use of acetone to dissolve the precipitate, suggested by Kolthoff and Bendix,[3] was discontinued when it was found that a slow reaction occurs between the dipicrylaminate ion, acetone and the alkali added to prevent hydrolysis. This reaction caused an initial rise in the extinction coefficient of the solution, which was detectable after 1 hour if the room temperature was high. The precipitate dissolves readily in hot water, and the solution so prepared ready for colorimetric determination is stable almost indefinitely, the extinction coefficient decreasing by less than 2 per cent per week.

OVER-ALL ACCURACY OF THE ANALYSES—

The reproducibility of the analytical procedure was determined by examining a series of standard potassium chloride solutions. With commercial filter-sticks, a standard deviation of about $\pm$ 1·5 per cent. was obtained: with micro filter-sticks and an asbestos pad, the standard deviation was slightly under $\pm$ 1 per cent. The total loss of potassium determined from the losses of potassium-42 found at each individual stage was 1·5 to 3·0 per cent.; these values agreed, within the limits of the colorimetric error, with those obtained by standardisation of the technique against recrystallised potassium dipicrylaminate. The best check of the absolute accuracy and reproducibility of the technique finally adopted was provided by the analysis of crab nerves that had been previously analysed by activation analysis. Four nerves were irradiated in the Harwell pile and their potassium contents (averaging 90 μg of potassium) determined, without any chemical treatment, by the method described by Keynes and Lewis.[10] These nerves were then incinerated and their potassium contents determined by the routine procedure, standardised against pure potassium chloride samples. The average difference between parallel determinations was only 2½ per cent., the dipicrylaminate technique giving values that were lower by 1·3 $\pm$ 1·3 per cent. (mean value and its standard error). The analysis of washed red cells by the routine procedure gave reproducible results that agreed, within 3 per cent., with later values obtained with an EEL flame photometer[13] (Evans Electroselenium Ltd.). Parallel determinations of an aqueous nerve extract by flame photometry and by the dipicrylaminate procedure without filtration also agreed within 3 per cent.

Some of the results mentioned in this paper formed part of other investigations carried out jointly with Dr. R. D. Keynes, whose help and advice I gratefully acknowledge. The expenses were met by grants from the Rockefeller and the Nuffield Foundations.

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Notes

THE DETERMINATION OF 3:5-DINITRO-*o*-CRESOL IN THE PRESENCE OF β-CAROTENE IN BIOLOGICAL TISSUES

3:5-DINITRO-*o*-CRESOL (DNOC) is widely used as a selective herbicide and, in Africa, in the control of locusts. The customary method of determination of DNOC in animal tissues is that of Parker,[1] in which an alkaline ethyl methyl ketone extract of a sample of the tissue, containing the yellow sodium salt of DNOC, is compared photometrically with a similar extract of a standard solution of DNOC. This method and modifications of it (Harvey[2] and Edson[3]) have been applied to blood samples of spray operators and the tissues of experimental animals.

Recently, however, in two instances, the original method has been found inadequate in that β-carotene, also, is partly extractable by ethyl methyl ketone. In the first case, it was required to determine DNOC in the blood of cows suspected to be suffering from DNOC poisoning (Edson and Fenwick[4]). Anomalous results were eventually traced to β-carotene, which normally occurs in cows' serum in concentrations of from 2 to 16 μg per ml, depending on the diet (Goodwin[5]). In the second instance, the amount of DNOC picked up by locusts that had been sprayed with this compound was required. Again, the presence of β-carotene, at levels of about 10 to 30 μg per g fresh weight (Goodwin[5]), invalidated the results.

Two ways of overcoming this difficulty are described in this Note.

METHOD A: COWS' BLOOD

The extraction procedure is that of Parker.[1] The method depends upon the fact that, whereas the sodium salt of DNOC is bright yellow, the free acid is almost colourless in ethyl methyl ketone. The optical density of β-carotene at 430 mμ, however, is the same in acid or alkaline solutions.

APPARATUS—

Spectrophotometer—Unicam SP600 or SP350.
Small centrifuge—e.g., Gallenkamp "Junior."

