An International Journal of Analytical Chemistry

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talanta

PERGAMON PRESS
OXFORD · LONDON · NEW YORK · PARIS

1963 VOLUME 10 DECEMBER

SUMMARIES FOR CARD INDEXES

The extraction and absorptiometric determination of titanium with 8-hydroxyquinoline. C. L. CHAKRABARTI, R. J. MAGEE and C. L. WILSON, *Talanta*, 1963, **10**, 1201. (Department of Chemistry, The Queen's University, Belfast, Northern Ireland.)

Summary—A study of the extraction of titanium into chloroform as the titanium oxinate complex, and its absorptiometric determination, using either the absolute spectrophotometric technique or the differential spectrophotometric technique, has been carried out. The relevance of the conclusions for the determination of titanium in steel is considered.

Studies on the mode of action of Fajans' adsorption indicators: E. PUNGOR and I. KONKOLY THEGE, *Talanta*, 1963, **10**, 1211. (Institute of Analytical Chemistry, University of Industrial Chemistry, Vesprem, Hungary.)

Summary—The mode of action of Fajans' adsorption indicators in the case of eosin with silver chloride and rhodizonate with barium sulphate is studied. It is established that because of the adsorption of the silver or barium ion the solubility product of silver eosinate and barium rhodizonate at the surface of the corresponding precipitate is reached sooner than it would be, calculated on the basis of the indicator concentration in the solution. The precipitation of silver eosinate or barium rhodizonate at the surface of the precipitate is influenced by factors affecting their solubility.

Spectrophotometric determination of indoles using a modification of the Ehrlich colour reaction: P. BYROM and J. H. TURNBULL, *Talanta*, 1963, **10**, 1217.

Summary—A sensitive spectrophotometric method for the determination of indole (1 μg/ml) in aqueous solution is described. It is also applicable to certain 3-substituted indoles. The method employs *p*-dimethylaminobenzaldehyde in aqueous trifluoracetic acid. With indoles this reagent gives stable colours which are more intense than those obtained in the conventional Ehrlich reaction. Factors affecting fading of the indole colour are discussed.

The determination of radium-226 in bone: NAOMI A. HALLDEN, ISABEL M. FISENNE and JOHN H. HARLEY: *Talanta*, 1963, **10**, 1223. (U.S. Atomic Energy Commission, Health and Safety Laboratory, New York, New York, U.S.A.)

Summary—A simple procedure is described for preparing 10-g samples of bone ash for measurement of ^{226}Ra by the ^{222}Rn emanation technique. In this case, the radon is measured in 2-litre ion chambers, but scintillation chambers could be used. Coprecipitation of ^{226}Ra with $BaSO_4$ gives separation from bulk constituents, and the final precipitate is soluble in a few ml of 30% EDTA. Chemical recovery is measured with ^{133}Ba tracer.

Mixed peroxy-complexes of titanium, niobium and tantalum—III: Chelatometric titration of niobium^{V}: ERIK LASSNER, *Talanta*, 1963, **10,** 1229. (Metallwerk Plansee, A.G., Aktiengesellschaft, Reutte, Tirol, Österreich.)

Summary—A chelatometric method for the titration of Nb^{V}, using nitrilotriacetic acid is described. This forms a 1:1 complex with the peroxy-Nb^{V} ion. NTA is added in excess, and the excess is back-titrated with Cu solution at pH 5·0–5·5 using the metallofluorescent indicator, Methylcalcein, under UV illumination. The reproducibility, for amounts of 4·56–23·68 mg of Nb is 0·07 mg of Nb. *N*-Hydroxyethylethylenediamine-*N,N′,N′*-triacetic acid forms a similar 1:1 complex, and can be used instead of NTA, but with little advantage.

Applications of infrared spectroscopy—XII: The behaviour of propoxyl and butoxyl groups in the Zeisel reaction: D. M. W. ANDERSON and S. S. H. ZAIDI, *Talanta*, 1963, **10,** 1235. (Department of Chemistry, The University, Edinburgh 9, Scotland.)

Summary—Vapour-phase infrared spectroscopy has been used to study the behaviour of n-propoxy, iso-propoxy, n-butoxy, iso-butoxy and sec-butoxy groups in Zeisel's reaction. Within each group, the reaction rate varies with the compound under study. The equilibrium iso-$C_3H_7I \rightleftharpoons HI + C_3H_6$ is involved in the determination of iso-propoxy compounds with hydriodic acid; low recoveries of iso-propyl iodide therefore result. Reflux with hydrobromic acid gives a more nearly quantitative analytical reaction, since iso-propyl bromide is more stable to reflux with hydrobromic acid than iso-propyl iodide is to reflux with hydriodic acid. n-Propoxy, n-butoxy and sec-butoxy groups can be determined successfully with hydriodic acid; in the determination of iso-butoxy groups a rearrangement occurs, and the reaction product is a mixture of iso-butyl and sec-butyl iodides.

Determination of silver in lead by neutron-activation analysis: F. ADAMS, J. HOSTE and A. SPEECKE, *Talanta*, 1963, **10,** 1243. (Laboratory for Analytical Chemistry, Ghent University, Belgium.)

Summary—Submicrogram amounts of silver have been determined in lead by neutron-activation analysis. The activity of the ^{110}Ag isotope was measured by following the decay of the 0·66-MeV gamma ray. Reproducibility was generally better than 10%. The smallest amount of silver determined was approximately 0·02 μg. The analyses were completed within 15 min.

A new oxidimetric reagent: Potassium dichromate in a strong phosphoric acid medium—I: Titrimetric determination of manganese^{II}: G. GOPALA RAO and P. KANTA RAO, *Talanta*, 1963, **10**, 1251. (Department of Chemistry, Andhra University, Waltair, India.)

Summary—A new method has been developed for the direct titrimetric determination of manganese^{II}, depending on its oxidation to manganese^{III} with potassium dichromate at room temperature in a strong phosphoric acid medium using a potentiometric or photometric end-point. Oxygen of the air does not interfere. The potentiometric method gives results to an accuracy within $\pm 0.3\%$ for 20–150 mg of manganese/50 ml of titration solution; with the photometric method 5–17 mg of manganese/40 ml of titration solution can be determined with an error of 0.3–1.0% depending on the amount present. Potassium dichromate in 12.0*M* phosphoric acid has a formal redox potential of about 1.5 *V* and this reagent appears to have great possibilities in titrimetric analysis.

Spectrophotometric determination of palladium with 1-thioglycerol and a study of palladium complex formation with four similar thio-organic compounds: R. W. BURKE and JOHN H. YOE, *Talanta*, 1963, **10**, 1267. (Pratt Trace Laboratory Department of Chemistry, University of Virginia, Charlottesville, Va., U.S.A.)

Summary—A study of the reactivity of five thio-organic compounds with palladium^{II} ions has been made and one of them, 1-thioglycerol, selected for use as a spectrophotometric reagent for palladium. Its sensitivity is 0·01 μg of palladium/cm^2 for log $I_0/I = 0{\cdot}001$. The sensitivity of the other four compounds is about the same. Beer's law is obeyed over the palladium concentration range of 0·5 to 9 ppm. The effects of pH, order of addition of reagents, temperature and diverse ions have been investigated.

The mode of operation and the uses of the oxidation-reduction indicator 2-hydroxy-4-amino-4′-methoxydiphenylamine: L. ERDEY and I. KÁSA, *Talanta*, 1963, **10**, 1273. (Institut für Allgemeine Chemie, Technische Universität, Budapest, Hungary.)

Summary—Either of two quinoidal structures might be formed in the oxidation of 2-hydroxy-4-amino-4′-methoxydiphenylamine (2-Oxyvariamine Blue). It is shown that it is the paraquinoidal holoquinone that is formed. The ascorbimetric determination of certain oxidising agents (I_2, Br_2, IO_3^-, BrO_3^-, CrO_4^{2-}), using 2-Oxyvariamine Blue and a hexacyanoferrate(II)-hexacyanoferrate(III) system is described.

Magnesium in biological samples by spectrophotometric measurement of the 8-quinolinolate extract: TAFT Y. TORIBARA, LARYSA KOVAL and JORGE F. P. OLIVE, *Talanta*, 1963, **10**, 1277. (Department of Radiation Biology, University of Rochester, School of Medicine and Dentistry, Rochester 20, New York, U.S.A.)

Summary—Conditions for the simple, rapid and sensitive determination of magnesium in biological samples have been determined. Commercially available solvents and reagents are used without further purification. Magnesium is separated and converted to a form which may be measured spectrophotometrically in a single extraction as the 8-quinolinolate in 1,1,2-trichloroethane. The extraction of other 8-quinolinolates is prevented by the addition of tartrate and cyanide to the buffer solution. Adequate sensitivity is obtained (absorbance of 0·4 for 20 μg of magnesium), and even greater sensitivity is possible by reducing the volumes of both aqueous and organic phases.

Contributions to the basic problems of complexometry—XIII: Determination of aluminium and tervalent chromium in the presence of chromate: RUDOLF PŘIBIL and VLADIMÍR VESELÝ, *Talanta*, 1963, **10**, 1287. (Analytical Laboratory, Polarographic Institute, Czechoslovak Academy of Sciences, Prague 1, Jilská 16, Czechosolvakia.)

Summary—Aluminium can be determined in the presence of tervalent chromium and chromate using 1,2-diaminocyclohexanetetra-acetic acid (DCTA), which forms a complex with aluminium even in the cold. This phenomenon enables a successive determination to be made of aluminium (iron) and tervalent chromium in the presence of hexavalent chromium. This procedure cannot be carried out with the commonly used ethylenediaminetetra-acetic acid (EDTA).

Polarographic determination of copper^{II}, arsenic^{III} and arsenic^{V} in copper arsenite: T. L. HUNTER, J. F. HAZEL and WALLACE MCNABB, *Talanta*, 1963, **10**, 1291. (Department of Chemistry, University of Pennsylvania, Philadelphia 4, Pennsylvania, U.S.A.)

Summary—A method is proposed in which Cu^{II}, As^{III} and As^{V} can be determined in copper arsenite without prior separation. It is based on the fact that Cu^{II} and As^{III} yield prominent, distinguishable, widely-separated cathodic polarographic waves in a 0·1*M* LiCl—0·01*M* EDTA—0·001*M* LiOH solution using a dropping mercury electrode, whereas As^{V} does not give a wave in this medium. The As^{V} is determined by difference after reduction with sulphurous acid.

Determination of submilligram amounts of cobalt by ferricyanide titration with photometric end-point detection: H. POPPE and G. DEN BOEF, *Talanta*, 1963, **10**, 1297. (Laboratory for Analytical Chemistry, University of Amsterdam, The Netherlands.)

Summary—The titrimetric determination of cobalt^{II} by oxidation with hexacyanoferrate(III) in ammoniacal solution, ordinarily carried out potentiometrically, can be extended to smaller amounts of cobalt by means of photometric end-point indication.

Titrimetric determination of tin^{II} with hexacyanoferrate(III) in the presence of redox indicators: HALINA BASINSKA and WIESLAW RYCHCIK, *Talanta*, 1963, **10,** 1299. (Department of Inorganic Chemistry, Copernicus University, Torun, Poland).

Summary—A titrimetric determination of tin^{II} in strong hydrochloric acid solution with standard potassium hexacyanoferrate(III) solution using 3,3′-dimethylnaphthidine or *o*-dianisidine as indicator is described.

New specific spot tests for barbituric and thiobarbituric acids, malonic acid and urea: V. ANGER and S. OFRI, *Talanta*, 1963, **10,** 1302. (Chemical Laboratory, Karl Beckgasse 35, Vienna 18, Austria.)

Summary—Barbituric and thiobarbituric acids may be detected with pyridyl pyridinium dichloride, giving, respectively, a reddish-blue and a blue colour. The limit of identification is 0.5 μg in each case. Malonic acid can be converted to barbituric acid by heating with urea, and is then detected in a similar manner. The limit of identification is 50 μg of malonic acid. Urea may be detected with a limit of identification of 200 μg by reversing the test for malonic acid. All four tests are specific.

The purification of calcein: C. J. KEATTCH, *Talanta*, 1963. **10,** 1303. (John Laing Research and Development Limited, Manor Way, Boreham Wood, Hertfordshire, England.)

Summary—Chromatographic techniques are described for purifying commercial Calcein. The purified product can be used successfully for titrating calcium solutions with EDTA, giving reproducible results at concentrations down to 0·00025*M*.

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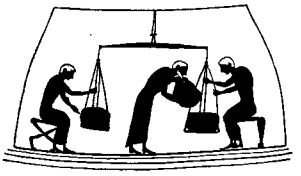

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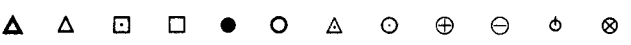

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[1] J. B. Austin and R. H. H. Pierce, *J. Amer. Chem. Soc.*, 1955, **57**, 661.
[2] S. T. Yoffe and A. N. Nesmeyanov, *Handbook of Magnesium-Organic Compounds.* Pergamon Press, London, 2nd Ed., 1956. Vol. 3, p. 214.
[3] A. B. Smith, *The Effect of Radiation on Strengths of Metals.* A.E.R.E., M/R 6329, 1962.
[4] W. Jones, *Brit. Pat.* 654321, 1959.

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Talanta, 1963, Vol. 10, pp. 1201 to 1210. Pergamon Press Ltd. Printed in Northern Ireland

THE EXTRACTION AND ABSORPTIOMETRIC DETERMINATION OF TITANIUM WITH 8-HYDROXYQUINOLINE

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(*Received* 19 *July* 1962. *Accepted* 1 *July* 1963)

Summary—A study of the extraction of titanium into chloroform as the titanium oxinate complex, and its absorptiometric determination, using either the absolute spectrophotometric technique or the differential spectrophotometric technique, has been carried out. The relevance of the conclusions for the determination of titanium in steel is considered.

IN 1951 Gardner[1] described the photometric determination of small amounts of titanium based on the yellow colour produced by titanium with hydrogen peroxide combined with 8-hydroxyquinoline (oxine) in chloroform. He measured the optical density of this colour with a Spekker absorptiometer, using an Ilford 601 filter (with maximum transmission at 4300 Å) and an H 503 filter, adding appropriate amounts of the interferents to the blank, and measuring the optical density of the test solutions against such a blank. However, no systematic study of the titanium-oxinate-chloroform system was made, nor was there any experimental evidence for the validity of the procedure when applied to titanium-bearing materials. Moreover, the addition of interferents to the blank to compensate for their effects on test solutions, as was done in that study, has limitations. In a later publication Morrison and Freiser[2] refer to the extraction of titanium (up to 200 μg) from a solution at pH 8–9, by oxine in chloroform, using EDTA as a masking agent for interferents, and measuring the optical density at λ400 mμ.

Apart from these references, no detailed information concerning the extraction behaviour of titanium oxinate into chloroform solution is available. The object of the present work was to make a study of this extraction, of the effect of interferents, and of the means of eliminating them.

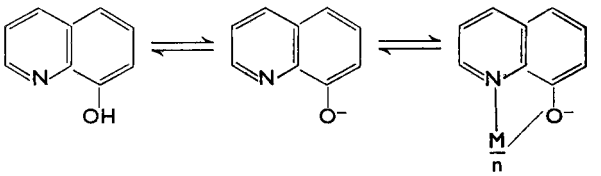
THEORETICAL CONSIDERATIONS

(*a*) *Oxine as a chelating agent*

Titanium^{IV} forms a yellow inner-complex salt with oxine which is precipitated from slightly acid or alkaline aqueous solution; it has the formula[3] $TiO(C_9H_6ON)_2 \cdot 2H_2O$. The extractability of this complex into organic solvents may well be associated with the removal of water molecules from it.

* Present address: Coates Chemical Laboratory, College of Chemistry and Physics, Louisiana State University, Baton Rouge, Louisiana, U.S.A.

Oxine is a bidentate chelating agent—the anion co-ordinates[4] with numerous metallic cations to give inner complexes as shown below, forming 5-membered rings:



In acid solutions the metal and hydrogen ions are in direct competition for the oxinate anion and in alkaline solutions the oxinate and hydroxyl ions are in competition for the metal cation. There is therefore a limited pH range within which the complex is stable; this is characteristic for each metal, and is dependent on the stability constant of the complex and the metal-hydroxyl ion association constant. The pH ranges over which the various metals form a complex with oxine differ sufficiently for useful quantitative separations to be possible by careful adjustment of the pH values, combined with the use of masking agents. By a judicious combination of masking agents further selectivity of the reaction can be achieved, and the reaction can, in fact, be made highly selective for a particular element. The combination of masking agents used in this study was EDTA and potassium cyanide.

(*b*) *Solvent extraction of titanium oxinate*

The electrical neutrality of inner complexes, which is responsible for their low solubility in water, frequently leads to an appreciable solubility in organic media; consequently, in the presence of an organic ligand which can form an inner complex, titanium can be extracted into a water-immiscible organic solvent, and especially into chloroform.

The solvent extraction of titanium as titanium oxinate into chloroform from an aqueous phase containing EDTA and cyanide as complexing agents involves consideration of the three equilibria existing between each of the ligands—oxine, EDTA, cyanide—and the metal ions in solution. Fundamentally, solvent extraction from aqueous solutions depends upon the competition of hydrogen ions and metal cations for the ligand anion (A^-):

$$H_2O + HA \rightleftharpoons H_3O^+ + A^- \quad (1)$$

$$M^{n+} + nA^- \rightleftharpoons MA_n \quad (2)$$

In the presence of more than one ligand in solution, there is a competition between the ligands for the metal ions. The ligand that forms the most stable complex at a given pH preferentially forms the complex with the metal ions.

The distribution of the metal between the phases thus depends on the acidity constant of the ligand acid and the stability constant of the inner complex, and is sensitive to changes in pH. It is for this reason that the most satisfactory results are obtained with the more acidic ligands when two ligands are in competition for the same metal cations. In the case of EDTA the relationship may be expressed as follows.

$$M^{n+} + H_2Y^{2-} \rightleftharpoons MY^{(n-4)} + 2H^+$$

With increasing value of pH there is an increasing tendency for the formation of

slightly soluble metal hydroxides. The over-all process may be represented by the reaction:

$$MY^{(n-4)} + nOH^- \leftrightharpoons M(OH)_n + Y^{(n-4)}$$

$$\frac{[M(OH)_n] \times [Y^{(n-4)}]}{[MY^{(n-4)}] \times [OH^-]^n} = K_h$$

Thus, an increase in the hydroxyl-ion concentration tends to shift the equilibrium to the right, *i.e.*, to cause the precipitation of the metal hydroxide. For this reason, a loss in complexing efficiency results with increasing pH. This effect may be overcome, at least in part, by using an excess of EDTA, the quantity needed increasing with increasing pH. Thus, in the absence of EDTA the pH range for the optimum extraction of titanium[2] is 3·8–5·0, whereas in the presence of EDTA the range for the complete extraction of titanium is raised to 7·9–9·0. Only when the pH is raised to 7·9–9·0 does the complex of titanium with EDTA become less stable than that with oxine, with the result that oxine will preferentially form a complex with titanium. The extraction of oxine alone is maximum[5] near a pH of 7.