REAGENTS—

Ethyl methyl ketone.
Sodium chloride - sodium carbonate mixture (9 + 1).
Concentrated hydrochloric acid.
3:5-*Dinitro*-o-*cresol, pure.*

PROCEDURE—

Add 5 ml of ethyl methyl ketone to 1 ml of cows' whole blood and shake to disperse the blood. Add 1 to 2 g of a mixture of sodium chloride and sodium carbonate (9 + 1). Shake vigorously for 30 seconds. Filter off the supernatant ethyl methyl ketone extract and measure its optical density at 430 mμ against a ketone blank. A Unicam SP350 spectrophotometer with a 10-mm optical path rectangular cell is suitable.

Transfer the extract to a small centrifuge tube, add one drop of concentrated hydrochloric acid and mix. If a slight clouding occurs, centrifuge the solution and again measure its optical density at 430 mμ. Subtract the second reading from the first and refer the difference to a standard DNOC curve, which may be prepared as follows.

Heat a known weight of free DNOC acid on a boiling-water bath under reflux in 0·5 per cent. w/v sodium carbonate solution at a concentration of about 1 mg per ml until it has all dissolved. Cool and make the solution up to a convenient volume with water. Make a series of dilutions with water of this solution of known concentration to produce solutions containing 5 to 50 μg of DNOC per ml.

Subject 1-ml aliquots of these solutions to the above extraction and acidification procedures; a 95 to 100 per cent. recovery of DNOC added to cows' blood is normally obtained.

METHOD B: LOCUSTS

Method A was not applicable to the determination of DNOC in locusts because of the production of highly stable emulsions after extraction with ethyl methyl ketone. In this method, the locust is homogenised in a mixture of chloroform and trichloroacetic acid. An aliquot of the chloroform extract is shaken with sodium carbonate solution, which extracts the DNOC but

not the carotene. A portion of the carbonate extract is shaken with ethyl methyl ketone in the presence of sufficient sodium chloride to "salt out" the DNOC. The optical density at 430 mμ of the ketone solution is compared with a standard curve.

APPARATUS—

"Nelco" homogeniser—Supplied by Hoslab of 12 Charterhouse Square, E.C.1.
Spectrophotometer—Unicam SP600 or SP350.

REAGENTS—

Trichloroacetic acid—A 10 per cent. w/v aqueous solution.
Sodium carbonate—A 2 per cent. w/v solution of the anhydrous solid.
Sodium chloride.
Chloroform.
Ethyl methyl ketone.

PROCEDURE—

Hold the locust in a pair of forceps over the glass vessel of the homogeniser and cut it into three or four pieces. Pour in 25 ml of chloroform, at the same time rinsing the tips of the scissors. Follow the chloroform with 25 ml of 10 per cent. trichloroacetic acid. Fit the vessel to the machine and homogenise for 1 minute at top speed (about 8000 r.p.m.).

Filter the mixture through four layers of gauze or cheese-cloth into a large test tube. Within a few minutes most of the chloroform separates from the top aqueous layer. Introduce a safety-pipette carefully into the bottom of the tube, avoiding inclusion of any of the aqueous phase. Withdraw a 5-ml aliquot by pipette and place it in a test tube. Add 10 ml of 2 per cent. sodium carbonate solution, close the tube with the thumb and shake vigorously for 10 seconds. Set the tube aside for at least 10 minutes so that the phases separate. The upper layer may remain cloudy, but this does not interfere with the result.

Remove an aliquot of the upper carbonate layer for the final step. Usually 6 ml is a reasonable amount to take, but inspection of the colour of the extract will indicate whether this should be increased or decreased. Add to this sample 5 ml of ethyl methyl ketone and about 1 g of sodium chloride and shake the mixture for a few seconds.

After a few minutes, remove the ketone layer by means of a drawn-out pipette and measure its optical density in a 10-mm cell at 430 mμ. The amount of DNOC corresponding to this value is read from a calibration curve prepared from standard DNOC solutions (see Method A). Multiply this value by the dilution factors to obtain the amount of DNOC on and in the locust.

A locust uncontaminated by DNOC, when put through this process, should produce a ketone solution whose optical density at 430 mμ is less than 0·01. A slight turbidity sometimes arises This can be removed by centrifuging.

DNOC added to 10 locusts, 162 μg of DNOC per locust, was recovered to an extent of 104 $\pm$ 4 per cent.; 50 to 500 μg of DNOC per locust were recovered to an extent of 97 $\pm$ 7 per cent.

DISCUSSION

The principles of both methods may obviously be applied to other aspects of the same problem, although the occurrence of β-carotene in tissues is comparatively rare owing to its rapid conversion to vitamin A. Carotene is reported (Goodwin[5]) to occur in the plasma of humans, horses, sheep, deer and hedgehogs, although not at levels that are likely to interfere with normal DNOC determinations. Carotenaemia has not been detected after high doses of carotene in goats, pigs, rats, rabbits or guineapigs. Xanthophylls (oxygen-containing carotenoid pigments) have been found in the blood of hens and other birds, but again only in small quantities. A possible application is to the determination of DNOC on green plant material.

Method A has been used on cows' liver and kidney, and pigs' stomach contents, the material being homogenised with ethyl methyl ketone in the presence of sodium chloride and sodium carbonate. The acidification principle is very simply applied whenever it is suspected that the sample contains pigments (of a non-acid nature) other than DNOC.

The locusts were supplied by the Natural History Museum, South Kensington, by the courtesy of Dr. Rainey of the Anti-Locust Research Centre.

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March 30th, 1955

THE NITROGEN CONTENT OF LEAN PORK

DURING recent months routine analyses of pork sausages in the author's laboratory have indicated a tendency for the total meat content as determined to be lower than the quantity used in the recipe. This has also been apparent in small-scale experimental sausage choppings. The discrepancy was not due to sampling error, since analysis of the sausages coincided with the theoretical analysis calculated from the composition of the individual components, as illustrated in Table I, which records two such sets of results. In both experiments, back fat was used to provide the bulk of the fat meat; in batch No. 1 the lean meat was derived from shoulder of pork and in batch No. 2 from pork loin. The ingredients of the recipe were chopped together as finely as possible to secure a uniform mixture before sampling for analysis.

TABLE I

ACTUAL AND THEORETICAL ANALYSIS OF PORK SAUSAGES

	Batch No. 1		Batch No. 2	
	Composition by analysis, %	Calculated composition, %	Composition by analysis, %	Calculated composition, %
Moisture	49·45	48·56	49·76	49·18
Fat	27·92	28·64	25·97	26·14
Ash	2·28	2·43	2·22	2·73
Carbohydrate	10·41	10·41	10·61	10·30
Total nitrogen	1·59	1·57	1·83	1·85
Nitrogen derived from lean meat	1·38	1·34	1·62	1·62

Calculation of the total meat content of the sausages, as found by analysis, employing the method of Stubbs and More,[1] with use of the corrected factor[2] of 100/3·6 based on the analytical data of Jackson and Jones[3] to convert meat nitrogen into defatted meat, gave 66·3 per cent. for the total meat (lean plus fat) content in batch No. 1 and 70·97 per cent. for the total meat content in batch No. 2. In both experiments the quantity of meat used in the recipe was 67·37 per cent.

The analyses of the shoulders and loins used in this experiment are recorded in Table III as sample (*a*). If the respective nitrogen contents (on a fat-free basis) of these meats are used for calculation of the lean meat in the sausages, the appropriate factors after correcting for the nitrogen derived from a small amount of lean adhering to the back fat are 100/3·43 and 100/3·90. This gives total meat contents, by analysis, of 68·16 per cent. and 67·51 per cent., respectively, in good agreement with the quantities of meat used.

It is apparent from the published data of Jackson and Jones, and also from those of Moulton,[4] that the nitrogen content of lean pork varies considerably throughout the carcase. Since the factor of 100/3·6 is based upon the average of Jackson and Jones's analyses, the meat content of pork sausages determined by analysis cannot be expected to coincide exactly with the actual amount of meat present. Although over a long period the differences might be expected to cancel, this has not occurred in our laboratory, where there has been a distinct tendency for the analyses to exhibit a deficiency in lean meat.