EXPERIMENTAL

Apparatus

Hilger Uvispek Photoelectric Spectrophotometer, model H 700.307, fitted with a quartz prism. 1-cm Silica cells.
Doran pH meter.

Reagents

All reagents were of Analytical-Reagent grade purity.

Oxine-chloroform solution: 1% w/v solution of oxine dissolved in chloroform.
EDTA solution: 0·1*M* aqueous solution of disodium salt of ethylenediaminetetra-acetic acid.
Potassium cyanide solution: 10% w/v aqueous solution of potassium cyanide.

RESULTS

Absorption spectrum of titanium-oxinate in chloroform

The yellow chloroform extract of the titanium-oxinate complex shows one broad absorption band with maximum absorption around 380 mμ. The ultraviolet absorption spectra of a 69×10^{-3} *M* solution of oxine against chloroform as blank, and of chloroform against distilled water as blank, are shown in Fig. 1. The broken part of the curve represents the maximum reading that is possible with the Uvispek spectrophotometer—the actual optical density being greater. In Fig. 2 the absorption curve of a $5{\cdot}6 \times 10^{-5}M$ solution of titanium oxinate in chloroform against a $69 \times 10^{-3}M$ solution of oxine in chloroform as blank, is shown.

It will be seen from these absorption spectra that the titanium oxinate complex absorbs strongly between 370 mμ and 400 mμ. The reagent oxine in chloroform absorbs strongly from 370 mμ to shorter wavelengths, and chloroform alone absorbs strongly below 250 mμ.

Molar extinction coefficient and sensitivity

The molar extinction coefficient of the titanium oxinate complex was found to be $6{\cdot}90 \times 10^3$ at 380 mμ, the peak of the absorption band, and $6{\cdot}36 \times 10^3$ at 400 mμ, the wavelength reported by Morrison and Freiser.[2]

It would appear from these values that the absorption band at 380 mμ probably

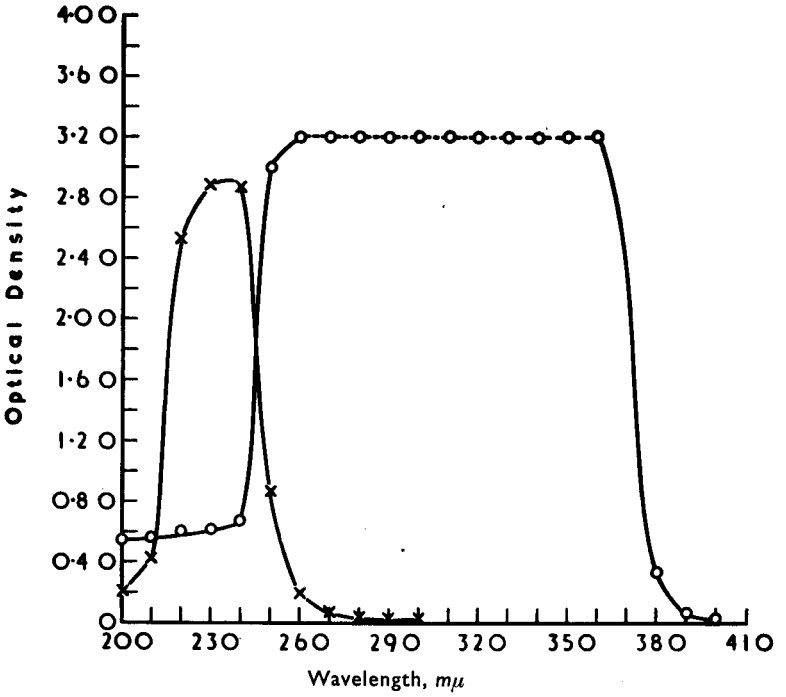


FIG. 1.—Ultraviolet absorption spectra:
○—69 × $10^{-3}M$ oxine in chloroform against chloroform as blank.
×—chloroform against distilled water as blank.

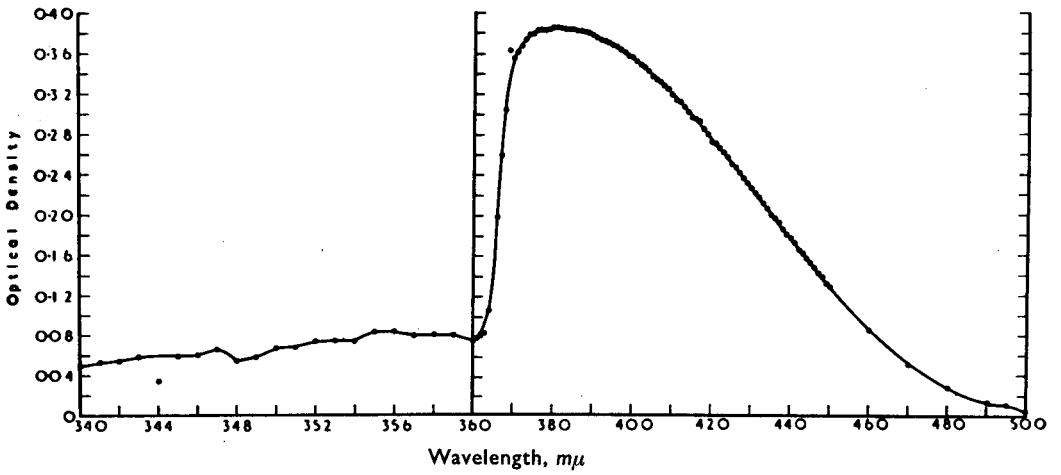


FIG. 2.—Absorption curve of titanium oxinate in chloroform
5·6 × $10^{-5}M$ titanium in chloroform against 69 × $10^{-3}M$ oxine in chloroform as blank.

arises from charge transfer, since the molar extinction coefficients are close to the usual range[6,7] of $E_{max} = 10^4$–10^5 for a fully allowed transition, and far removed from those for a forbidden transition, such as a d–d transition, with its range of $E_{max} =$ 0·01–200, and a value usually less than 50.

The sensitivities of the colour reaction, based upon the Sandell notation,[5] are, at 380 mμ, 0·007 μg/cm^2 for log $I_0/I = 0{\cdot}001$; and at λ 400 mμ, 0·008 μg/cm^2 for log $I_0/I = 0{\cdot}001$.

These values demonstrate the advantage of making measurements at 380 mμ rather than at 400 mμ. The present authors have therefore used 380 mμ in this study, despite the report in the literature[2] of the use of 400 mμ.

Interferences

One of the objects of the present study was to evaluate the suitability of titanium oxinate in chloroform for the absorptiometric determination of very low percentages of titanium in steel. Interference studies were therefore restricted to metals commonly found in steel.

Oxine forms complexes with all metals other than the alkali metals, and these complexes are extractable into chloroform. As already noted, selectivity can be controlled by pH and selective masking agents. When the interferents are in small quantities they can be effectively masked; those present in large quantities are obviously best eliminated by solvent extraction.

The metals that react in the selected pH range of 8–9 are: aluminium, antimony, bismuth, cadmium, cerium^{VI}, cobalt, copper, iron^{II}, iron^{III}, lead, manganese, mercury^{I}, mercury^{II}, nickel, tin^{II}, titanium, uranium and zinc. Of these, only aluminium, copper, iron^{III}, manganese, nickel and cobalt need be considered for the purpose of steel analysis. Chromium^{III} must also be considered since it forms a complex with EDTA. EDTA also forms complexes with the other interfering elements but its complexes with amphoteric metals, such as aluminium and chromium, and with weak electropositive metals like iron, are not stable in basic solutions.[8] Also, although the formation of EDTA complex with most metals is practically instantaneous, the amphoteric and weakly electropositive metals mentioned above are exceptional. Chromium^{III} reacts scarcely at all with EDTA at normal temperature and in acidic medium, but when heated to boiling forms a very stable violet complex,[8] CrY^-. Iron^{III} and aluminium also react slowly with EDTA at room temperature, and their complexes are not stable in basic solution in the absence of a large excess of EDTA, probably because hydroxyl ion displaces EDTA as ligand.

Although chromium^{III} does not form an extractable complex with oxine, it does interfere by forming a voluminous gelatinous precipitate of $Cr(OH)_3$ in the basic medium, even in the presence of EDTA, and thus interferes in the separation of the phases in solvent extraction. Hence chromium^{III} should be removed by oxidation to chromium^{VI} followed by solvent extraction.

A study of the relative stability constants of the complexes formed by the above metals with EDTA and with oxine indicates that EDTA is not a satisfactory masking agent for any of the above metals other than manganese^{II}. A few examples of stability constants of the respective complexes shown below in Table I will make this point clear.

Although the above values do not relate to strictly comparable media in the experimental determination of the constants, they do, however, indicate the general

TABLE I

Cations	Log stability constants	
	EDTA	Oxine
Cu^{2+}	18·4	26·22
Fe^{3+}	25·1	33·9
Fe^{2+}	14·33	15·0
Mn^{2+}	13·52	12·6
Ni^{2+}	18·62	18·7

unsuitability of EDTA for preventing all but manganese from forming a complex with oxine.

Cyanide may, however, be used as a masking agent in conjunction with EDTA. The cyanide ion forms very stable complexes with cadmium, cobalt^{II}, copper^{I}, iron^{II}, manganese^{II}, mercury^{II}, nickel, silver, uranium and zinc. All these complexes, with the exception of that with manganese, are stronger than the corresponding complexes with EDTA. Even with the combined use of EDTA and KCN, however, uranium interferes, and also aluminium in excess of ~50 μg, because of co-extraction and absorbance at about the same wavelengths.

In dealing with the problem of interferences in the analysis of steel, an example of a titanium steel *e.g.*, 18/8 stainless steel, B.C.S. No. 235/1, may be considered. This steel has the following percentage composition: Fe 71·92%; C 0·42%; Si 0·60%; S 0·019%; P 0·020%; Mn 0·60%; Ni 8·21%; Cr 18·36%; Mo 0·04%; Cu 0·13%; V 0·035%; W <0·02%; Ti 0·36%. A steel with a composition of this type presents an elimination problem in which the ratio iron: titanium is 200:1 and the ratio chromium:titanium is 50:1. Since this steel has a low titanium percentage of 0·36, it would normally be necessary to take for final determination of titanium, a sample-weight of 60 mg which, when extracted into a volume of 60 ml of extractant, would give a titanium concentration of 3·6 ppm, with optical density ~0·4 when measured in a 1-cm cell at 380 mμ. Such a sample weight would involve elimination of about 43·2 mg of iron and about 10·8 mg of chromium. Selective masking, while feasible and economic for a maximum of about 2 mg of individual interferent, is clearly impractical and uneconomic for such large amounts of interferent. In any case, in practice iron^{III} solutions masked with EDTA and KCN produced a bulky iron^{III} oxinate precipitate, only partially extracted into chloroform. The only practical solution for such major constituents as interferents is to eliminate them by a preliminary solvent extraction. Iron^{III} and chromium^{VI} can be readily extracted respectively from a 5–7*M* HCl solution at room temperature and 3*M* HCl solution at ~0°, by iso-butyl methyl ketone.

Adherence to the Beer-Lambert law of the titanium oxinate solution

The titanium oxinate-chloroform system obeys the Beer-Lambert law at the wavelengths 380 mμ and 400 mμ in the concentration range of 0–12 ppm, and a linear curve is obtained by plotting the optical density as a function of concentration. The system deviates markedly from the Beer-Lambert law above the limiting concentration 12 ppm. Fig. 3 shows the calibration curve of titanium for titanium concentrations of 0–6 ppm at the wavelengths 380 mμ and 400 mμ, using an absolute spectrophotometric technique, *i.e.*, by using the reagent blank as the check solution. Fig. 4 is the calibration curve for titanium at 380 mμ, using a differential spectrophotometric technique, *i.e.*, by using a standard titanium solution of a slightly lower concentration

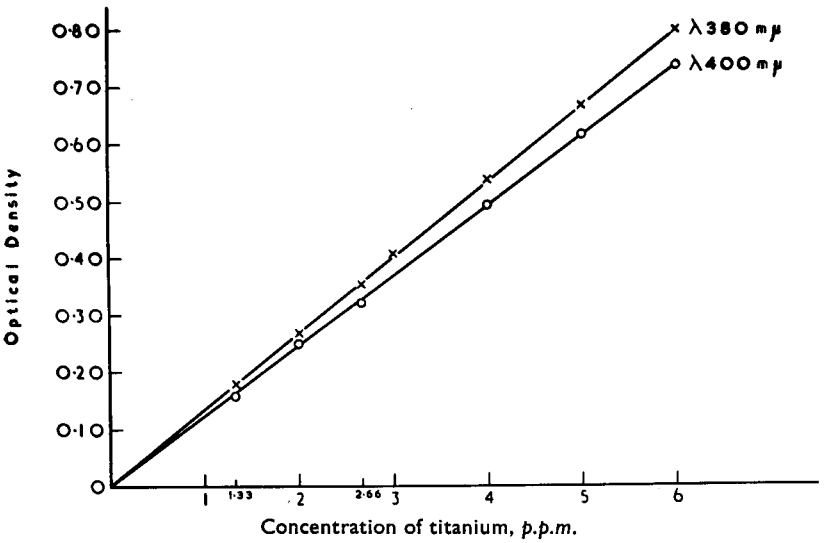


FIG. 3.—Calibration curves of titanium oxinate in chloroform at wavelengths 380 mμ and 400 mμ
Molar absorptivities: at 380 mμ = $6{\cdot}90 \times 10^3$; at 400 mμ = $6{\cdot}36 \times 10^3$.

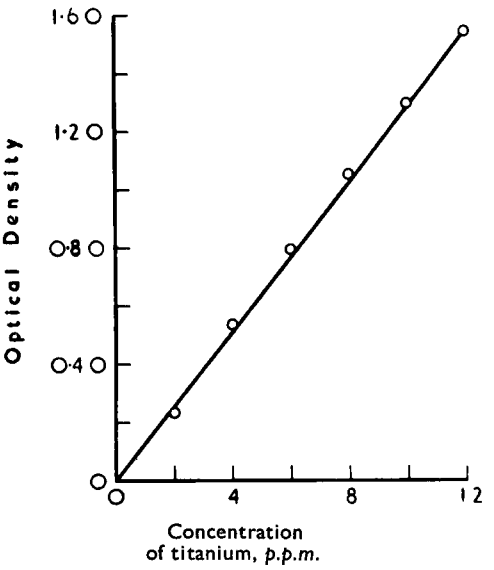


FIG. 4.—Calibration curve of titanium oxinate in chloroform.

as check solution. It will be seen that the curve at 380 mμ is slightly steeper than the curve at 400 mμ, which confirms the finding that the molar extinction coefficient at 380 mμ is slightly greater than that at 400 mμ. Because of the increased error in the absolute spectrophotometric method when the reading of optical density exceeds ~0·70, it is necessary to use the differential spectrophotometric method for more concentrated solutions to keep the error within reasonable limits. Also, the differential spectrophotometric method gives better accuracy and precision, as predicted from theoretical considerations.

Control of pH

In the presence of EDTA, the range for complete extraction of titanium is raised to pH 7·9–9·0. Copper and uranium interfere in this pH range when EDTA is used as a masking agent. Copper can be masked by KCN. Aluminium in small amounts can be masked by EDTA, but if present in excess of ~50 μg, would interfere by being co-extracted with titanium. In the pH range 8–9, which gives satisfactory extraction coefficients, the pH 8·5 has been found to be the most satisfactory, and is, therefore, used in this study.

Stability of colour of the oxinate complex

The colour formed by titanium with oxine in chloroform is fairly stable for a few hours, if the chloroform extract of titanium oxinate is kept protected from light. The solution of oxine in chloroform is also stable when kept protected from light. Both should, therefore, be kept in amber-coloured bottles, and the optical density of the chloroform extract of titanium oxinate should be measured as soon as possible after the development of colour. In the differential spectrophotometric technique, any slight change in the intensity of colour of the test solution is largely cancelled by a similar change in the intensity of colour of the check solution, the titanium concentrations of both being nearly the same.

Precision of instrument readings

A study of the precision of the readings of the Uvispek Photoelectric Spectrophotometer, when used as an absorptiometer, gave the results shown in Table II. In two series of experiments with two different test solutions, multiple readings of optical density of each solution were taken consecutively, keeping all instrumental settings and operational conditions constant throughout the experiments.

TABLE II.—PRECISION OF INSTRUMENT READINGS

Wavelength, *mμ*	Optical density	Number of readings	Average standard deviation, $S = \sqrt{\frac{\Sigma x^2}{n-1}}$	Coefficient of variation, %
400	0·650	21 readings in 5 series	0·004	0·60
400	0·292	11 readings in 1 series	0·001	0·34
380	0·292	11 readings in 1 series	0·001	0·34

The low values of the standard deviation indicate that the instrument itself, when used as an absorptiometer, has high precision. The cause for any larger deviations in the experimental results of titanium determinations should, therefore, be sought elsewhere, *i.e.*, in the various steps of separations and extraction, and in other photometric steps (other than setting and reading the spectrophotometer scale).

ANALYSIS OF A TITANIUM STEEL

Procedure

Take a suitable aliquot of steel sample in hydrochloric acid solution so as to give about 3 ppm of titanium with an optical density of about 0·4. Adjust the acidity of the solution to make it about ~1*M* in HCl. Oxidise chromium^{III} and iron^{II} to chromium^{VI} and iron^{III} with a small quantity of solid potassium bromate, or any other suitable oxidant (which must not interfere with subsequent solvent extraction and colour reaction) and boil off bromine. Adjust the acidity of the solution to 3*M* in HCl.

Cool in an ice-bath, transfer to a 250-ml separating funnel, and extract with two successive 25-ml portions of iso-butyl methyl ketone by continuous inversion for 2 min. Chromium^{VI} is quantitatively extracted into the organic phase. Allow the phases to separate. Draw off the aqueous phase into another 250-ml separating funnel. Adjust the acidity to 5–7*M* in HCl, and extract with two successive 25-ml portions of iso-butyl methyl ketone by continuous inversion for 2 min. Iron is quantitatively extracted into the organic phase. Allow the phases to separate. Draw off the aqueous phase into a 250-ml beaker. Neutralise the solution with concentrated NaOH solution. Add 10 ml of 0·1*M* EDTA solution and 10 ml of 10% KCN solution. Cool, and adjust the pH to 8·5, using a pH-meter. Transfer to a 250-ml separating funnel, and dilute the volume to 100 ml with distilled water. Extract with three successive 20-ml portions of 1% oxine-chloroform extractant, equilibrating manually for 2 min. Allow the phases to separate. Draw off the organic phase into a small amber-coloured stoppered bottle. Remove traces of water from the organic extract and the reagent blank by shaking with anhydrous sodium sulphate and filter the extracts. Measure the optical density of the test solution against the reagent blank (for the absolute method) or a known titanium of concentration slightly less than that of the test solution (for the differential method). Determine a standard curve by carrying known amounts of titanium—0, 60, 120, 180, 240, 360, 480, 600, 720 μg through the whole extraction procedure. Read off the concentration of titanium in the test solution from the standard curve, which should be prepared daily to avoid slight variations.