In order to account for this deficiency, the distribution of the nitrogen in a headless side of home-killed pork has been examined. A side weighing 76 lb. was selected for boning and cutting into joints according to standard factory practice. Excess of fat was trimmed from all the lean joints, which were then weighed and minced through a fine plate. Each sample was subsequently thoroughly mixed and minced three times in the laboratory before analysis. Two samples, which showed good agreement on analysis, were taken from each joint. The average composition of each cut is shown in Table II, together with the weight expressed as a percentage of the total weight of the lean meat in the whole side.

The nitrogen content of the whole of the lean meat (on a fat-free basis) calculated by proportion was 3·39 per cent.; a higher figure of 3·43 per cent., which is less accurate, is obtained by direct averaging of the analyses.

TABLE II

THE NITROGEN CONTENT OF LEAN MEAT IN SIDES OF PORK

	Weight of each cut expressed as percentage of total weight of lean meat	Fat, %	Water, %	Nitrogen, %	Nitrogen on fat-free basis, %
(a) *Home-killed pork*—					
Leg.. ..	32·63	11·51	68·38	3·02	3·41
Loin ..	8·20	12·35	66·01	3·30	3·77
Belly ..	21·33	28·55	54·45	2·34	3·28
Back ..	11·04	25·72	57·25	2·48	3·34
Shoulder ..	26·80	20·51	62·04	2·68	3·37
(b) *Irish pork**—					
Leg.. ..	30·61	16·99	64·36	2·83	3·41
Loin ..	9·20	19·03	61·85	2·88	3·56
Belly ..	13·14	42·47	44·44	1·97	3·43
Back ..	30·14	31·62	54·89	2·36	3·45
Shoulder ..	16·91	25·23	58·13	2·53	3·38

* Further analyses carried out in October, 1954, after this Note had been submitted for publication.

In view of the low nitrogen figures obtained in the work on the first side of pork, it was considered expedient to perform analyses on further samples. Since it was not possible at the time to repeat the entire operation on several sides of pork, these analyses were confined to shoulders and loins only, which were selected as being representative of joints containing low and high nitrogen contents, respectively. The results are shown in Table III.

Some further analyses were carried out in October, 1954, on a side of Irish pork. These results are also included in Table II. The nitrogen content of the lean meat on a fat-free basis calculated both by proportion and by direct averaging was 3·43 per cent.

The method of cutting the sides and separation into the different cuts was purely arbitrary and was left to the discretion of the butcher. This accounts for differences between similar cuts (expressed on a percentage basis) in parts (*a*) and (*b*) of the Table.

TABLE III

NITROGEN CONTENT OF LEAN SHOULDERS AND LOINS FROM HOME-KILLED PORK

	Shoulders				Loins			
Sample	Fat, %	Water, %	Nitrogen, %	Nitrogen on fat-free basis, %	Fat, %	Water, %	Nitrogen, %	Nitrogen on fat-free basis, %
(*a*) Hog ..	12·84	67·47	2·97	3·41	6·90	68·96	3·64	3·91
(*b*) Hog ..	42·38	44·70	1·91	3·31	32·42	50·85	2·37	3·51
(*c*) Hog ..	22·10	59·87	2·69	3·45	20·80	59·17	2·96	3·74
(*d*) Hog ..	14·17	67·27	2·77	3·23	12·70	64·78	2·96	3·39
(*e*) Sow ..	24·26	57·98	2·52	3·33	21·75	57·89	2·88	3·68
			Average ..	3·35			Average ..	3·65

The average nitrogen contents of the defatted meats were lower than those obtained by Jackson and Jones for similar cuts (shoulders, 3·57 per cent. of nitrogen; backs, 3·84 per cent. of

nitrogen) and established beyond doubt an explanation for the discrepancies that have previously been mentioned. It is possible that changes in methods of feeding during recent years may account for the differences between our data for nitrogen content of lean pork and those of Jackson and Jones. Breed may also have a contributory effect, for it can be calculated from Moulton's data, compiled before 1929, that the average nitrogen content of defatted pork used in American pork sausages was as low as 3·32 per cent. These data raise issues of some significance, which are beyond the scope of the present investigation but would appear to merit careful consideration.

I am indebted to Excel Co. Ltd. for permission to publish this Note.

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2. Analytical Methods Committee, "The Analysis of Meat Products," *Ibid.*, 1952, **77**, 543.
3. Jackson, F. W., and Jones, O., *Ibid.*, 1932, **57**, 562.
4. Moulton, C. R., "Meat through the Microscope," University of Chicago Press, 1929, Table 141, p. 330.

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July 26th, 1954
Additional results submitted, *July 5th*, 1955

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B.S. 2621–5 : 1955. Glycerine (Glycerol). Price 10s.

Book Reviews

METHODS OF BIOCHEMICAL ANALYSIS. Volume II. Edited by DAVID GLICK. Pp. vi + 470. New York and London: Interscience Publishers Inc. 1955. Price $5.50; 40s.

This is the second volume of a new annual series, and the standard set by the first volume (reviewed in *Analyst*, 1954, **79**, 658) is well maintained.

H. Rosenkrantz contributes a 57-page article on the analysis of steroids by infra-red spectrophotometry. This includes a useful description of the preparation of samples and a thoroughly serviceable account of the correlation of structure and absorption. H. Persky discusses the determination of adrenaline and noradrenaline in the body fluids and tissues, and shows how newer chemical methods can be used to determine very small (mμg) quantities more accurately than by bio-assays.

Warren Sperry deals with lipide analysis "in a fashion that will furnish the qualified biochemist with all the information required to carry out the analyses," but gives "sufficient detail so that they may be used successfully by a biochemist who is a novice in the lipide field." This is a good chapter based on great experience, but it does not take for granted that the qualified biochemist knows much about filtering solutions or using separating funnels!

R. T. Holman summarises crisply his experience on the measurement of lipoxidase activity. T. H. Jukes describes chemical and microbiological methods for the assay of compounds with folic acid activity and includes the citrovorum factor and the interesting use of the protozoon *Tetrahymena galeii* as a test organism for folic acid.

The determination of vitamin E is dealt with by R. W. Lehman in a conversational "I and you" style that makes the reader feel that he is on a conducted tour with a reliable and breezy guide.

G. D. Novelli's article on coenzyme A is concise and clear but does not cater for the novice. The section by N. C. Davies and Emil L. Smith on the assay of proteolytic enzymes is an admirable account, in 40 pages, of a large field; it covers titrimetric, gasometric and spectrophotometric methods as well as colorimetric and other techniques.

J. W. Patterson and A. Lazarow cover the determination of glutathione in a manner that reflects much personal experience.

* Obtainable from the British Standards Institution, Sales Department, 2 Park Street, London, W.1.

R. J. Winzler contributes an interesting essay on serum glycoproteins and their determination.

New colour reactions for the determination of sugars in polysaccharides are discussed by Z. Dische in a substantial and very readable chapter.

Three authors offer a detailed chapter (66 pages) on recent developments in techniques for terminal and sequence studies in peptides and proteins. The fluorodinitrobenzene method is described by the late A. L. Levy, the phenyl*iso*cyanate method by H. Fraenkel-Conrat and the carboxypeptidase method by J. E. Harris. Finally, Lucile Smith describes the spectrophotometric assay of cytochrome-*c* oxidase.

This volume displays clearly the importance of analytical methods in present-day biochemical research. It also illustrates how great are the contributions made in the U.S.A. not only to biochemistry but to books about biochemistry. These different books are well edited and produced and, in spite of a good deal of overlapping, they clearly meet various needs, the greatest of which seems to be a minimal use of the original literature. It is as though the biochemist may navigate the ocean himself but must take a pilot on board when in sight of land. R. A. MORTON

ELECTROMETRIC pH DETERMINATIONS. THEORY AND PRACTICE. By ROGER G. BATES, Ph.D. Pp. xiv + 331. London: Chapman & Hall Ltd.; New York: John Wiley & Sons Inc. 1954. Price 60s.; $7.50.