Notes

(*a*) At higher acidities HCl tends to reduce Cr^{VI} to Cr^{III}.

(*b*) The optimum extraction of Cr^{VI} occurs at 3*M* HCl.

(*c*) Lowering the temperature of the test solution to $\sim 0°$ increases greatly the extraction of Cr^{VI} into iso-butyl methyl ketone.

(*d*) Shaking, manually or mechanically, produces a water—iso-butyl methyl ketone emulsion. The emulsification delays phase separation and makes quantitative separation of phases difficult and questionable.

(*e*) The optimum extraction of Fe^{III} occurs at 5–7*M* HCl.

(*f*) The amounts of EDTA and KCN required as masking agents depend on the amount of interferents to be masked. Since EDTA and KCN also compete with oxine to form complexes with titanium, they should not be added in amounts more than necessary to mask interferents, to prevent a shift of the equilibrium

$$Ti^{+4} + Y^{-4} \rightleftharpoons TiY$$

to the right. (Y represents EDTA or cyanide anions.)

(*g*) The amount of oxine-chloroform extractant required depends on the amount of titanium and the excess of EDTA and KCN present. For higher concentrations of titanium, a more concentrated solution of oxine in chloroform, *i.e.*, 5% or 10% oxine w/v, will tend to shift the above equilibrium (Y now represents oxinate anions) to the right, thereby favouring the formation and extraction of titanium oxinate. For the same volume of the extractant, a larger number of extractions with smaller portions of the extractant is more effective than a smaller number of extractions with larger portions of the extractant.

DISCUSSION

The validity of this absorptiometric method for the analysis of steel containing less than 1% of titanium, is substantiated by the results shown in the Table III, which are in satisfactory agreement with the accepted values.

TABLE III.—REPRODUCIBILITY AND ACCURACY OF TITANIUM ANALYSIS

Description of sample	Number of tests	Ti content certified, %	Ti content found, %	Mean Ti content found, %	Standard deviation, %	Coefficient of variation, %	Error as % of Ti content
B. C. S. 235/1,18/8 stainless steel + Ti	7	0·36	0·37 0·33 0·33 0·34 0·37 0·34 0·37	0·35	0·019	5·4	2·8

The increasing importance of titanium in the field of steels and high temperature alloys has prompted a continuous search for simple and sensitive methods of determining this element. While this procedure can be used with satisfactory results for steel containing less than 1% of titanium, the magnitude of the standard deviation suggests some lack of precision. While a lack of high precision does not affect seriously the determination of titanium contents less than 1%, it would affect seriously those with a higher percentage of titanium. This lack of high precision originates in the various steps in the process of solvent extraction and masking. The actual absorptiometric determination, especially by the differential absorptiometric technique, is, however, very precise as has been found when working with pure titanium in examining the adherence of the titanium-oxinate-chloroform system to Beer-Lambert's Law.

Acknowledgment—One of the authors (C. L. C.) wishes to thank The Queen's University of Belfast for the research scholarship which enabled him to undertake this research.

Zusammenfassung—Die Extraktion von Titanoxinat mit Chloroform wurde studiert. Der Extrakt wird entweder absolut oder diferentiell spektrophotometriert. Die Anwendung der Methode auf die Bestimmung von Titan in Stählen wird diskutiert.

Résumé—Les auters ont étudiés l'extraction du titane à l'état d'oxinate dans chloroforme at son dosage spectrophotométrique par colorimétrie classique et par colorimétrie différentielle. La méthode est appliquée au dosage du titane dans les aciers.

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STUDIES ON THE MODE OF ACTION OF FAJANS' ADSORPTION INDICATORS

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(*Received* 17 *April* 1963. *Accepted* 23 *July* 1963)

Summary—The mode of action of Fajans' adsorption indicators in the case of eosin with silver chloride and rhodizonate with barium sulphate is studied. It is established that because of the adsorption of the silver or barium ion the solubility product of silver eosinate and barium rhodizonate at the surface of the corresponding precipitate is reached sooner than it would be, calculated on the basis of the indicator concentration in the solution. The precipitation of silver eosinate or barium rhodizonate at the surface of the precipitate is influenced by factors affecting their solubility.

THE adsorption indicators introduced by Fajans[1–3] have been widely used in analytical chemistry. Recent studies on their mode of action have suggested that Fajans' theory should be modified. The adsorption indicator theory of Schulek and Pungor[4,5] explains, in general, the colour change of these indicators at the end-point of a titration by an alteration in their physico-chemical constants in consequence of adsorption. By applying this general consideration, Pungor and Schulek[6] divided adsorption indicators into the following groups:

1. Surface precipitation indicators,
2. Surface acid-base indicators,
3. Surface redox indicators (redox adsorption indicators),
4. Surface fluorescent indicators,
5. Surface complexometric indicators.

In the present paper only the original Fajans indicators, namely, surface precipitation indicators, will be considered. They are characterised by themselves giving a precipitate with one of the components of the precipitation titration. The end-point of the titration is indicated by the adsorption of a slightly soluble salt of the dye (dye salt) on the precipitate formed in the reaction. The adsorption indicator theory developed by Schulek and Pungor explains the mode of action of this group of indicators, the most typical members of which are derivatives of fluorescein, in terms of the silver salt of the indicator being less soluble in a system containing a silver halide precipitate than in the absence of the latter.

Taking into consideration the new theory, various authors have investigated the mechanism of action of adsorption indicators. Sierra and Sánchez-Pedreno[7] reported that the indicator action of fluorescein derivatives on the surface of silver halides was influenced by acidifying the solution. In his original investigations Fajans[3] had already shown that some fluorescein derivatives were only suitable for the titration of iodides, while others could be used for both iodides and bromides; some derivatives

could also be employed in the determination of chlorides. Fajans' results are summarised in Table I. Sierra and Sánchez-Pedreno found that by acidifying the solution eosin was a suitable indicator for the titration of chloride. On the basis of the Lewis theory they attempted to explain the mode of action of surface precipitation indicators by extending the acid-base part of the Schulek-Pungor conception.

TABLE I. FLUORESCEIN DERIVATIVES AS ARGENTOMETRIC INDICATORS

Indicator	Titration of		
	Cl^-	Br^-	I^-
Fluorescein	+	+	+
Dimethyl/R/fluorescein	+	+	+
Dichloro/P/fluorescein	+	+	+
Dichloro/R/fluorescein	+	+	+
Tetrachloro/P/tetrabromo/R/ fluorescein (Phloxim)	−	+	+
Dibromo/R/fluorescein	−	+	+
Dichloro/P/tetrabromo/R/ fluorescein	−	+	+
Tetrabromo/R/fluorescein (Eosin)	−	+	+
Di-iodo/R/fluorescein	−	−	+
Dimethyl/R/di-iodo/R/fluorescein	−	−	+
Dichloro/P/tetra-iodo/R/ fluorescein (Rose bengale)	−	−	+
Tetra-iodo/R/fluorescein (Erythrosin)	−	−	−

The knowledge of surface precipitation indicators was greatly advanced by the studies of Bognár and Sárosi.[8] They very ingeniously made all fluorescein derivatives suitable for the determination of chloride by altering the dielectric constant and acid content of the solution. Bognár and Sárosi's results are shown in Table II.

We regard as very important the finding of Bognár and Sárosi that an alteration in the solvent and a consequent change in the dielectric constant caused no extension of the indicator applicability of the surface acid-base indicator *p*-ethoxychrysoïdine.

TABLE II.—USE OF ERYTHROSIN FOR THE DETERMINATION OF HALIDES WITH SILVER NITRATE

Halide ion	Concentration, %			Error, %
	Water	Acid	Organic solvent	
I^-	37	13% of CH_3COOH	50% of dioxan	±0·0
	36	14% of CH_3COOH	50% of dioxan	+0·4
	30	20% of CH_3COOH	50% of acetone	−0·1
	48	2% of H_2SO_4	50% of dioxan	+0·1
	48	2% of H_2SO_4	50% of acetone	+0·1
	48	2% of HNO_3	50% of acetone	±0·0
	30	7% of $HClO_4$	63% of acetone	−0·1
	44	6% of $HClO_4$	50% of ethanol	+0·1
Br^-	25	25% of CH_3COOH	50% of dioxan	±0·0
	49	1% of H_2SO_4	50% of acetone	+0·2
	48	2% of H_2SO_4	50% of acetone	+0·3
Cl^-	11	22% of CH_3COOH	67% of dioxan	+0·2

In our opinion the general statement of Sierra and Sánchez-Pedreno about the acid-base theory is not satisfactorily confirmed by the above observations. In order to settle the problem, we have carried out experiments to ascertain whether numerical data can or cannot be obtained to justify the mode of action of surface precipitation indicators.

EXPERIMENTAL

The experiments were carried out with sodium eosinate and sodium rhodizonate dyes as indicators. For eosin silver chloride was used as the adsorbing precipitate; the silver salt of eosin was examined separately. For rhodizonate a barium sulphate precipitate was used. The solubility of barium rhodizonate was also examined separately.

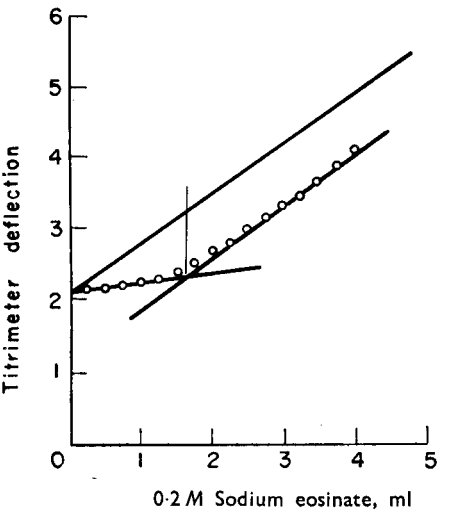

FIG. 1.—Conductometric titration of 5 ml of 0·1*M* silver nitrate.

The solubility of the dye salts (silver eosinate and barium rhodizonate) was determined conductometrically. Each dye was titrated with the corresponding salt (silver nitrate or barium chloride) in a neutral system, then from points situated more distant from the equivalence point on the titration curve, the ideal curve was drawn. A straight line was drawn from the starting point of the titration curve parallel with that part of the curve following the end-point of titration, indicating the zero reaction. Considering the deviation from the ideal curve at the end-point, the solubility of the dye salt was calculated (see Fig. 1).

The solubility product of the dye salts at the surface of the alien precipitates (silver chloride and barium sulphate) was determined as follows. Silver chloride and barium sulphate were precipitated by *Winkler's* method, then washed three times with distilled water by centrifugation. The precipitates were dried and resuspended in distilled water. To the suspensions the corresponding ions that gave precipitation with the dye were added in known quantities (silver ion to silver chloride and barium ion to barium sulphate). These systems were titrated with suitably diluted indicator solutions. The concentration of the indicators causing the characteristic coloration of the precipitates at the end-point was noted. From the ionic concentrations and the amounts of dyes added, the alien surface solubility product of the dye salts (hereafter referred to as the virtual solubility product) can be calculated.

The adsorption isotherms for the corresponding ions of the two precipitates were measured at room temperature. Silver was measured photometrically by use of *p*-dimethylaminobenzylidene-rhodanine. The adsorption of barium was determined flame photometrically. A given concentration of the corresponding ion was shaken for 20 min with 50 mg of the precipitate, then the solution was filtered and an intermediate aliquot was used in the experiments.

The size of the specific surface of the precipitates was estimated statistically from electron micrographs.

RESULTS AND DISCUSSION

The results obtained with the two systems are shown in Table III. In the case of surface precipitation indication the solubility of the dye salt is seen to suffer no alteration when the dye salt is in contact with the alien precipitate. Why the indicator and the corresponding ion seem to form a slightly soluble salt on the surface of the precipitate may be explained as follows. In consequence of the high ionic adsorption of the precipitate-forming ion, the solubility product of the dye salt at the surface of the precipitate is exceeded. The smaller concentration of the precipitate-forming ion in the solution, however, is insufficient for the formation of a precipitate.

TABLE III

Precipitate	Indicator	Solubility product of dye salt as determined conducto-metrically	Specific surface of precipitate, m^2/g	Virtual solubility product of dye salt on alien precipitate	Equilibrium concentration of precipitate-forming ion, M		Real solubility product of dye salt on alien precipitate
					In solution	In surface layer[a]	
AgCl	Eosin	$1{\cdot}4 \times 10^{-9}$	0·3	$2{\cdot}5 \times 10^{-15}$	2×10^{-6}	6×10^{-2}	9×10^{-10}
$BaSO_4$	Rhodizonate	4×10^{-9}	0·4	$2{\cdot}5 \times 10^{-10}$	4×10^{-4}	$3{\cdot}0 \times 10^{-2}$	$7{\cdot}5 \times 10^{-9}$

[a] The thickness of the solution layer forming a dynamic unit with the precipitate surface has been arbitrarily taken as 1 μ.

The above conclusion makes unnecessary the various hypotheses explaining the mechanism of indication. In our opinion Sierra and Sánchez-Pedreno's general adsorption indicator theory cannot be applied to surface precipitation indicators, because, according to the present examination, it does not explain the facts.

Taking into consideration the effects described by Sierra and Sánchez-Pedreno and by Bognár and Sárosi, the findings may be interpreted as follows. Being weak acids, the dissociation of fluorescein derivatives depends on the hydrogen ion concentration and the dielectric constant of the solution.

Hence, because the silver salt is formed with the fluoresceinate or fluorescein derivative anion, the solubility of the former is a function of the electrolytic dissociation of the dye. Further, according to the Walden rule the dissociation constant of electrolytes changes with the third power of the dielectric constant of the medium, so that with a decrease in value of the latter, in an acidic solution the amount of dissociated dye also decreases considerably. Thus if either the acid content is increased or the dielectric constant is lessened at the same acid concentration, the solubility of the silver salt of the dye virtually increases, *i.e.*, the silver salt of the dye is precipitated only at higher silver concentrations. For example, in a neutral aqueous system eosin is unsuitable as an indicator in the titration of chloride because under such conditions silver eosinate dissolves very poorly and thus its precipitation occurs before completion of the chloride titration. By increasing the acid concentration or decreasing the dielectric constant the solubility of silver eosinate at the same concentration of eosin may be increased so that silver eosinate will be precipitated only after completion of the chloride titration. Under the increase of acid concentration, the real solubility of silver chloride is altered only slightly; the alteration is primarily the effect of ionic strength on the solubility of the precipitate.

The present investigations indicate that surface precipitation indicators, in reality, do not belong to the group of adsorption indicators. Including them in this

group may be justified only for historical reasons. This consideration is confirmed by a difference existing between these indicators and those belonging to the other groups. The latter are characterised by a considerable dye adsorption on the surface of the precipitate at the excess of both kinds of precipitate-forming ions. On the other hand, surface precipitation indicators, as already shown by Fajans, are not adsorbed practically when the ions not precipitating with the dye are in excess. In the case of an excess of the ion producing a precipitate with the dye, adsorption increases very rapidly because of the precipitate formation.

The mode of action of surface precipitation indicators can thus be summarised as follows. Because of the high degree of adsorption of the precipitate-forming ion (silver or barium in the present investigations) on the surface of the alien precipitate, the solubility product of the dye salt is exceeded at very small excesses of the titrating ion. Hence, as a consequence of centre effect of crystal building, no supersaturation appears and the colored product of the dye and the precipitate-forming ion is formed at the equivalence point on the surface of the precipitate. Accordingly, the Schulek-Pungor theory is invalid for surface precipitation indicators, because with these indicators there is no adsorption in the usually accepted sense.

Acknowledgement—The authors are grateful to Dr. M. Bauman and Á. Szász for the electronmicrographs and their evaluation.

Zusammenfassung—Verfasser untersuchten den Wirkungsmechanismus der Fajans' schen Adsorptionsindikatoren und stellten fest, dass das Löslichkeitsprodukt des Indikatorniederschlages im Falle von Eosin an AgCl und Rhodisonate on $BaSO_4$ wegen der bedeutenden Adsorption des Eigenions früher erreicht wird als es nach deren Konzentration in der Lösung zu erwarten ist. Das Ausfallen des Indikatorniederschlages an der Niederschlagsoberfläche kann durch die Löslichkeit bestimmenden Faktoren beeinflusst werden.

Résumé—On a étudie le mode d'action des indicateurs d'absorption de Fajan dans le cas de l'éosine avec le chlorure d'argent et du rhodizonate avec le sulfate de baryum. On a pu montrer qu'à cause de de l'éosinate d'argent et du rhodizonate de baryum à la surface du précipité correspondant est atteint plus tôt que prévu d'après le calcul basé sur la concentration de l'indicateur dans la solution. La précipitation de l'éosinate d'argent ou du rhodizonate de baryum à la surface du précipité est influenceé par les facteurs affectant leur solubilité.

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Talanta, 1963, Vol, 10, pp. 1217 to 1221. Pergamon Press Ltd. Printed in Northern Ireland

SPECTROPHOTOMETRIC DETERMINATION OF INDOLES USING A MODIFICATION OF THE EHRLICH COLOUR REACTION

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(*Received* 3 *May* 1963. *Accepted* 13 *August* 1963)

Summary—A sensitive spectrophotometric method for the determination of indole (1 μg/ml) in aqueous solution is described. It is also applicable to certain 3-substituted indoles. The method employs *p*-dimethylaminobenzaldehyde in aqueous trifluoracetic acid. With indoles this reagent gives stable colours which are more intense than those obtained in the conventional Ehrlich reaction. Factors affecting fading of the indole colour are discussed.

DIRECT spectrophotometric determination of indoles in the presence of substances which absorb in the same region of the ultraviolet spectrum is difficult. In these circumstances, particularly where high sensitivity is required, a specific colorimetric method is desirable. A colour reaction which is virtually specific for indoles having a vacant 2- or 3-position is the well known Ehrlich test. This consists of condensation of the indole with *p*-dimethylaminobenzaldehyde in hydrochloric acid solution, to give a violet colour. In practice, accurate determination of colour intensity is difficult because the colour tends to fade. Modification of the reaction have been described[1,2,3] for colorimetric use, but no method has been found which is entirely satisfactory for spectrophotometric analysis.

We have now developed a reliable method in which indoles are treated with *p*-dimethylaminobenzaldehyde in trifluoracetic acid solution under carefully controlled conditions. The colours obtained show reproducible characteristic absorption maxima in the visible region of the spectrum. In this manner microgram quantities of indoles may be conveniently determined using the spectrophotometer.

EXPERIMENTAL

Reagents

p-Dimethylaminobenzaldehyde (AnalaR grade) was supplied by British Drug Houses Ltd., England, and used without further purification. Trifluoracetic acid (B.D.H.) was redistilled before use (b.p. 72°). Indole and skatole were purified by sublimation under reduced pressure, and gramine, indole-3-acetic acid and indole-3-(β)propionic acid by recrystallisation from cyclohexane/acetone, chloroform and water, respectively.

Procedure

The reagent was a 1% w/v solution of *p*-dimethylaminobenzaldehyde in 10% v/v aqueous trifluoracetic acid. All indoles examined were in aqueous solution. Reactions were carried out in stoppered test-tubes immersed in a thermostat at 25·0°, and screened from direct light. The procedure adopted was to add 2 ml of reagent to 2-ml portions of test solution. After shaking and allowing a standard time interval to elapse, the mixtures were examined in a Uvispek spectrophotometer (H. 700 Hilger & Watts Ltd., England) fitted with a thermostatic cell-holder maintained at 25·0°. The blank solution consisted of a mixture of equal volumes of reagent and water. Stoppered Vitreosil spectrophotometer cells of 10-mm path-lengths were used throughout.

Colour development

The indoles examined (Table I) comprised indole itself and several 3-substituted derivatives. Indole gave a stable violet colour with a well-defined maximum at 563 mμ (Fig. 1). Colour development was initially very rapid, slowing down and eventually fading. The curve for colour development (Fig. 2) shows a characteristic threshold plateau which can be conveniently utilised for the rapid determination of indole. The use of trichloracetic in place of trifluoracetic acid did not appreciably impair colour formation or intensity. Hydrochloric and perchloric acids gave lower colour intensities.

TABLE I.—CHARACTERISTICS OF THE EHRLICH COLOURS OBSERVED

Compound	Colour	λ Max., mμ
Indole	Violet	563
Skatole	Blue	578
N-Acetyltryptamine	Blue	575
Indole-3-(β)propionic acid	Blue	575
N-Acetyltryptophan	Blue	570

The violet colour faded rapidly on anaerobic illumination with green light (λ_{max} 530 mμ). The addition of excess *p*-dimethylaminobenzaldehyde increased the rate of fading. Immediate bleaching occurred on the addition of sodium dithionite. On the other hand, fading was inhibited by oxygen or hydrogen peroxide.

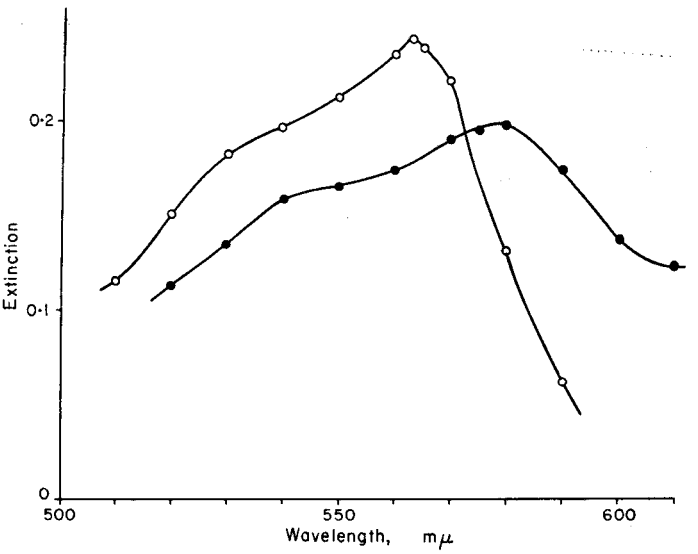


FIG. 1.—Visible absorption spectra of colours produced by indole and skatole upon treatment with the modified Ehrlich reagent:
○—Indole (4 μg/ml),
●—Skatole (4 μg/ml).

The 3-substituted indoles reacted rather differently. Skatole (3-methylindole) gave a clear blue colour with an absorption maximum at 578 mμ (Fig. 1). This colour developed slowly over a period of hours, no plateau being evident (Fig. 2). *N*-Acetyltryptamine, indole-3-(β)propionic acid and *N*-acetyltryptophan behaved in a similar way, yielding less intense blue colours (λ_{max} 570–575 mμ) which developed slowly. The colour intensities obtained after a standard reaction time (15 hr) plotted as a function of the indole concentration are shown in Fig. 3.

Gramine (3-dimethylaminomethylindole), tryptamine, indole-3-acetic acid, tryptophan, glycyltryptophan, serotonin (5-hydroxytryptamine) and bufotenin (5-hydroxy-*N*,*N*-dimethyltryptamine) yielded no colour in the reaction.

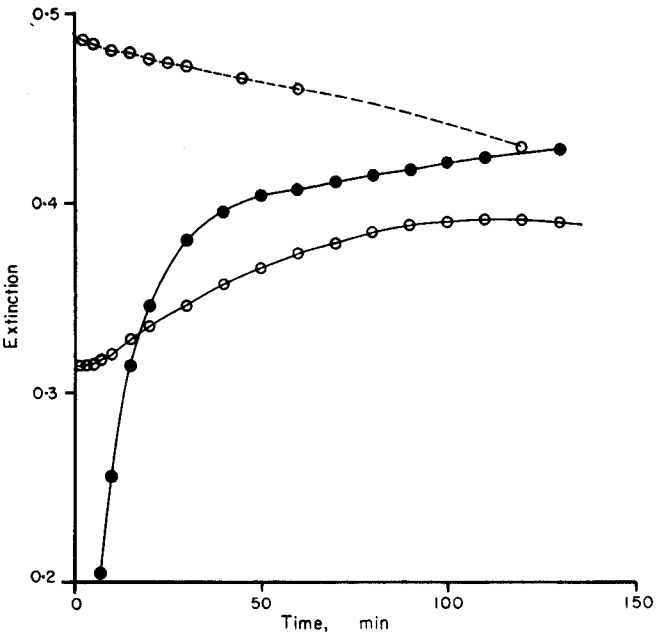


FIG. 2.—Typical rate of colour development curves obtained upon treating indole and skatole with the modified Ehrlich reagent:
○—Indole (10 μg/ml),
●—Skatole (10 μg/ml),
- ⊖ - - —Indole (10 μg/ml; 2% w/v *p*-dimethylaminobenzaldehyde in 10·5% v/v trifluoroacetic acid).

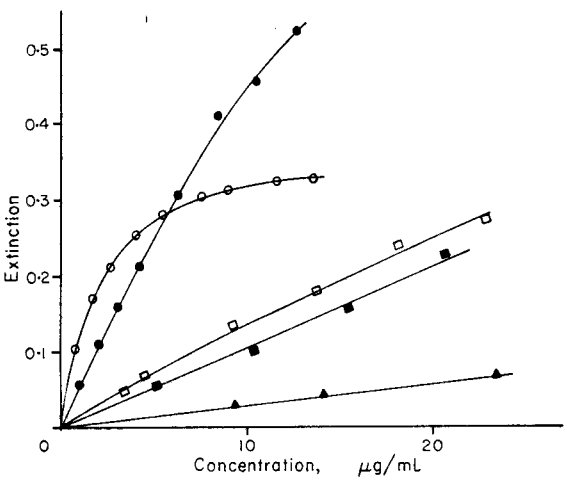


FIG. 3.—Calibration curves for the determination of indoles and certain 3-substituted derivatives using the modified Ehrlich reagent:
○—Indole,
●—Skatole,
□—Indole-3-(β)propionic acid,
■—*N*-Acetyltryptamine,
▲—*N*-Acetyltryptophan.

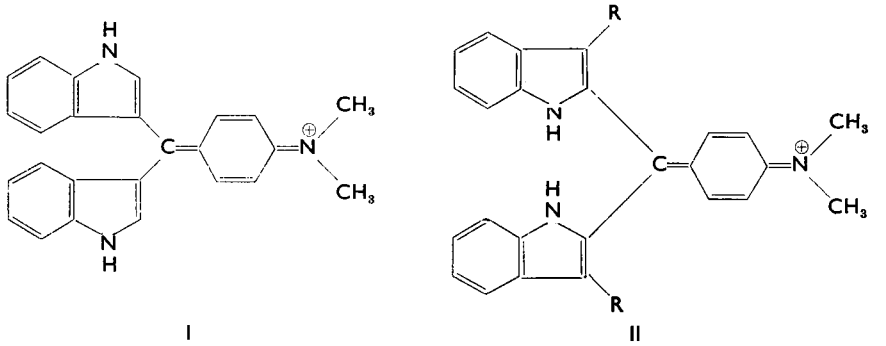
Effect of interfering substances

The colour reaction with indole was conducted in the presence of individual salts: sodium chloride, sodium phosphate, sodium acetate and "tris" hydrochloride. None of these had any marked effect on the observed colour intensity in concentrations up to 0·1*M*. Histidine, phenylalanine and tyrosine had no effect at 0·01*M* concentration. Tryptophan, however, caused rapid fading of the colour even when added after colour development had taken place. Urea (0·1*M*), often used in the study of protein systems, and anthranilic acid (0·1*M*) a potential oxidation product of indole, each considerably reduced the colour intensity. This may have been caused by competition with indole for the reagent, because the blank solutions were observed to be yellow indicating that some reaction with the reagent had taken place.

The effect of riboflavin was also examined. An attempt to determine indole in the presence of flavin mononucleotide (0·01*M*) appeared to increase the intensity of the indole colour, at the same time shifting the absorption maximum to longer wavelengths (575 mμ). The characteristic indole plateau was absent from the extinction/time curve in this case.

DISCUSSION

The colour given by indole in the original Ehrlich reaction is generally ascribed to condensation between the electrophilic *p*-dimethylaminobenzaldehyde and the reactive 3-position of the indole nucleus, finally yielding the strongly absorbing



I II

violet cation (I). In skatole, in which the 3-position is blocked by a methyl group, condensation occurs at the (less-reactive) 2-position giving the blue cation (II, R = Me),[4] colour development in this case being slower. 3-Substituents which deactivate the indole nucleus by electron withdrawal will reduce the reactivity of the 2-position, thereby further decreasing the rate of colour development. At the same time, however, lower colour intensities would be expected, because fading of the colour will be a competing process. Our results are consistent with this mechanism. Indole-3-(β)propionic acid, *N*-acetyltryptamine and *N*-acetyltryptophan reacted slowly with the reagent, giving relatively weak blue colours, diminishing in intensity according to the nature of the 3-substituent groups. The 3-substituted bases gramine, tryptamine, tryptophan, glycyltryptophan, serotonin and bufotenin in which strong cationic deactivation of the system would obtain in acid solution, gave no colour with the reagent. The negative reaction given by indole-3-acetic, acid in contrast to the propionic acid indicates considerable deactivation of the indole 2-position by the adjacent carboxyl group, possibly from a field effect.

The fading observed is an important factor in relation to colour development and sensitivity. The results obtained with indole clearly indicate that fading of the violet colour involves a photochemical reductive bleaching of the dye. The reactive intermediate would appear to be the excited (triplet) state of the dye which is reduced to a colourless product by a hydrogen donor in the solution. Fading is therefore

increased by the addition of excess *p*-dimethylaminobenzaldehyde or tryptophan[5] which act as reducing agents, or (more rapidly) by dithionite. This process is favoured by anaerobic conditions, but is inhibited by the presence of oxygen which quenches the reactive triplet state of the dye. It is significant that higher colour intensities are obtained with trifluoracetic and trichloracetic than with hydrochloric and perchloric acids. The trihalogenated acids are weaker,[6] but they have the property of quenching excited states.[7,8] Their effect then would be to inhibit the fading process, thereby increasing the apparent colour intensity.

The effect of riboflavin on the colour reaction with indole is interesting. It is conceivable that the change in position and intensity of the absorption maximum which we observed, is caused by actual oxidation of the indole photosensitised by the flavin. Flavins have been shown to act in this way in several cases.[9,10,11]

This phenomenon is being studied more closely.

Acknowledgment—We are indebted to the Department of Scientific and Industrial Research for a Maintenance Allowance (P. B.).

Zusammenfassung—Die vorliegende Arbeit bespricht ein empfindliche Spektrophotometrische Methode für die Abschätzung des Indolegehaltes (1 μg/ml) in einer Wässerigen Lösung. Sie ist auch auf gewisse 3-substituierte Indolen anwendbar. Die Methode verwendet p-dimethylaminobenzaldehyd in wässeriger Trifluoressigsäure. Dieses Reagens gibt beständige Farben mit Indoles; diese Farben sind intensiver als, jene, die man bei der allgemein ubliche Ehrlich-reaktion erhält. Faktoren, die das Blaßwerden der Indolesfarben beeinflußen, werden gleichfalls besprochen.

Résumé—On décrit une méthode sensible spectrophotométrique pour l'estimation de l'indol (1 μg/ml) dans une solution aqueuse. On peut appliquer cette methode à certains indols 3-substitués. Elle emploie p-diméthylaminobenzaldehyde dans acide trifluoracétique aqueuse. Ce réactif donne des couleurs qui sont plus intenses que celles obtenues par le réactif Ehrlich conventionnel. Les facteurs qui affectant l'altération d'indol-couleur sont discutés.

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Talanta, 1963, Vol. 10, pp. 1223 to 1227. Pergamon Press Ltd. Printed in Northern Ireland

THE DETERMINATION OF RADIUM-226 IN HUMAN BONE

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(*Received* 15 *May* 1963. *Accepted* 20 *June* 1963)

Summary—A simple procedure is described for preparing 10-g samples of bone ash for measurement of ^{226}Ra by the ^{222}Rn emanation technique. In this case, the radon is measured in 2-litre ion chambers, but scintillation chambers could be used. Coprecipitation of ^{226}Ra with $BaSO_4$ gives separation from bulk constituents, and the final precipitate is soluble in a few ml of 30% EDTA. Chemical recovery is measured with ^{133}Ba tracer.

INTRODUCTION

THIS laboratory had a requirement for a simple, accurate method for determining the radium-226 content of human bone for correlation with corresponding studies of radium in the diet. Actually, only a chemical procedure was required, since the classical emanation technique of measuring the radon-222 daughter is sensitive and specific.

It was desirable that the chemical preparation be able to handle 10 g of bone ash, since this amount of material can usually be obtained from autopsy specimens. Second, it was necessary that the preparation procedure should bring the sample into complete solution in a small volume, since the emanation technique is more reliable under these conditions. Third, the low levels of radium-226 in human bone made it imperative to reduce any blank contribution from reagents and handling. Previous methods have been lacking in one or more of these requirements.

The procedure described fulfils the requirements. The radium is co-precipitated as sulphate with barium carrier in buffered solution to remove most of the calcium, the alkali metals and phosphate. Further purification is achieved by taking up any acid-soluble material in the precipitate with HCl and repeating the precipitation in the presence of undissolved $(RaBa)SO_4$. The precipitate is dissolved in EDTA to prepare the emanating solution and final measurement is made by alpha counting the emanated radon in large ion chambers.[1] The chemical yield of barium is determined with the gamma emitting tracer ^{133}Ba.

EXPERIMENTAL

Reagents

Ba^{133} tracer solution: About 3000 cpm per 0·1-g aliquot prepared in 0·1*N* HCl.
Barium carrier solution: About 20 mg of Ba^{2+} per ml.
Ammonium acetate solution: 15 g/litre.
Acetic acid solution: 20 ml of glacial acetic acid/litre.
Ammonium sulphate solution: 100 g/litre.
Aerosol OT solution: 0·1%.
EDTA solution: 300 g/litre.
EDTA wash solution: 30 g/litre.

Sample preparation

The bone samples are scraped to remove as much residual tissue as possible, and are then ashed in a silica tray at 600° for 16 hr, or until a white or light grey ash is obtained. Most of the residual carbon can be removed by a brief re-ashing after the standard treatment with a small amount of ammonium nitrate. The bone ash is then ground to a fine powder and blended; 10 g of this material are used in the analysis for ^{226}Ra.

Chemical procedure

1. Weigh 10 g of bone ash into a 90-ml glass centrifuge tube or a 100-ml polypropylene centrifuge tube.
2. Add to the ash an accurately weighed aliquot of ^{133}Ba tracer solution.
3. Add 1 ml of Ba^{2+} carrier (20 mg of Ba^{2+}).
4. Add 20 ml of concentrated HCl (slowly at first to prevent foaming).
5. Stir, and warm in an 85° steam bath for about 5 min.
6. Add 10 ml of H_2O.
7. Add 8–10 ml of concentrated aqueous ammonia (until a dense white permanent hydroxide flock forms).
8. Dissolve the flock in concentrated HCl (about 4 ml are required).
9. Add 2 ml of ammonium acetate solution.
10. Add 1 ml of acetic acid solution.
11. Cool in a water bath to room temperature.
12. Add 1 ml of ammonium sulphate solution.
13. Stir and let stand for 0·5 hr.
14. Add 1 drop of Aerosol OT solution to reduce creeping of precipitate.
15. Balance pairs of centrifuge tubes with centrifuge cups.
16. Centrifuge at 2000 rpm for 1 hr. (3000 rpm if polypropylene tubes are used).
17. Decant carefully and discard the supernate.
18. Add 5 ml of concentrated HCl and warm in an 85° steam bath for 5 min. (Most of the $BaSO_4$ remains as a precipitate).
19. Add 10 ml of distilled H_2O and add aqueous ammonia until a permanent flock forms.
20. Dissolve the flock in concentrated HCl and repeat steps 9–17.
21. Heat the solution of EDTA and the EDTA wash solution in an 85° steam bath.
22. Break up the $BaSO_4$ precipitate with a stirring rod.
23. Add 1 ml of concentrated monoethanolamine and 5 ml of the hot EDTA solution, and stir.
24. After 5 min, wash down the sides of the tube with about 10 ml of hot EDTA wash solution.
25. Let the tube remain in the steam bath for 15 min, stirring occasionally.
26. If some residual carbon particles are present, filter the hot solution through a small Whatman No. 41 filter paper into a 1-oz polyethylene bottle.
27. Wash the tube and the filter paper with hot EDTA wash solution. Discard the paper and residue.
28. Dilute the sample to the same liquid level as a known ^{133}Ba solution (0·1 g of ^{133}Ba tracer solution diluted to 25 ml in a 1-oz polyethylene bottle). Cap, and gamma count on a flat crystal to determine the chemical yield.
29. Transfer the EDTA solution to a standard 40-ml radon bubbler[2] with a fine frit.
30. De-emanate by bubbling with "forming gas" (85% N_2 + 15% H_2) for about 10 min at 200 ml/min. (Record time as zero time for radon build-up).

With a four position centrifuge head, one person can run a total of 8 samples per day.