A work on this subject from the pen of Dr. Roger Bates cannot fail to excite the greatest interest. It may be said at once that none will be disappointed in it. The book is roughly divided into two sections, theoretical and practical, each of which is further subdivided. The theoretical treatment is thermodynamic rather than classical, but the mathematical aspects are treated so simply and lucidly as to present no difficulties to the non-mathematician. Valuable discussions of the definitions of pH scales and pH standards, of junction potentials and of ionic activities are prominent features, and buffer solutions and non-aqueous media are excellently treated. About half the book deals with the practical aspect of pH measurement, and the chapters on cells, electrodes and techniques, particularly the one on the glass electrode, are most rewarding. The chapter on pH meters is, in comparison, perhaps a little less satisfactory, but understandably so in the available space. British and continental meters are well, if briefly, covered, and excellent advice on care and maintenance is given. The short chapter on automatic pH control affords an adequate introduction to this large and important subject. The author's claim that, while the book is an integrated whole, each chapter is complete in itself, is fully justified, and each chapter may be consulted as a treatise on its particular aspect.

Specialists may find points in the earlier chapters to argue about, but the general reader can confidently welcome this work as authoritative and definitive. The whole work bears the obvious imprint of personal experience and mature thought. It is characteristic, though regrettable, that Dr. Bates's modesty has led him to exclude his own name from the author index. The author's avowed objectives of presenting the theoretical and experimental basis of electrometric measurement and of providing a practical handbook for all who measure pH have certainly been admirably fulfilled; and all who have anything at all to do with pH can aquire this book with the assurance that they will find much of value to them in its pages.

Dr. Bates commands a flow of language that is at once scholarly without being pedantic, and terse yet eminently readable. Printing, lay-out and binding are first class, and the few errors, such as "d_0 is the density of the solvent" instead of "solution" on p. 10, are minor and obvious. Dr. Bates is to be congratulated on making a major and enduring contribution to the literature of the subject. E. BISHOP

POLAROGRAPHIC TECHNIQUES. By LOUIS MEITES. Pp. xiii + 317. New York and London: Interscience Publishers Inc. 1955. Price $6.00; 48s.

This volume by Dr. Meites of Yale University provides for American students a concise theoretical and practical introduction to inorganic polarography: it supplements, but does not try to rival, more comprehensive books on the subject, such as Kolthoff and Lingane's well known monograph.

Dr. Meites introduces the student in turn to the nature and scope of polarographic measurements, instrumentation, polarographic limiting current, theory of the current - voltage curve, interpretation of half-wave potential data, maxima and their suppression, techniques of quantitative polarographic analysis, amperometric titrations and special techniques, and he concludes with appendices on "trouble-shooting" in polarographic circuits and inorganic half-wave-potential and diffusion-current data. The appendix on "trouble-shooting" would, in my opinion, be more

appropriate in a polarograph instruction manual, but since polarograph manufacturers seldom provide such information, its presence here is welcome. So far as it goes, the method of presentation is satisfactory.

As an introduction to modern polarography the book has limitations. The newer techniques, such as differential, oscillographic and square-wave polarography, are not discussed, and derivative polarography is dismissed as "still too new to have found any important practical applications." The examples mentioned in the text and the experiments described at the end of each chapter are largely restricted to inorganic polarography; few organic compounds are mentioned. In the chapter on instrumentation and elsewhere in the book American instruments and equipment are alone described.

The book can therefore be recommended only as an introductory volume. The analyst who wishes either to investigate new methods or to study organic compounds will soon have to look elsewhere for additional information. The book is well printed and bound, but the price appears to be excessive for one of its limited scope. J. E. PAGE

Publications Received

ANNUAL REPORTS ON THE PROGRESS OF CHEMISTRY FOR 1954. Pp. 456. London: The Chemical Society. 1955. Price 30s.

METHODS FOR DETERMINING LEAD IN AIR AND IN BIOLOGICAL MATERIALS. Pp. 69. New York: American Public Health Association Inc. 1955. Price $1.25.