Counting procedure

1. Allow the sample to build up for a measured time, usually about 1 week (75% of equilibrium).
2. Connect the bubbler to the counting system with rubber tubing, and insert a small drying tube to prevent accidental transfer of liquid into the system.
3. Evacuate the external system and chamber. Flush "forming gas" through the sample at a rate of 200 ml/min for 10 min and collect the exit gas in a pulse-type ionisation chamber. The HASL 2-litre chambers are constructed of electropolished stainless steel, and the background on 6 chambers varies between 6 and 10 cph. One pico curie (pc) of ^{222}Rn gives 225 cph 5 hr after the sample has been introduced into the chamber.
4. Count all samples for a period of 14 hr after the initial 5-hr build up of radon daughters in the

chamber. Since ^{222}Rn decays appreciably during this counting interval, compute the results in the following manner:

$$\frac{\text{pc } ^{226}\text{Ra}}{\text{g ash}} = \frac{\left[\frac{\lambda}{1 - e^{-\lambda t}}\right]\left[\frac{A - B}{F_B}\right] - C}{[225]\,[\text{g ash}]\,[R]}$$

where

A = total counts during count interval (usually 14 hr),
B = backgound counts for an equivalent count interval,
C = average blank value in cph at equilibrium,
F_B = build-up factor for ^{222}Rn from ^{226}Ra to equilibrium,
225 = cph at 5 hr after introduction of 1 pc ^{222}Rn into chamber,
$\frac{\lambda}{1 - e^{-\lambda t}}$ = factor to convert net counts to cph at the beginning of the count interval (0·07531 for a 14-hr count).
λ = ^{222}Rn decay constant = $7{\cdot}5532 \times 10^{-3}$ hr^{-1},
t = count interval, and
R = fractional recovery determined with ^{133}Ba.

RESULTS AND DISCUSSION

Barium is a good analytical carrier for radium if the two elements are properly equilibrated. In this procedure, the equilibration is not quite so important, since insolubles that might contain a significant amount of radium are not discarded. The recovery for the procedure was tested with added ^{226}Ra as well as with the ^{133}Ba. The results for three sample matrices—human bone, calcium phosphate and animal bone—are shown in Table I. In all cases, the ^{133}Ba recovery seems to be adequate for estimating the ^{226}Ra recovery.

TABLE I.—^{226}Ra RECOVERY IN THE PROCEDURE
(Total radium in 10-g samples)

Sample type	^{226}Ra added, *pc*	Total ^{226}Ra, *pc*	^{133}Ba recovery, %	^{226}Ra found, *pc**	Total ^{226}Ra recovery, %
Human bone	0		93·3	0·298	—
	0		93·6	0·312	—
	0		93·6	0·270	—
	0		91·9	0·287	—
	0		91·3	0·308	—
	0		88·9	0·323	—
	0·051	0·351	90·4	0·354	101
	0·052	0·352	92·3	0·344	98
	0·052	0·352	88·7	0·338	96
	0·094	0·394	94·7	0·394	100
	0·101	0·401	94·3	0·398	99
	0·102	0·402	93·4	0·411	102
$Ca_3(PO_4)_2$	0		94·6	0·276	—
	0		95·3	0·271	—
	0		91·4	0·272	—
	0·301	0·574	92·2	0·570	99
	0·342	0·615	94·0	0·611	99
	0·317	0·590	94·1	0·599	102
	3·04	3·31	94·8	3·42	103
	3·09	3·36	91·2	3·50	104
	3·05	3·32	91·1	3·41	103

Table I (contd.)

Animal bone	0		92·8	3·77	—
	0		96·6	3·61	—
	0		93·9	3·75	—
	0·320	4·03	96·8	4·02	100
	0·304	4·01	96·4	3·95	99
	0·299	4·01	96·9	3·95	99
	3·03	6·74	99·3	6·73	100
	3·04	6·75	97·2	6·73	100
	3·06	6·77	96·6	6·86	101

* Corrected for ^{133}Ba recovery.

In the routine analysis of 120 bone-ash samples,[3] the ^{133}Ba recovery averaged 92%, which is quite comparable with the values shown in Table I.

Since the levels of ^{226}Ra in human bone are quite low, the reagent blank can be a critical factor in the analysis. A series of 12 blanks were run, with duplicate measurement of the radon in each case. The results, corrected for ^{133}Ba recovery, are shown in Table II, and give a mean of 0·020 ± 0·007 pc of radium. This blank

TABLE II.—^{226}Ra REAGENT BLANK DETERMINATIONS
(Corrected for ^{133}Ba Recovery)

Blank number	^{133}Ba recovery, %	^{226}Ra, *pc*
1	95·7	0·024, 0·005
2	92·8	0·015, 0·019
3	95·5	0·018, 0·017
4	91·0	0·016, 0·016
5	97·1	0·022, 0·024
6	92·5	0·020, 0·023
7	98·3	0·026, 0·011
8	95·8	0·028, 0·037
9	96·0	0·021, 0·018
10	97·9	0·011, 0·025
11	96·4	0·011, 0·024
12	92·8	0·023, 0·029
Mean	95·1	0·020 ± 0·007

may be compared with a mean radium content of about 0·1 pc per sample for the 120 bones mentioned above.

In general, distilled reagents like monoethanolamine, HCl and NH_4OH contribute negligible amounts of ^{226}Ra. The contribution of the other reagents is shown in Table III. These values were obtained from measurements on large quantities of the original reagents by emanation from solution, and should be more accurate than the direct blank runs. The reagents available actually dictate the procedure. Fusions with sodium carbonate, for example, are not possible, since that compound contains about 0·01 pc ^{226}Ra per g.

Another source of contamination which could be troublesome is the possible introduction of ^{226}Ra during ashing, even in silica trays. Samples of reagent $Ca_3(PO_4)_2$ were analysed for ^{226}Ra before and after ashing, and there was no detectable activity

in 4 pairs of samples arising from the ashing process, over the naturally occurring level of ^{226}Ra in the reagent.

In the case of a bone-ash sample which retains a small amount of carbon, it is desirable to filter off this residue so that ^{222}Rn is always emanated from a clear solution. Checks were performed to ensure that no significant amount of ^{226}Ra was adsorbing on the carbon particles. Ten different bone samples and 1 bone sample treated with 30 pc of ^{226}Ra were run through the procedure and filtered on 1-inch Whatman filter paper. This was covered with a 1-inch disc of alpha phosphor and counted after 30 days build-up, using a scintillation counting system.[4] The backgrounds on these counters average 0·75 cph and the efficiencies 50%. The measured ^{226}Ra activity of the untreated samples was about 1% of the original bone activity, or 0·001 pc from a mean of 0·110 pc per sample. On the treated sample, the activity in the carbon was only 0·05% of that added to the sample. The correction is negligible, and the filtration is not necessary if a carbon-free ash is obtained.

TABLE III.—^{226}Ra CONTENT OF REAGENTS USED IN THE PROCEDURE

Reagent	Amount used in procedure	^{226}Ra/g or ml found, *pc*	^{226}Ra/sample, *pc*
$BaCl_2$–$2H_2O$	0·036 g	0·19	0·0068
EDTA (0·3 g/ml)	6 ml	0·001	0·006
$(NH_4)_2SO_4$	0·2 g	0·005	0·001
Glacial acetic acid	0·04 ml	0·002	0·0001
Ammonium acetate	0·06 g	0·005	0·0003
^{133}Ba tracer solution	0·1 g	0·02	0·002
Total			0·016

Zusammenfassung—Eine einfache Methode zur Herstellung von 10g-Proben von Knochenasche zur Messung von ^{226}Ra über die ^{222}Rn-Emanation wird beschrieben. Das Rn wird hier in 21-Ionenkammern gemessen, aber man könnte auch Szintillationskammern verwenden. Mitfällung von ^{226}Ra mit $BaSO_4$ führt zur Abtrennung von der Hauptmenge und der letzte Niederschlag löst sich in ein paar ml EDTA. Die chemische Ausbeute wird mit ^{133}Ba als Tracer gemessen.

Résumé—Les auteurs décrivent un procédé simple de préparation d'échatillons de 10 grammes de cendres d'os pour le dosage de ^{226}Ra par la technique d'émanation de ^{222}Rn. Dans ce cas, le radon est mesuré dans une chambre d'ionisation de 2 litres, ou dans un compartiment à scintillation. La coprécipitation de ^{226}Ra avec $BaSO_4$ permet une séparation des constituants globaux et le précipité final est soluble dans une faible quantité d'EDTA à 30%. La récupération chimique est mesurée au moyen du traceur ^{133}Ba.

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Talanta, 1963, Vol. 10, pp. 1229 to 1233. Pergamon Press Ltd. Printed in Northern Ireland

ÜBER PEROXO-MISCHKOMPLEXE VON TITAN, NIOB UND TANTAL—III

DIE CHELOMETRISCHE TITRATION VON FÜNFWERTIGEM NIOB*

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(*Eingegangen am* 24 *Mai* 1963. *Angenommen am* 25 *Juni* 1963)

Zusammenfassung—Es wird über eine chelometrische Methode zur Titration von fünfwertigem Niob berichtet. Als Chelon wird Nitrilotriessigsäure verwendet, welche mit Peroxoniob(V)-Ionen einen 1:1-Komplex bildet. NTA wird im Überschuß zugesetzt und der Überschuß an Chelon wird sodann bei pH 5,0–5,5 mit Kupferlösung gegen den Metallfluoreszenzindikator Methylcalcein unter UV-Beleuchtung zurücktitriert. Aus Beleganalysen ergibt sich die Reproduzierbarkeit für 4,56 mg bis 23,68 mg Niob mit ±0,07 mg Niob. *N*-Hydroxyäthyl-äthylendiamin-*N*,*N*′,*N*′-triessigsäure bildet ebenfalls einen 1:1-Komplex mit Peroxoniob(V)-Ionen und kann anstelle von NTA bei der Niobtitration verwendet werden, bietet aber keinerlei Vorteile.

SEIT man die Möglichkeit erkannt hat, die dem Niob und Tantal benachbarten Elemente Titan, Zirkon, Hafnium, Thorium, Vanadium, Chrom, Molybdän und Uran mittels chelometrischer Titration zu bestimmen, wurde versucht, diese elegante Bestimmungsart auch auf Niob und Tantal zu erweitern.

Frühere Untersuchungen[1] haben gezeigt, daß sowohl fünfwertiges, als auch dreiwertiges Niob wegen der starken Tendenz von Lösungen dieses Metalls zur Hydrolyse mit EDTA nicht titrierbar sind.

Neuere polarographische Messungen[2–7] weisen jedoch auf eine Chelatbildung von Niob mit EDTA hin, doch scheint eine chelometrische Titration auf dieser Grundlage praktisch undurchführbar, weil konzentriertere Lösungen als sie in der Polarographie verwendet werden, bei pH-Werten über 3,4 zur Hydrolyse neigen.

In der bisherigen Literatur findet man nur eine Arbeit, in der eine Niobbestimmung mittels EDTA-Titration beschrieben wird.[8] Es handelt sich dabei jedoch um eine indirekte Bestimmung, bei der das Niob als Zink-Peroxoniobat gefällt wird. Der Niederschlag wird abfiltriert, gewaschen und aufgelöst, und das in Lösung gegangene, dem Niob entsprechende Zink wird anschließend chelometrisch titriert. Zur Berechnung dient ein empirischer Faktor.

Die in den ersten beiden Teilen dieser Serie veröffentlichten Untersuchungen haben ergeben,[9,10] dass sowohl Niob, als auch Tantal in Gegenwart von Wasserstoffperoxid mit einer Reihe von Chelonen Peroxo-Chelonate bilden, die gegen Hydrolyse wesentlich beständiger sind als die einfachen Chelonate dieser Metalle. Unter allen untersuchten Chelonen bilden offenbar Nitrilotriessigsäure (NTA)

* Die ersten beiden Beiträge dieser Serie finden sich in *Z. anorg. Chem.* und *Mikrochim. Acta* (im Druck).

Diäthylentriaminpentaessigsäure (DTPA) und *N*-Hydroxyäthyl-äthylendiamin-*N*,*N*',*N*'-triessigsäure (HEDTA) die stabilsten Chelate mit den Peroxokationen dieser Metalle.

Es erschien daher am aussichtsreichsten, die Eignung dieser Substanzen als Titriermittel für Niob und Tantal zu untersuchen.

Versuche zur direkten Titration von Niob wurden frühzeitig aufgegeben, da kein geeigneter Indikatorfarbstoff gefunden werden konnte. Wohl bilden Methylthymolblau (MTB) und Pyridylazoresorcin (PAR) gefärbte Nb-H_2O_2-Chelate.[10,11] Letzter Farbstoff reagiert auch mit Ta-H_2O_2.[10] Die Bildung der MTB-Peroxometall-Chelate verläuft zu langsam, während PAR erst bei Zugabe eines erheblichen Überschusses an NTA von Rot nach Gelb umschlägt.

Aus diesem Grunde schien der Versuch aussichtsreicher, das Niob mittels einer Rücktitration zu erfassen. Voraussetzung dazu ist, daß man zur Rücktitration des zugegebenen Chelon-Überschusses die Lösung eines Kations verwendet, das Niob nicht aus dem Chelonat verdrängt. Wismut, Thorium, Eisen u.a., die in saurem Medium Chelonate hoher Stabilität bilden, scheiden daher zur Rücktitration aus.

In einer neueren Arbeit hat Wilkins[12] in einem ähnlichen Fall (der Titration von Aluminium mit HEDTA) Kupfermaßlösung als geeignet gefunden, wenn die Rücktitration bei Zimmertemperatur vorgenommen wird und man Indikatoren verwendet, deren Kupferchelate von relativ geringer Stabilität sind, wie z.B. Methylcalcein (MC) und Methylcalceinblau (MCB). Methylcalcein und Methylcalceinblau sind Kondensationsprodukte von n-Methylglycin, Formaldehyd und Fluorescein bzw. 4-Methylumbelliferon.[12]

Versuche unter den von Wilkins angegebenen Bedingungen reine Lösungen von NTA und HEDTA mit Kupfermaßlösung zu titrieren, zeigten befriedigende Ergebnisse. Die Titration von DTPA mit Kupfer ergab sehr schleppende Umschläge und wurde daher nicht weiter verfolgt.

EXPERIMENTELLER TEIL

Die Titration von reiner Niob-Lösung mit NTE

Versetzt man eine saure, wasserstoffperoxidhaltige Nioblösung (Nb–H_2O_2) mit einem gemessenen Überschuß an 0,05 m NTE-Lösung, stellt anschließend den pH-Wert durch Zugabe von Natriumacetat auf 5,0–5,5 ein und titriert man dann bei Zimmertemperatur mit 0,05 m Kupferlösung gegen MC als Indikator bis zum Verlöschen der Fluoreszenz zurück, so entspricht der Kupferverbrauch dem über die vorgelegte Niobmenge hinausgehenden Uberschuß an NTA. Daraus ergibt sich die Folgerung, daß Nb–H_2O_2 mit NTA im Verhältnis 1:1 reagiert. Die Titration wird unter UV-Beleuchtung in einer bereits früher beschriebenen Apparatur durchgeführt.[13]

Stellt man den pH-Wert *vor* Zugabe von NTA ein, so erhält man einen überhöhten Kupferverbrauch und einen sehr unscharfen Indikatorumschlag. Das heißt, daß die Chelatbildung zwischen Nb–H_2O_2 und NTA nur unvollständig erfolgt. Erhitzt man aber nach pH-Einstellung und NTA-Zugabe bis zum Sieden, kühlt dann ab und titriert bei Zimmertemperatur mit Kupfer zurück, so stellt man quantitative Chelatbildung fest. Führt man die Rücktitration mit Kupfer bei höherer Temperatur durch, stellt man einen zu hohen Kupferverbrauch und außerdem einen sehr undeutlichen Indikatorumschlag fest, was darauf hindeutet, daß bei höherer Temperatur Nb oder Nb–H_2O_2 durch Cu aus dem Chelanat verdrängt werden.

Aus diesen Versuchen kann man folgendes erkennen:

(1) Quantitative Chelatbildung von Nb–H_2O_2 mit NTA erhält man:
  (a) durch Versetzen der sauren Lösung mit NTA und nachherigem Erhöhen des pH-Wertes auf 5
  (b) indem man die Lösung auf pH 5 bringt, NTA zusetzt und zum Sieden erhitzt.

(2) Die Rücktitration des NTA-Überschusses muß bei Zimmertemperatur erfolgen.

Komplexbildner wie Fluorid, Phosphat, Oxalat, Tartrat oder Zitrat maskieren das Niob mehr oder weniger vollständig und dürfen daher nicht in der Probelösung anwesend sein.

Im weiteren zeigte sich, daß man zur Pufferung anstelle von Natriumacetat zweckmäßiger Urotropin verwendet, weil man so am Titrationsendpunkt einen schärferen Indicatorumschlag erhält.

Lösungen und Reagenzien

0,05 *m Kupferlösung:* 12,49 g $CuSO_4.5\ H_2O$ werden in Wasser gelöst und im Meßkolben auf 1000 ml aufgefüllt. Die genaue Gehaltsermittlung erfolgt durch Titration mit EDTA-Maßlösung.

0,05 *m NTA-Lösung:* 9,55 g NTA werden mit etwas Wasser aufgeschlemmt und durch Zugabe von Natronlauge in Lösung gebracht. Nach dem Auffüllen auf 1000 ml wird die Lösung mit obiger Kupferlösung nach der weiter unten angegebenen Arbeitsvorschrift gestellt.

0,05 *m Nioblösung:* 6,645 g Nb_2O_5 werden mit Kaliumhydrogensulfat aufgeschlossen und der Schmelzkuchen in 100 ml Schwefelsäure 1:1 unter Zusatz von 20 ml Perhydrol gelöst. Man füllt auf 500 ml auf. Die Lösung ist Monate hindurch haltbar.

Hexamethylentetramin: gesättigte wässrige Lösung.

Methylcalcein: feste Verreibung mit Kaliumnitrat im Verhältnis 1:200. (Der Farbstoff ist bei der Fa. G. Frederick Smith Chemical Co., Columbus, Ohio erhältlich.)

Arbeitsvorschrift

Die saure, wasserstoffperoxidenthaltende Nioblösung versetzt man mit einem gemessenen Überschuß NTA-Lösung und verdünnt mit Wasser auf etwa 100–150 ml. Durch Zugabe von Urotropinlösung stellt man einen pH-Wert von 5,0–5,5 ein (Spezialindikatorpapier) und gibt eine Spatelspitze Indikatorpulver zu. Sodann titriert man in der früher beschriebenen Apparatur[13] mit Kupferlösung bis zum Verlöschen der grünen Fluoreszenz.