MODERN APPARATUS FOR STERILISATION. By J. H. BOWIE, M.B., Ch.B., M.R.C.P.E. Pp. 24. London: The Pharmaceutical Press. 1955. Price 2s. 6d.
Reprinted from The Pharmaceutical Journal, June 11th and 18th, 1955.

THE CHEMISTRY AND FERTILITY OF SEA WATERS. By H. W. HARVEY, Sc.D., F.R.S. Pp. viii + 224. Cambridge University Press. 1955. Price 30s.

THE QUANTITATIVE ANALYSIS OF DRUGS. By D. C. GARRATT, B.Sc., Ph.D., F.R.I.C. Second Edition. Pp. xvi + 670. London: Chapman & Hall Ltd. 1955. Price 70s.
The First Edition was published under the title "Drugs and Galenicals: their Quantitative Analysis."

QUALITATIVE ORGANIC ANALYSIS. By A. MCGOOKIN, D.Sc., Ph.D., A.R.I.C. Pp. viii + 155. London: Chapman & Hall Ltd. 1955. Price 15s.

THE EXTRA PHARMACOPOEIA (MARTINDALE). Published by direction of the Council of the Pharmaceutical Society of Great Britain. Twenty-third Edition. In two Volumes. Volume II. Pp. xxxii + 1501. London: The Pharmaceutical Press. 1955. Price 57s. 6d.

LABORATORY INSPECTION OF CANNED AND BOTTLED FOODS. By DENIS DICKINSON, Ph.D., M.Sc., F.R.I.C., M.R.San.I., and P. GOOSE, A.C.I.I. Pp. x + 148. London and Glasgow: Blackie & Son Ltd. 1955. Price 10s. 6d.

GENETICS AND METABOLISM. By ROBERT P. WAGNER and HERSCHEL K. MITCHELL. Pp. xii + 444. London: Chapman and Hall Ltd.; New York: John Wiley & Sons Inc. 1955. Price 60s.

TEXTBOOK OF PHARMACOGNOSY. By T. E. WALLIS, D.Sc., F.R.I.C., F.P.S., M.I.Biol., F.L.S. Third Edition. Pp. xii + 578. London: J. & A. Churchill Ltd. 1955. Price 42s.

Erratum

MAY (1955) ISSUE, p. 374, line 21. *After* "then 2·0 ml in excess" *insert* "Add 0·5 ml of bromine water, boil off completely all excess of bromine and cool."

PRINCIPAL SCIENTIFIC OFFICER OR SENIOR EXPERIMENTAL OFFICER required by ATOMIC WEAPONS RESEARCH ESTABLISHMENT, ALDERMASTON, BERKS., to lead a team engaged on analytical problems associated with research and development work in both organic and inorganic chemistry. To be responsible for laboratory organisation and training of junior staff.

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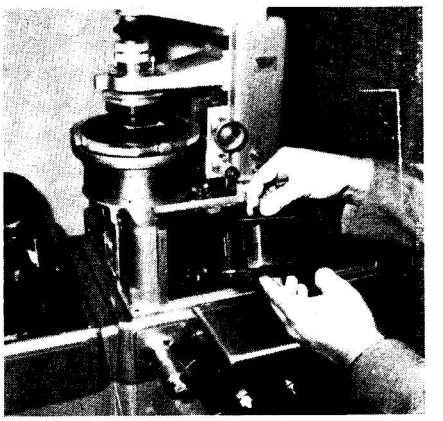

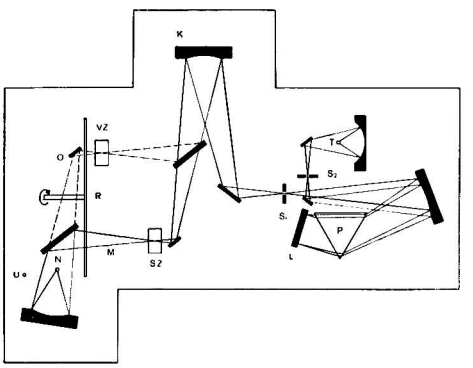

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