ERGEBNISSE

Nach obiger Arbeitsvorschrift wurde eine Reihe von Niobtitrationen durchgeführt, deren Ergebnisse in Tabelle I festgehalten sind. Die Resultate stellen

TABELLE I.—TITRATIONSERGEBNISSE BEI VERWENDUNG REINER NIOBLÖSUNG (DREIFACHE ENDPUNKTSEINSTELLUNG DURCH "PENDELN")

Nb vorgelegt, *ml*	NTA verbraucht, *ml*	Differenz, *ml*	mg Nb, *mg*		
			gegeben	gefunden	Differenz
0,98	1,00	+0,02	4,56	4,65	+0,09
0,98	0,99	+0,01	4,56	4,61	+0,05
1,96	1,97	+0,01	9,12	9,16	+0,04
1,96	1,95	−0,01	9,12	9,07	−0,05
1,96	1,94	−0,02	9,12	9,03	−0,09
2,94	2,93	−0,01	13,68	13,63	−0,05
2,94	2,93	−0,01	13,68	13,63	−0,05
4,90	4,89	−0,01	22,80	22,75	−0,05
5,09	5,08	−0,01	23,68	23,62	−0,06

jeweils das Mittel aus drei Endpunktseinstellungen dar. Da man mit zwei Maßlösungen (NTA und Cu^{++}) arbeitet, kann man den Endpunkt beliebig oft wiederholen. Flaschka[14] hat gezeigt, daß dieses "Pendeln des Endpunktes" zu einer Verminderung des Titrierfehlers führt. Wie ersichtlich, liegt die Streuung des Verbrauches innerhalb der bei derartigen Methoden üblichen Grenze, nämlich Bruchteilen eines Tropfens Maßlösung.

Die statistische Auswertung und die Reproduzierbarkeit des Verfahrens ist aus den Werten der Tabelle II ersichtlich.

Man erkennt, daß der Fehler nicht größer ist, als bei anderen bekannten und bewährten chelometrischen Titrationen.

Versuche mit HEDTA

In gleicher Weise wie mit NTA bildet $Nb\text{-}H_2O_2$ auch mit HEDTA ein Chelat,

TABELLE II.—STATISTISCHE SCHWANKUNGEN DER NIOB-TITRATION MIT NTA

Nb vorgelegt, *ml*	Anzahl der Messungen	Mittelwert gefunden, *ml*	Standardabweichung, *ml*	Variationskoeffizient, *Rel.* %
1,95	5	$1{,}94_8$	$\pm 0{,}011$	0,56
2,94	5	$2{,}93_8$	$\pm 0{,}008$	0,27

welches die titrimetrische Nb-Bestimmung erlaubt. Quantitative Komplexbildung tritt nur ein, wenn die Lösung nach dem HEDTA-Zusatz und pH-Einstellung auf 5,5 aufgekocht wird. Die Rücktitration des HEDTA-Überschusses darf in gleicher Weise wie bei den Versuchen mit NTA beschrieben, nur bei Zimmertemperatur erfolgen, da sonst Niob durch Kupfer aus dem Chelat verdrängt wird. Der günstigste pH-Wert für die Rücktitration liegt auch hier bei 5,5, wobei mit MC als Indikator wiederum die besten Ergebnisse erhalten wurden. Wie mit NTA reagiert das Nb-H_2O_2 auch mit HEDTA unter Bildung eines Mischkomplexes, der Nb und Chelon im Verhältnis 1:1 enthält. Versetzen der sauren Nb-H_2O_2-Lösung mit HEDTA und nachherige pH-Einstellung führen hier zu keiner quantitativen Komplexbildung.

Die Verwendung von HEDTA bringt im Vergleich zur oben beschriebenen NTA-Titration keinerlei Vorteile. Vielmehr ist der Indikatorumschlag am Titrationsendpunkt eher schlechter und das Chelon HEDTA nicht allgemein im Handel erhältlich. Daher wurden keine weiteren Versuche in dieser Richtung unternommen.

Die oben beschriebenen Verfahren gestatten es somit erstmals, Niob auf chelometrischem Wege zu bestimmen. Es sei vorweggenommen, daß Tantal in ähnlicher Weise reagiert. Die Störung der Titration seitens von Fremdionen, deren eventuelle Maskierung, sowie die Titration des Tantals sind Gegenstand laufender Untersuchungen und werden in Bälde mitgeteilt werden.

Für die Überlassung eines Musters HEDTA möchte ich auch an dieser Stelle der Fa. GEIGY/Basel bestens danken.

Summary—A chelatometric method for the titration of Nb^V, using nitrilotriacetic acid is described. This forms a 1:1 complex with the peroxy-Nb^V ion. NTA is added in excess, and the excess is back-titrated with Cu solution at pH 5·0–5·5 using the metallofluorescent indicator, Methylcalcein, under UV illumination. The reproducibility, for amounts of 4·56–23·68 mg of Nb is 0·07 mg of Nb. *N*-Hydroxyethylethylenediamine-*N*,*N*′,*N*′-triacetic acid forms a similar 1:1 complex, and can be used instead of NTA, but with little advantage.

Résumé—On décrit une méthode de dosage du Nb (V) par formation de chélates. L'acide nitrilotriacétique, qui forme un complexe 1/1 avec les ions peroxoniobium (V) est utilisé comme agent chélatant. On ajoute du NTA en excès er l'on dose en retour par une solution de cuivre en utilisant comme indicateur de fluorescence la méthylcalcéine sous irradiation ultra-violette. Les analyses d'essais portant sur des échantillons variant de 4,56 mg à 23,68 mg de niobium fournissent une reproductibilité de $\pm 0{,}07$ mg. L'acide N-hydroxyéthyléthylénediamine-N,N′,N′-triacétique forme également un complexe 1/1 avec les ions peroxoniobium (V) et peut être utilisé à la place du NTA mais ne présente pas d'avantages particuliers.

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Talanta, 1963, Vol. 10, pp. 1235 to 1242. Pergamon Press Ltd. Printed in Northern Ireland

APPLICATIONS OF INFRARED SPECTROSCOPY—XII*

THE BEHAVIOUR OF PROPOXYL AND BUTOXYL GROUPS IN THE ZEISEL REACTION†

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(*Received* 27 *May* 1963. *Accepted* 30 *June* 1963)

Summary—Vapour-phase infrared spectroscopy has been used to study the behaviour of n-propoxy, iso-propoxy, n-butoxy, iso-butoxy and sec-butoxy groups in Zeisel's reaction. Within each group, the reaction rate varies with the compound under study. The equilibrium iso-$C_3H_7I \rightleftharpoons HI + C_3H_6$ is involved in the determination of iso-propoxy compounds with hydriodic acid; low recoveries of iso-propyl iodide therefore result. Reflux with hydrobromic acid gives a more nearly quantitative analytical reaction, since iso-propyl bromide is more stable to reflux with hydrobromic acid than iso-propyl iodide is to reflux with hydriodic acid. n-Propoxy, n-butoxy and sec-butoxy groups can be determined successfully with hydriodic acid; in the determination of iso-butoxy groups a rearrangement occurs, and the reaction product is a mixture of iso-butyl and sec-butyl iodides.

ALTHOUGH there have been many investigations of Zeisel's reaction (see, for example, literature cited in ref. 1) very few of these have considered its application to the determination of propoxyl and butoxyl groups. In continuation of our previous investigations of aspects of the Zeisel reaction (see earlier parts of this series) we have therefore studied the behaviour of propoxy and butoxy compounds, which are now very frequently used, *e.g.*, in plastics, resins, paints and agricultural chemicals, and also as anticholinesterases and solvent extractants.[2]

Problems encountered in the analysis of the *tertiary* butoxyl isomer were found to merit individual attention: a method of analysis, involving the use of hydrobromic acid, has been published.[3]

In an extensive study, Kirsten and Nilsson[4] confirmed (*cf.* ref. 5) that iso-propoxyl groups gave low results, but these authors did not establish the cause clearly and did not suggest a remedy. They did observe, however, that reaction rates for propoxyl and for butoxyl compounds varied[4] from compound to compound; previously Inglis[6] had reported a similar effect for methoxyl compounds, which, comparatively, react much more quickly.

Several previous papers on propoxyl and butoxyl compounds can therefore be criticised on the grounds that only a few (<3) compounds were studied, or that no analytical results were quoted (*cf.* refs. 5–9). Furthermore, although these investigations used the Vieböck titrimetric finish, the other reaction conditions employed varied so widely that it is difficult to draw any critical conclusions; for example, the reaction-time recommended for n-butoxy groups varied from $7\frac{1}{2}$ min[6] to 3 hr.[8] This

* Part XI: D. M. W. Anderson and S. S. H. Zaidi, *Talanta*, 1963, **10**, 691.

† Presented at a Joint Meeting of the Scottish and North of England Sections of the Society for Analytical Chemistry, held in Belfast on 28/29 June, 1962.

lack of agreement has been maintained in more recent papers in which non-aqueous titration[10] and gas chromatography[11] have been used to determine the alkyl iodides formed in Zeisel reactions; reaction periods of 1 hr [11] (at N_2 flow-rate of 1–2 bubbles per sec) and 2 hr[10] (at 2–3 bubbles per sec) were proposed for iso-propoxy groups.

Rearrangements and decompositions of alkyl groups are known to be caused by the action of hydriodic acid,[12–16] but the extent to which these effects occur under the conditions operative in a Zeisel determination had not been investigated. Since standard analytical reference compounds for propoxyl and butoxyl groups have not yet been proposed, the present study was based on an investigation of as wide a range of compounds as could conveniently be obtained.

EXPERIMENTAL

Apparatus and general reaction conditions

These have been described,[1] together with details of the techniques for trapping volatile products and for their determination by vapour-phase infrared spectroscopy.[17,18]

Reagents

Hydriodic acid, phenol, Anhydrone and *soda asbestos* were all as previously described.[18]
Hydrobromic acid: AnalaR, about 48%, sp. gr. 1·46–1·49 (B. D. H. Ltd.)

Reference compounds

Specimens of *propane* and *propylene* (purity > 99% by vapour phase chromatography) were kindly provided by Dr J. H. Knox.

Propyl and *butyl alcohols, iodides:* Reagent grade (B.D.H. Ltd.) samples were re-distilled, then re-distilled immediately before use. Sec-butyl iodide was particularly difficult to obtain sufficiently dry (infrared spectroscopy) for calibration purposes; distillation of the vapour through a packing of Anhydrone was required.

Compounds investigated

The range of compounds investigated included alcohols, esters and ethers; their origin is shown in footnotes to the Tables. The research specimens kindly given by Messrs. I.C.I. (Dyestuffs Division) Ltd., by Dr. W. J. Kirsten, and by Dr. E. S. Lane were used as received. Commercial samples were recrystallised or redistilled carefully before use.

Procedure

Samples (2–6 mg.) were refluxed with 6 ml of hydriodic acid (constant-boiling azeotrope, pre-conditioned as described,[1] or with hydrobromic acid (48% w/w). The flow-gas was nitrogen (N. O. F. grade) at 6–8 ml per min. Crystalline samples were added to the reaction-flask after *careful* dissolution in molten phenol;[1] for liquid samples, phenol (200 mg) was added to the reaction acid before the addition of the sample. For some samples (*e.g.*, di-n-propyl ether, tri-iso-propyl phosphate, di-n-butyl ether, tri-sec-butyl phosphate) the addition of a few drops of propionic anhydride was also required to prevent their distillation, unreacted, from the reaction-flask.

In all the experiments, kinetic runs were timed from the start of ebullition of the reaction mixture and a steady flow of cold water was passed through the reaction flask condenser. The volatile reaction products were collected in a cold-trap after passage through soda asbestos and Anhydrone.[1]

RESULTS

1. *Iso-propoxy compounds*

(*a*) *Recovery of iso-propyl iodide from reflux with hydriodic acid:* When iso-propyl iodide (freshly redistilled, 2–5 mg) was refluxed with constant boiling hydriodic acid, the recovery of iso-propyl iodide was 76% after 30 min and 92% after 1 hr (averages of several runs). The use of (1) reflux for longer periods, (ii) increasing the nitrogen flow-rate to 15 ml per min (iii) passing warm water through the condenser jacket (*cf.* refs. 8, 19), and (iv) adding xylene[10] to the reaction-flask as a "carrier" did not in separate experiments, increase the percentage recovery.

(*b*) *Reaction of iso-propoxy compounds with hydriodic acid:* In experiments with iso-propanol, the molar recovery of iso-propyl iodide did not exceed 92%, and the infrared spectrum of the vapour products showed that iso-propanol had not distilled unchanged from the reaction-flask. Similar results were obtained from experiments with the esters and ethers listed in Table I.

This low but constant recovery of iso-propyl iodide indicated that some decomposition or rearrangement was involved, rather than incomplete reaction of the compounds with hydriodic acid. To facilitate the detection and identification of any decomposition product, experiments involving larger samples (50–60 mg) of iso-propyl iodide were carried out; small amounts of propylene were indicated in the infrared spectrum of the reaction products, and this was confirmed independently by vapour phase chromatography (experiments by courtesy of Dr. J. H. Knox). (In order to eliminate the possibility of thermal decomposition of iso-propyl iodide to propylene in the chromatography column, the propylene was separated from the iso-propyl iodide in the Zeisel reaction products before the chromatographic examination).

(*c*) *Recovery of iso-propyl bromide from reflux with hydrobromic acid*: Kinetic experiments showed that the recovery of iso-propyl bromide when refluxed with hydrobromic acid (48%, w/w) was quantitative in 1 hr. The recovery was 99·2% (average of several runs).

(*d*) *The reaction of iso-propoxy compounds with hydrobromic acid*: A number of iso-propoxy compounds were refluxed with constant-boiling hydrobromic acid. As shown in Table I, quantitative recovery of iso-propyl bromide was given in 1 hr for many of the compounds studied, although iso-propanol required a reaction period of 3 hr and di-iso-propyl ether required 4 hr. Analysis of the last compound was achieved by the procedure already described[1] for analysis of volatile compounds; the addition of a few drops of propionic anhydride to the reaction mixture eliminated the tendency for traces of di-iso-propyl ether to distil unreacted.

TABLE I.—YIELDS OF ISO-PROPYL BROMIDE (AS PERCENTAGE OF THEORETICAL) FROM REFLUX OF ISO-PROPOXY COMPOUNDS WITH 48% AQ. HYDROBROMIC ACID

Compounds	Reaction period, *hr*			
	1	2	3	4
1. Propan-2-ol	90·7	94·5	99·6 (max.)	
2. iso-Propyl-β-glucoside tetra-acetate	100·2			
3. Tri-iso-propyl phosphite	99·5			
4. Tri-iso-propyl phosphate	99·4			
5. iso-Propyl-*N*-(α-naphthyl)-carbamate	99·6			
6. iso-Propyl-(2,4,5-trichlorophenyl) acetate	100·2			
7. iso-Propyl-*N*,phenyl-carbamate	97·4			
8. iso-Propyl-(2,4-dichlorophenyl) acetate	100·2			
9. 2-iso-Propoxyethanol	98·3			
10. p-iso-Propoxydiphenyl	100·2			
11. 2-iso-Propoxybenzthiazole	100·2			
12. Di-iso-propyl ether	77·8	89·4	95·6	98·0 (max.)

Origin of samples: Commercial samples—1, 3, 4, 6, 8, 12.
Research specimens—5, 7, 10.
Given by Messrs. I.C.I. Ltd.—9, 11.
Given by Dr. W. Kirsten—2.

2. *n-Propoxy compounds*

(*a*) *The recovery of n-propyl iodide from reflux with hydriodic acid*: Kinetic experiments showed the recovery of n-propyl iodide to be 83·5% (0·5 hr); 89·5% (1 hr); 95% (2 hr); 98·0% (3 hr, max.)

(*b*) *Reaction of n-propoxy compounds with hydriodic acid*: The recoveries of n-propyl iodide from several compounds are shown in Table II.

TABLE II.—YIELDS OF n-PROPYL IODIDE (AS PERCENTAGE OF THEORETICAL) FROM REFLUX OF n-PROPOXY COMPOUNDS WITH 55% aq. HYDRIODIC ACID

Compound	Reaction period, *hr*				
	1	2	3	4	5
1. Propan-1-ol	92·8	95·5	99·4(max.)		
2. n-Propyl-2-chloro-3,5-dinitrobenzoate			99·6		
3. *p*-n-Propoxydiphenyl			99·4		

Origin of samples: Commercial samples—1.
Research specimens—2, 3.

(*c*) *Recovery of* n-*propyl bromide from reflux with hydrobromic acid*: The recoveries of n-propyl bromide from reflux with hydrobromic acid were:— 83·5% (1 hr); 92·6% (2 hr); 98·0% (3 hr, max.)

(*d*) *Reaction of n-propoxy compounds with hydrobromic acid*: The recoveries of n-propyl bromide from the compounds studied are shown in Table III.

TABLE III.—YIELDS OF n-PROPYL BROMIDE (AS PERCENTAGE OF THEORETICAL) FROM REFLUX OF n-PROPOXY COMPOUNDS WITH 48% aq. HYDROBROMIC ACID

Compound	Reaction period, *hr*				
	1	2	3	4	5
1. Propan-1-ol	69·8	76·5	88·2	94·5	99·8
2. Di-n-propyl ether	57·6	68·9	77·5	86·8	93·7
3. *p*-n-Propoxydiphenyl	82·2	89·3	99·6(max.)		
4. n-Propoxyacetic acid	90·0	94·0	100·2(max.)		
5. n-Propyl-α-naphthylurethane	84·0	90·3	99·3(max.)		
6. n-Propyl-3,5-dinitrobenzoate			97·6(max.)		
7. n-Propyl-2-chloro-3,5-dinitrobenzoate			99·7(max.)		
8. 2-n-Propoxybenzthiazole			99·5(max.)		
9. Di-n-propoxypentaerythritol			100·0(max.)		

Origin of samples: Commercial samples—1, 2.
Given by Messrs. I.C.I. Ltd.—4, 8, 9.
Research samples—3, 5, 6, 7.

3. *Butoxy compounds*

(*a*) *Recovery of the isomeric butyl bromides when refluxed with hydrobromic acid:* The recoveries obtained were:—

n-butyl bromide: 86·5% (0·5 hr); 90·2% (1 hr); 95·5% (2 hr); 98·2% (3 hr, max.)
iso-butyl bromide: 96·3% (1 hr, max.)
sec-butyl bromide: 96·6% (1 hr, max.)

(*b*) *Reaction of butyl alcohols with hydrobromic acid*: The recoveries of butyl bromides (as percentage of theoretical yield) were:—

n-butyl bromide from n-butanol: 68·8% (1 hr); 70·5% (2 hr); 75·6% (3 hr, max.)
sec-butyl bromide from sec-butanol: 77·5% (1 hr); 83·3% (2 hr, max.)
iso-butyl bromide from iso-butanol: 66·5% (1 hr); 67·7% (2 hr, max.)

These low recoveries indicated that reflux with hydrobromic acid does not give quantitative analytical reactions for n-, iso- and sec-butyl compounds. There was no increase in yield in experiments in which small amounts of the catalysts[14] zinc chloride and sulphuric acid were added to the reaction mixture. Further experiments using the "carrier" technique[10] were also made; toluene, mesitylene, α-methylnaphthalene, nitrobenzene and carbon tetrachloride were all tested for carrier activity, but with no significant success.

(*c*) *Reaction of butyl alcohols with phosphoric acid + potassium iodide*: A mixture of *ortho*-phosphoric acid + potassium halide is well-known[20] as a reagent for converting ethers into the corresponding halide: the use of this reagent was proposed recently[21] for the determination of methoxyl and ethoxyl groups. This reaction mixture gives a much higher reflux temperature than HI or HBr, and it appeared that its use might lead to quantitative recoveries of the butyl bromides. This was found not to be the case. It is noteworthy that Stone and Shechter[20] also reported yields of only 80–90% for the conversion of dibutyl ether and di-iso-propyl ether to the corresponding iodides.

(*d*) *Recovery of butyl iodides from reflux with hydriodic acid*: The recoveries obtained were:—

n-butyl iodide: 74·1% (0·5 hr); 80·3% (1 hr); 91·2% (2 hr); 96·4% (3 hr, max.)
iso-butyl iodide: 90·2% (0·5 hr); 94·5% (1 hr, max.)
sec-butyl iodide: 81·3% (0·5 hr); 85·5% (1 hr); 94·1% (2 hr, max.)

(*e*) *Reaction of n-butoxy compounds with hydriodic acid*: The recoveries of n-butyl iodide from a number of compounds are recorded in Table IV, which shows that the reaction-time required for maximum recovery of iodine depends on the compound under study and varies from 1–4 hr.

(*f*) *Reaction of iso-butoxy compounds with hydriodic acid*: When iso-butoxy compounds were refluxed with hydriodic acid it was observed that reaction was complete in 1 hr and that the volatile reaction product was a mixture of iso-butyl and sec-butyl iodides. The relative yields varied with the compound studied, as shown in Table V. Since iso-butyl iodide can be recovered unchanged from reflux with hydriodic acid, partial rearrangement involving a carbonium ion probably occurs during a step-wise reaction mechanism with the formation of a mixture of iso- and sec-butyl iodides, which then distil unchanged.

(*g*) *Reaction of sec-butyl compounds with hydriodic acid*: Only two compounds of sufficient purity were available for study. The molar yields of sec-butyl iodide were as follows:—

tri-sec-butyl phosphate: 85·7% (1 hr); 89·8% (2 hr); 96·7% (3 hr):
sec-butanol: 89·7% (1 hr); 95·8% (2 hr); 97·9% (3 hr).

A rearrangement of sec-butyl groups to tert-butyl has been reported to occur,[16] but it was not observed to take place to any significant extent in the present study. The sec-butanol used was purified according to Failes and Stimson.[22]

TABLE IV.—YIELDS OF n-BUTYL IODIDE (AS PERCENTAGE OF THEORETICAL) FROM REFLUX OF n-BUTOXY COMPOUNDS WITH 55% aq. HYDRIODIC ACID

Compound	Reaction period, hr			
	1	2	3	4
1. Piperonyl butoxide	98·6 (max.)			
2. 2-Butoxyethanol†	97·4 (max.)			
3. Tri-butyl phosphite	93·9 (max.)			
4. Allyl dibutyl phosphate*	95·5 (max.)			
5. Diethyl dibutyl ether	93·5	95·6 (max.)		
6. Dibutyl hydrogen phosphonate	96·8	97·7 (max.)		
7. Dibutyl butyl phosphonate	95·7	98·8 (max.)		
8. Butyl lactate	72·8	92·9 (max.)		
9. Butyl salicylate	72·4	93·2 (max.)		
10. Butyl vinyl ether†	85·2	92·8 (max.)		
11. 1-Butoxy-3-*N*,*N*-diethylcarbamoylbenzene	84·3	96·2 (max.)		
12. Butyl phenyl ether	86·6	99·0 (max.)		
13. Butyl chloromethyl ether	82·7	93·6	97·5 (max.)	
14. Butan-1-ol	78·6	91·3	97·6 (max.)	
15. 2-Amino-2′-butoxydiethyl ether†	71·5	91·3	97·2 (max.)	
16. Titanium tetra-butoxide	65·6	82·0	93·2 (max.)	
17. *N*,*N*′-Bisbutoxymethylurea	66·1	88·6	97·8 (max.)	
18. 1,1′-Dibutoxy-n-butane	56·5	63·5	78·2	92·6 (max.)

* Also gives iso-propyliodide.
† Also gives ethylene + ethyl iodide.

Origin of samples: Commercial samples—1, 2, 3, 8, 9, 10, 14.
Research samples—12.
Given by Messrs. I.C.I. Ltd.—11, 13, 15, 16, 17.
Given by U.K.A.E.A., Harwell—4, 5, 6, 7.

TABLE V.—YIELDS OF BUTYL IODIDES (AS PERCENTAGE OF THEORETICAL) FROM REFLUX OF ISO-BUTOXY COMPOUNDS FOR 1 HR WITH 55% aq. HYDRIODIC ACID

Compound	Recovery, %	
	As iso-butyl iodide	As sec-butyl iodide
1. Vinyl iso-butyl ether	63	35
2. iso-Butyl-chloromethyl ether	54	45
3. Boron-tri-iso-butoxide	58	42
4. iso-Butanol	86	12
5. Tri-iso-butyl phosphate	60	38

Origin of samples: Commercial samples—1, 2, 3, 4.
Given by U.K.A.E.A., Harwell—5.

DISCUSSION

These studies have confirmed earlier evidence[4,6] that reaction rates in the Zeisel reaction vary considerably from compound to compound; for a particular alkoxyl group, no single reference compound can be taken as a standard. This is particularly important for propoxyl and butoxyl groups; for methoxyl and ethoxyl groups the reaction rates involved are very much faster, and the effect is therefore not of such great practical significance. The lack of agreement in earlier papers[6–9] regarding the

reaction-periods required can now be ascribed, at least in part, to the fact that the range of samples studied in each case was too restricted.

The thermal decomposition of iso-propyl iodide to propylene has been extensively studied[23,24] in the gas phase, but the reaction conditions involved are not directly applicable to the comparatively low temperature of the Zeisel reaction. Evidence has been obtained that under our Zeisel reaction conditions the equilibrium

$$\text{iso-propyl iodide} \rightleftharpoons \text{HI} + \text{propylene}$$

exists to a small extent which is, however, sufficient to cause low analytical recoveries of iso-propyl iodide. The molar amount of propylene present is equivalent to 6–8 moles per cent of iso-propyl iodide, and this small amount of propylene could only be detected and estimated satisfactorily when the weight of iso-propyl iodide taken was increased from 5 to 50 mg. [This is unlikely to cause any significant change in the reaction mechanism since this range of sample weights is small compared with the large excess of hydriodic acid used (6 ml of 55% w/w).]

The increased thermal stability of iso-propyl bromide permits nearly quantitative recoveries from refluxing hydrobromic acid azeotrope. This constitutes the third important application of hydrobromic acid in modified Zeisel reactions—earlier papers have outlined its use in simultaneous determinations of mixed ethoxyl-methoxyl groups[1] and in the determination of tert-butoxyl groups.[3] Unfortunately, the comparative thermal stability of iso-butyl bromide cannot be invoked—because of the inadequate reactivity of hydrobromic acid—to give an alternative method of analysis for iso-butoxyl groups. The rearrangement to sec-butoxyl which occurs in hydriodic acid would not, of course, cause any difficulty in a determination using the Vieböck iodometric finish; the rearrangement would, however, invalidate an attempted determination, by the vapour phase infrared method, of a mixture of iso-butoxy and sec-butoxy compounds.

The difficulty of securing pure commercial samples of propyl and butyl halides, and of avoiding thermal and acidic rearrangements to other isomeric forms during purification, has recently received attention.[25,26] The standard samples used for calibration purposes in this study were probably of 98–99% purity: a paper[27] on the purification of propyl and butyl alcohols was published as this report was being prepared for publication. Many of the compounds studied were probably of 94–99% purity, as indicated by the maximum recoveries of alkyl halides recorded in the Tables; this is not considered to effect the validity of the conclusions which have been reached.

Acknowledgements—We are grateful to Professor E. L. Hirst, C.B.E., F.R.S., for his interest in these analytical studies, and we thank the P.C.S.I.R., Karachi, for awarding study leave and financial support (to S. S. H. Z.). Assistance in some of the preliminary experiments, on which this work was based, was given by J. L. Duncan, B.Sc., Ph.D., and (the late) J. F. Smith, B.Sc. We offer grateful thanks to Dr. W. J. Kirsten (Sweden), Dr. E. S. Lane (U.K.A.E.A., Harwell) and Messrs. I.C.I. Ltd. (Dyestuffs Divn.) for providing suitable specimens for examination.

Résumé—La spectroscopie infra-rouge en phase vapeur a été utilisée pour étudier le comportement des groupes n-propoxy, iso-propoxy, n-butoxy, iso-butoxy et sec-butoxy, dans la réaction de Zeisel. Pour chaque groupe, le taux de réaction varie avec le composé étudié. L'équilibre iso $C_3H_7I \rightleftharpoons HI + C_3H_6$ est compris dans la détermination des composés iso-propoxy avec l'acide iodhydrique; par conséquent,

il en résulte un taux de récupération assez bas d'iodure d'isopropyle. Le reflux avec de l'acide bromhydrique donne une réaction analytique plus quantitative, car le bromure d'isopropyle est plus stable au reflux avec l'acide bromhydrique, que ne l'est l'iodure d'isopropyle avec l'acide iodhydrique. Les groupes n-propoxy, n-butoxy et sec-butoxy peuvent être dosés avec succès au moyen d'acide iodhydrique; dans le dosage du groupe iso-butoxy, il apparait un réarrangement et le produit de réaction est un mélange d'iodure d'isobutyle et d'iodure de butyle secondaire.

Zusammenfassung—Das Verhalten von n-Propoxy-, iso-Propoxy-, n-Butoxy-, iso-Butoxy- und sek-Butoxygruppen bei der Zeisel-Reaktion wurde mittels Dampfphasen-Infrarotspektroskopie untersucht. Innerhalb jeder Gruppe hängt die Reaktionsgeschwindigkeit von der untersuchten Verbindung ab. Bei der Bestimmung von Isopropoxyverbindungen mit HJ spielt das Gleichgewicht $C_3H_7J \rightleftarrows C_3H_6 + HJ$ eine Rolle, daher ist die Ausbeute an Isopropyliodid zu klein. Der Rückfluß mit HBr gibt bessere quantitative Ergebnisse, da Isopropylbromid unter Rückfluß mit HBr stabiler ist als das Iodid mit HJ. n-Propoxy-, n-Butoxy- und sek-Butoxygruppen können mit HJ bestimmt werden. Bei der Bestimmung von iso-Butoxygruppen findet Umlagerung statt; es resultiert ein Gemisch von Isobutyliodid und sek-Butyliodid.

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Talanta, 1963, Vol. 10, pp. 1243 to 1249. Pergamon Press Ltd. Printed in Northern Ireland

DETERMINATION OF SILVER IN LEAD BY NEUTRON-ACTIVATION ANALYSIS

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(*Received* 13 *May* 1963. *Accepted* 12 *June* 1963)

Summary—Submicrogram amounts of silver have been determined in lead by neutron-activation analysis. The activity of the ^{110}Ag isotope was measured by following the decay of the 0·66-MeV gamma ray. Reproducibility was generally better than 10%. The smallest amount of silver determined was approximately 0·02 μg. The analyses were completed within 15 min.

INTRODUCTION

Activation analyses of silver have been made on many types of materials using the long-lived ^{110m}Ag species. The disadvantage of the technique is the long activation time necessary to obtain a sufficient sensitivity. A rapid activation analysis using ^{110}Ag can shorten the time necessary for the analysis. Taken into account the short half-live of ^{110}Ag (24,2 sec), rapid transfer and measurement facilities are required, a chemical separation not being feasible. Anders[1] used this isotope for analytical purposes, activating with a neutron generator. Okada[2] analysed silver in lead and other materials using ^{110}Ag. He accumulated the spectrum during a preselected time, and computed the silver content from peak height measurements of the 0·66-MeV peak. Schindewolf *et al.*[3] preferred ^{108}Ag and a rapid chemical separation for their silver analyses.

NUCLEAR PROPERTIES AND INTERFERENCES

Natural silver gives rise to three radio-isotopes by neutron irradiation, as shown in Table I. As appears from this, ^{110}Ag is more advantageous for a gamma spectrometric measurement than ^{108}Ag, which is a pure beta emitter in 97·3% of its decay mode. Consequently ^{110}Ag was chosen for the silver determination.

Since both short-lived silver isotopes have high cross-sections and considerable resonance peaks in the epithermic region,[4] care must be taken in preparing the standards necessary for the analyses. The absolute amount of silver present was always kept below 50 μg, except for the lead samples in which silver is homogenously distributed in the matrix element.

Since there is no possibility of performing a chemical separation on an isotope

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TABLE I.—NUCLEAR PROPERTIES OF SILVER ISOTOPES

Stable isotopes	Occurrence, %	Activation cross-section, *barn*	Isotope formed	Half-life	Radiation and energy, *MeV*
^{107}Ag	51·35	44	^{108}Ag	2·3 min	β: 1·77(97·3%) 1·15 γ: 0·620; 0·600; 0·430
^{109}Ag	48·65	110	^{110}Ag	24·2 sec	β: 2·84; 2·16 γ: 0·660 others weak
			^{110m}Ag	270 d	I.T.β: 0·530; 0·080 γ: 0·656; 0·706; 0·936; others

TABLE II.—INTERFERING ACTIVITIES

Isotope	Abundance, %	Activation cross-section, *barn*	Isotope formed	Half-life	Gamma energy, *MeV*
^{206}Pb	23·6	0·026	^{207m}Pb	0·84 sec	I.T. 1·06; 0·55;
^{65}Cu	30·9	2·1	^{66}Cu	5·1 min	0·83; 1·04
^{121}Sb	57·25	6·4	^{122m}Sb	3·5 min	I.T. e^- 0·059
^{123}Sb	42·75	2·5	^{124m}Sb	1·3 min	I.T. e^- (γ) 0·012
^{124}Sn	5·98	0·2	^{125m}Sn	9·5 min	I.T. 0·326
^{70}Zn	0·62	0·085	^{71}Zn	2·2 min	1·09; 0·900; 0·510; 0·120

with an half-life of 24·2 sec, the ^{110}Ag activity must be measured specifically. Most long-lived isotopes give rise to a negligible activity for short irradiation periods. Some minor and trace constituents present in lead alloys can, however, give rise to a number of short-lived isotopes. The nuclear properties of possible interfering nuclides are summarised in Table II. It appears that by restricting the measurements

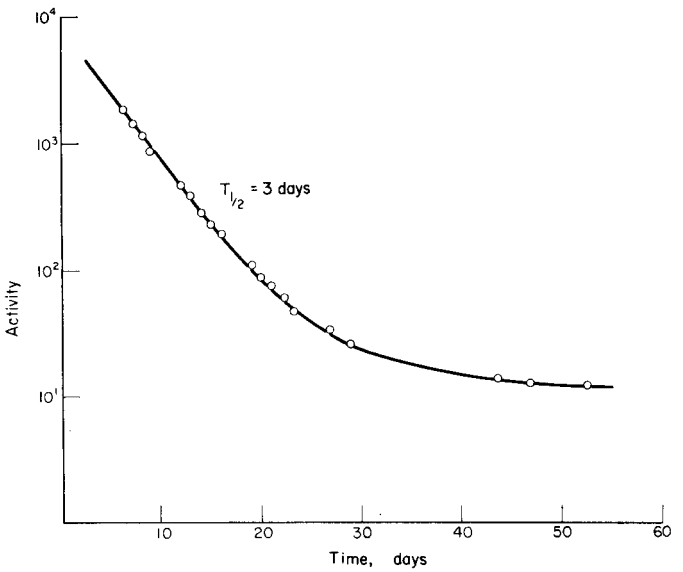

FIG. 1.—Decay curve of an irradiated flux monitor.

to the 0·66-MeV peak and analysing the decay curve, all these interferences can be avoided.

MONITORING PROCEDURES

Because samples and standards had to be measured separately, it seemed desirable to use a flux monitor to correct for neutron-flux variations during the activations. Although gold foil is generally used for this purpose, platinum was used here. The latter offers the advantage over gold that the cross-section is much lower, hence self-shielding effects are negligible.[4] Besides, because of the lower Pt cross-section, more easily weighable amounts can be used than is the case with gold monitors. Pieces of platinum wire (diam. 0·3 mm) weighing 10 mg were used. Such amounts yield an easily measurable activity of approximately 20,000 cpm for an irradiation time of 10 sec after a decay period of 24 hr. After a waiting period of 6 hr the predominant activity is that of ^{199}Au, daughter of ^{199}Pt. A decay curve of an irradiated flux monitor is shown in Fig. 1. Flux variations appeared however to be within 3% over a 12-hr period, so that an occasional check only is required.

SAMPLE PREPARATION

The analyses were tested on a series of synthetic and commercial lead samples. The synthetic samples were obtained by adding a standardised solution of $AgNO_3$ to lead powder of known purity. The silver is quantitatively precipitated on the lead powder, as could be shown in tracer experiments using ^{110m}Ag. It appeared that 99% of the silver was deposited on the lead powder after 48 hr, whereas the homogenity of the samples was better than 2% for a silver concentration of 100 ppm.

EXPERIMENTAL

The samples were irradiated in the pneumatic tube system of the BR-1 reactor. This irradiation system, developed by Campbell and Fetweiss,[5] permits short and reproducible irradiation periods at a neutron flux of approximately 10^{12} neutrons. $cm^{-2}.sec^{-1}$, and was mainly constructed for the study of isomeric nuclear states. The samples are irradiated automatically for short periods of time with a pre-set timer, which actuates the pneumatic tube system. The transfer time to and from the core of the reactor is very short (less than 0·2 sec). After irradiation, the samples are automatically positioned before a 2 × 2″ NaI(Tl) detector. The measurements can thus be started immediately after the end of the irradiation. The reproducibility of sample positioning was measured by means of a carrier-free ^{137}Cs source in the same circumstances as the real samples. No additional activity is induced in this sample during the short irradiation time of 3 sec. The reproducibility proved to be 1·5% for 10 successive runs.

The decay of the lead samples was followed with a Technical Measurement Corporation 400-channel multiscaler arrangement. The activity was accumulated in each channel during 3 sec. The 0·66-MeV photo-peak of ^{110}Ag (0·61–0·70 MeV) was selected using an external single-channel analyser. The samples were packed in Nylon containers, which gave a small short-lived activity contribution in the energy region used. A systematic error of approximately 1 μg of silver was thus introduced. It was hence necessary to remove the container for silver contents lower than 10 μg. Although 30–45 sec (1·5–2 half-lives) were lost in transferring the irradiated sample from the container, the use of a well-type NaI(Tl) detector extended the detection limit into the 0·05-μg region, because of a more favourable detection geometry.

RESULTS

The results of a number of determinations of silver in lead samples with high silver content are given in Table III. The synthetic samples with increasing percentage of silver were prepared by the method described above. The silver activity (y) is plotted against the added amount of silver (x); the slope of the straight line and its intercept with the ordinate allow the calculation of the original silver content

TABLE III.—ACTIVATION ANALYSIS OF SILVER IN LEAD

Ag added, *ppm*	Sample weight, *g*	Calculated Ag content, *ppm*	Ag found, *ppm*
540	0·1513	562	533
	0·1702		523
	0·0819		507
	0·3097		591
	0·4509		578
	0·2613		567
			550 ± 33
140	0·05125	162	164
	0·1557		178
	0·12365		182
	0·25835		168
	0·39455		164
			171 ± 8·5
133	0·16510	155	168
	0·16490		169
	0·15830		163
	0·17190		156
	0·15012		154
			160 ± 7
30	0·48025	52	59
	0·49880		49
	0·39755		53
	0·53785		54
	0·47838		52
			53 ± 4
0	0·67750	22	21
	0·74430		21
	0·86265		22
	0·93475		26
	0·96330		21
	1·02100		20
	0·99858		22
			22 ± 2

in the investigated lead alloy. This was found to be 21 ppm with a standard deviation of 9%.

The results of a number of analyses of commercial lead samples are summarised in Table IV. These were obtained with the second instrumental set-up described above. A typical calibration curve in the concentration range of 5–0·1 ppm is presented in Fig. 2. The samples for this calibration curve were obtained by mixing, in different proportions, two commercial lead samples with silver contents of 11·1 and 0·1 ppm. The content of the latter was found by extrapolating the calibration curve to zero addition of the former. The extrapolated value of 0·10 ppm of silver

TABLE IV.—ACTIVATION ANALYSIS OF SILVER IN COMMERCIAL LEAD

Commercial lead	Ag added, *ppm*	Irradiation time, *sec*	Activity, *cp* 3″	Weight, lead, *g*	Ag found, *ppm*
1	9	3	8,241	0·5192	10·70
			7,229	0·4699	10·37
			5,598	0·3166	11·92
			5,632	0·3020	12·57
			5,106	0·2796	12·30
			15,545	0·9703	10·80
			14,865	1·002	10·00
			13,983	0·9550	9·87
					11·1 ± 1·0
2	1	30	421	1·251	0·032
			234	1·169	0·019
			176	1·195	0·014
			305	0·997	0·029
			261	1·242	0·020
					0·023 ± 0·007
3	0·1	30	443	0·5386	0·078*
			1,021	1·0651	0·091
			1,190	1·0002	0·113
			1,607	0·8574	0·178
			2,013	0·9412	0·203
					0·13 ± 0.05
4	0·1	30	634	0·6013	0·100
			979	0·9031	0·103
			908	0·7697	0·112
					0·105 ± 0·006

* As the results fall distinctly in two groups, it is evident that the lack of homogenity of the sample is the reason for the important standard error on the determination.

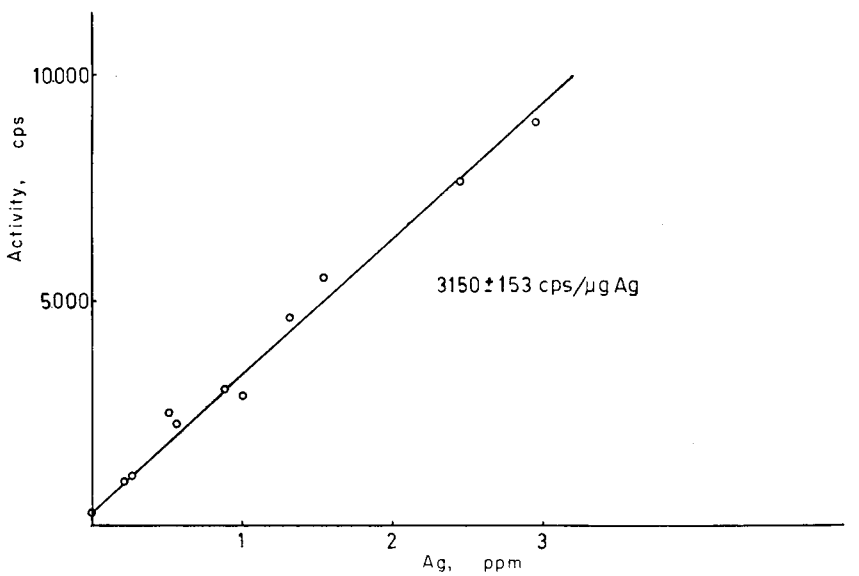

FIG. 2.—Calibration curve for the determination of silver in lead.

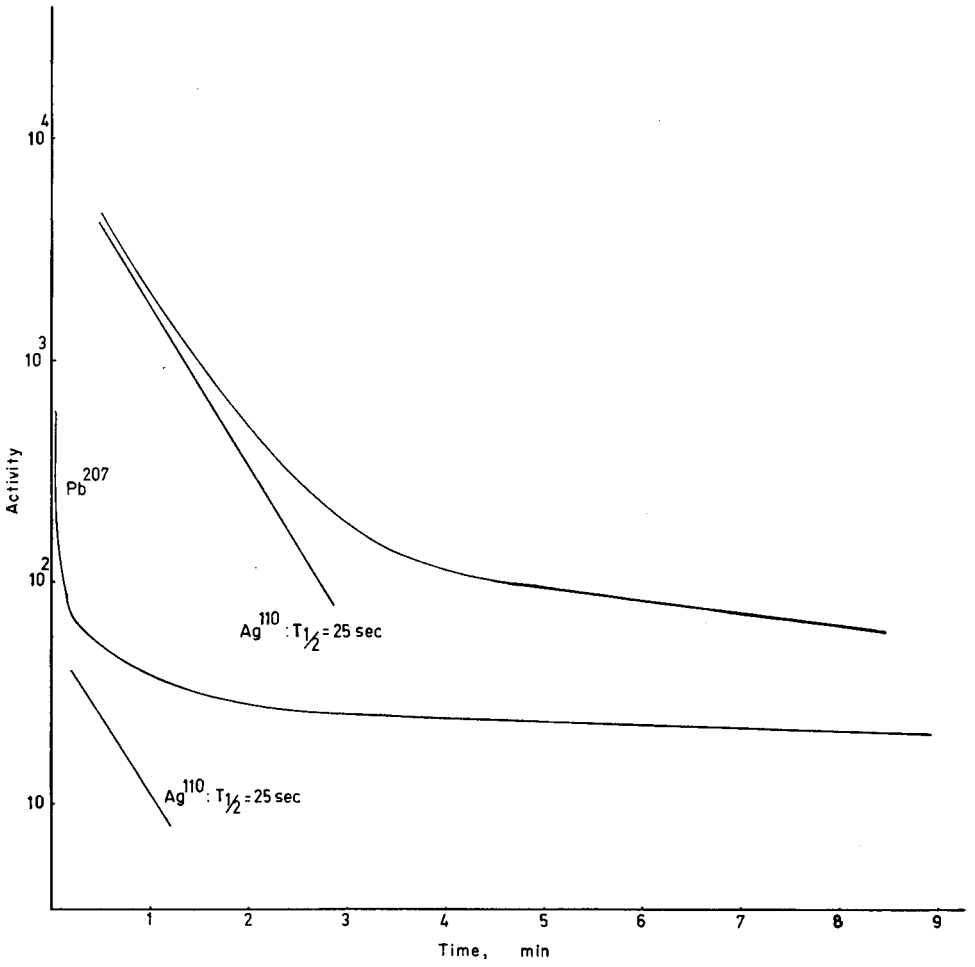

FIG. 3.—Two typical decay curves of lead samples.

gives the silver content of the purest lead sample (VM 3). The other sample was previously analysed, and showed a silver concentration of 11·1 ppm.

The half-lives found (23–25·5 sec) are in close agreement with the values given in the literature (24·2 sec). Two typical decay curves are shown in Fig. 3. The interference of antimony and copper, both of which give rise to short-lived species, was tested experimentally. Under the given experimental conditions these elements do not interfere with the accuracy of the analyses even when the antimony or copper concentration is 50 times the silver concentration.

Acknowledgements—This work is part of the research sponsored by the "Interuniversitair Instituut voor Kernwetenschappen". The hospitality offered to one of the authors (F. A.) by the "Studiecentrum voor Kernenergie" is gratefully acknowledged.

Zusammenfassung—Submikrogrammengen Ag wurden neutronenaktivierungsanalytisch in Pb bestimmt. Die ^{110}Ag-Aktivitat wurde durch Verfolgung des Abklingens der 0,66 MeV-Gammastrahlung gemessen. Die Reproduzierbarkeit war im allgemeinen besser als 10%, die kleinsten nachgewiesenen Silbermengen erwa 0.02 μg. Die Analyse dauert 15 Minuten.

Résumé—Des quantités d'argent inférieures au microgramme ont pu être dosées dans des échantillons de plomb, au moyen de l'analyse par activation de neutrons. L'activité de l'isotope Ag^{110} a été mesurée en suivant l'affaiblissement du rayonnement gamma à 0,66 MeV. La reproductibilité est généralement supérieure à 10%. Les quantités les plus faibles d'argent analysées sont de l'ordre de 0,02 microgramme et le temps d'analyse ne dépasse pas 15 minutes.

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A NEW OXIDIMETRIC REAGENT: POTASSIUM DICHROMATE IN A STRONG PHOSPHORIC ACID MEDIUM—I

TITRIMETRIC DETERMINATION OF MANGANESE^{II}

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(*Received* 3 *June* 1963. *Accepted* 20 *July* 1963)

Summary—A new method has been developed for the direct titrimetric determination of manganese^{II}, depending on its oxidation to manganese^{III} with potassium dichromate at room temperature in a strong phosphoric acid medium using a potentiometric or photometric end-point. Oxygen of the air does not interfere. The potentiometric method gives results to an accuracy within $\pm 0.3\%$ for 20–150 mg of manganese/50 ml of titration solution; with the photometric method 5–17 mg of manganese/40 ml of titration solution can be determined with an error of 0.3–1.0% depending on the amount present. Potassium dichromate in 12.0*M* phosphoric acid has a formal redox potential of about 1.5 *V* and this reagent appears to have great possibilities in titrimetric analysis.

BECAUSE of its great importance to the steel industry, the determination of manganese has engaged the attention of analytical chemists for nearly a century, resulting in the accumulation of a vast and varied literature. Many of the procedures recommended for the titrimetric determination of manganese^{II} consist of treating the sample with an excess of an oxidising agent at room temperature or at an elevated temperature, with or without catalyst, removing the excess of the oxidising agent, then titrating the manganese^{VII} formed with a standard solution of a suitable reducing agent. The use of excess bismuth tetroxide for the oxidation of manganese^{II} at room temperature was suggested by Schneider;[1] the method has been improved by, among others, Reddrop and Ramage,[2] Ibbotson and Brearley,[3] Blum,[4] Cunningham and Coltman,[5] who employed sodium bismuthate in a nitric acid medium in place of bismuth tetroxide. Bart Park[6] introduced a further improvement in the method by establishing conditions for the oxidation of manganese^{II} with excess of sodium bismuthate in 2–4*N* sulphuric acid, because the nitric acid used by earlier workers interferes during the titration of the permanganic acid formed with iron^{II} sulphate. One of the chief disadvantages of the bismuthate method is that even traces of chloride ion interfere. Cerium^{III}, cobalt^{II}, chromium^{III}, vanadium^{IV} and other ions also interfere. Further, the method is cumbersome on account of the difficulty of washing all of the permanganic acid from the excess of sodium bismuthate.

A widely employed method consists of boiling the sample with excess of persulphate in a mixture of sulphuric and phosphoric acids in the presence of silver nitrate as catalyst, cooling to room temperature, and titrating the permanganic acid formed with a standard solution of a suitable reducing agent. Several variants of this procedure have been proposed, consisting in variations of reaction medium, heating schedules, and reducing agent (arsenic^{III}, iron^{II}, mercury^{I} nitrate, hydrogen peroxide, oxalic acid, nitrite-arsenite mixture, potassium iodide, *etc*). All of these

methods suffer from the disadvantage that some of the permanganic acid may decompose when the solution is boiled to destroy the excess of persulphate. Recently, Finkel'shteĭn and Petropavlovskaya,[7] and Gaĭdarzhi[8] proposed the use of a mixture of cobalt and copper (or nickel) salts as a catalyst in place of silver nitrate when dealing with small amounts of manganese.

Willard and Thomson[9] boiled the manganese^{II} sample with an excess of potassium periodate in a medium containing sulphuric and phosphoric acids. The mixture was cooled to room temperature, treated with excess of mercury^{II} nitrate solution to precipitate the unreacted periodate and most of the iodate as the sparingly soluble mercury^{II} salts, then the permanganic acid in the filtrate was titrated with iron^{II} sulphate solution. The method is subject to the drawback that permanganic acid is completely washed from the precipitate only with difficulty. Moreover, chloride, cobalt^{II}, cerium^{III} and chromium^{III} interfere.

Willard and Merritt[10] proposed ozonised oxygen for the oxidation of manganese^{II} perchlorate in a perchloric acid medium at room temperature, using a silver salt as catalyst. In spite of the apparent simplicity of the method, it has the disadvantage that the completeness or otherwise of the oxidation depends on the concentration of ozone in the gas stream, time of reaction and concentration of silver catalyst. Moreover, the perchloric acid concentration must be maintained within certain critical limits. If the acid concentration is above 2.32*M*, the results are erratic; if the concentration is below 1.16*M*, manganese dioxide precipitates. Further, the method is subject to interferences by several ions.

Tanaka[11] and Lingane and Davis[12] oxidised the manganese^{II} with excess of silver^{II} oxide in acid solution at room temperature, subsequently heating to destroy the excess of silver^{II} oxide and titrating the permanganic acid formed with iron^{II} sulphate. The heating of the solution must not be unduly prolonged, otherwise some of the permanganic acid also decomposes. The method is also subject to interference from chromium^{III}, cerium^{III} and vanadium^{IV}.

The method of Issa and Hewaidy[13] consists in the oxidation of manganese^{II} to manganese^{IV} by alkaline hydrogen peroxide in the presence of an excess of tellurate which stabilises the manganese^{IV}. Apart from the fact that this is an indirect procedure, it suffers from other disadvantages.

The methods proposed by Metzger and Marrs,[14] Muller and Wahle[15] and Zvenigorodskaya and Gotsdiner,[16] involving the oxidation of manganese^{II} to manganese^{III} by permanganate in a hydrofluoric acid medium to stabilise the manganese^{III}, have not come into vogue for obvious inconveniences. Watters and Kolthoff[17] reported that manganese^{II} can be oxidised to manganese^{III} by treatment with excess lead dioxide in a pyrophosphate medium. After filtration of the excess lead dioxide, the manganese^{III} is titrated with standard iron^{II} solution. The method is subject to interference by chloride ion, chromium and other elements which are oxidised by lead dioxide. Ingamells[18] proposed oxidising manganese^{II} to manganese^{III} at about 145° with nitric acid in a strong phosphoric acid-pyrophosphate medium, then titrating the manganese^{III} with iron^{II} sulphate solution using diphenylamine or diphenylamine sulphonate solution as redox indicator. This method is somewhat difficult to carry out because of the great care to be exercised in heating, cooling and diluting before the titration is made. Chloride, vanadium^{IV} and cerium^{III} interfere.

A method which is now generally recognised to be very convenient is that developed by Lingane and Karplus.[19] The manganese^{II} is titrated in a 0.2–0.3*M* pyrophosphate medium at a controlled pH of 6 to 7 with a standard solution of potassium permanganate. Because of the strong colour of the manganese^{III}-pyrophosphate complex, a potentiometric end-point is used. Large amounts of chloride, cobalt, chromium, iron, nickel, copper, *etc.*, are reported not to interfere. The pH of the titration mixture is, however, somewhat critical. At a pH above 8 the manganese^{III} disproportionates into manganese dioxide and manganese^{II}, while the potential break decreases considerably with decreasing pH.

Lang and Kurtz[20] developed a new procedure for the determination of manganese based on the oxidation of manganese^{II} to manganese^{III} by chromic acid induced by the reaction between arsenic^{III} and chromic acid in the presence of hydrofluoric and phosphoric acids, which evidently help the stabilisation of manganese^{III}. The manganese^{III} formed in the reaction is determined by titration with standard iron^{II} solution using diphenylamine sulphonic acid as indicator. This method is unsatisfactory in the presence of aluminium, calcium and magnesium, which form insoluble fluorides and the precipitates absorb a small amount of manganese^{II} ion.

During our investigation of iron^{II} as a reductimetric reagent in a strong phosphoric acid medium, it has been observed accidentally that the phosphoric acid obtained from one manufacturer gave a pink colour when used as a medium for the titrimetric determination of chromium^{VI} with iron^{II} sulphate. Detailed experiments have shown that the pink colour is due to manganese^{III} formed by the reaction of chromium^{VI} with the manganese^{II} present as an impurity in the phosphoric acid. It was observed that vanadium^{IV}, cerium^{III}, uranium^{IV}, molybdenum^{V}, iron^{II}, arsenic^{III} and antimony^{III} are also oxidised by chromium^{VI} in a strong phosphoric acid medium. We have now been able to establish conditions under which manganese^{II} can be titrated directly at room temperature with a standard solution of potassium dichromate using a potentiometric end-point. Because the new reaction offers interesting possibilities for the use of potassium dichromate in a concentrated phosphoric acid medium as a powerful oxidimetric reagent, a systematic investigation of this reagent is now under way in this laboratory.

We have determined the formal redox potentials of the chromium^{VI}/chromium^{III} and manganese^{III}/manganese^{II} couples in media of varying phosphoric acid concentration. The data presented in Tables I and II and the curves given in Fig. 1 show that while the redox potential of the manganese^{III}/manganese^{II} couple is nearly constant (*ca.* 1.31 V) with changing phosphoric acid concentration, the redox potential of the chromium^{VI}/chromium^{III} couple steeply increases with increasing phosphoric acid concentration from 1.016 V in 1*M* phosphoric acid to 1.483 V in 12*M* phosphoric acid. It will be noted that the chromium^{VI}/chromium^{III} couple has a lower potential than that of the manganese^{III}/manganese^{II} couple below 7.7*M* phosphoric acid concentration, but it is higher above this concentration. In a 12*M* phosphoric acid medium the potential of the chromium^{VI}/chromium^{III} couple is greater than that of the manganese^{III}/manganese^{II} couple by 0.175 V. Using this value the equilibrium constant of the reaction

$$6Mn^{2+} + Cr_2O_7^{2-} + 14H^+ \rightarrow 6Mn^{3+} + 2Cr^{3+} + 7H_2O$$

is 3.89×10^{17}($\log_e K = nF \times E_{cell}/RT$). From the data obtained during a number

of potentiometric titrations of manganese^{II} with potassium dichromate in phosphoric acid, potential *vs.* volume curves have been plotted. The potentials of the manganese^{III}/manganese^{II} and chromium^{VI}/chromium^{III} couples computed from these curves are given in Table III and they show sufficient agreement with the determined

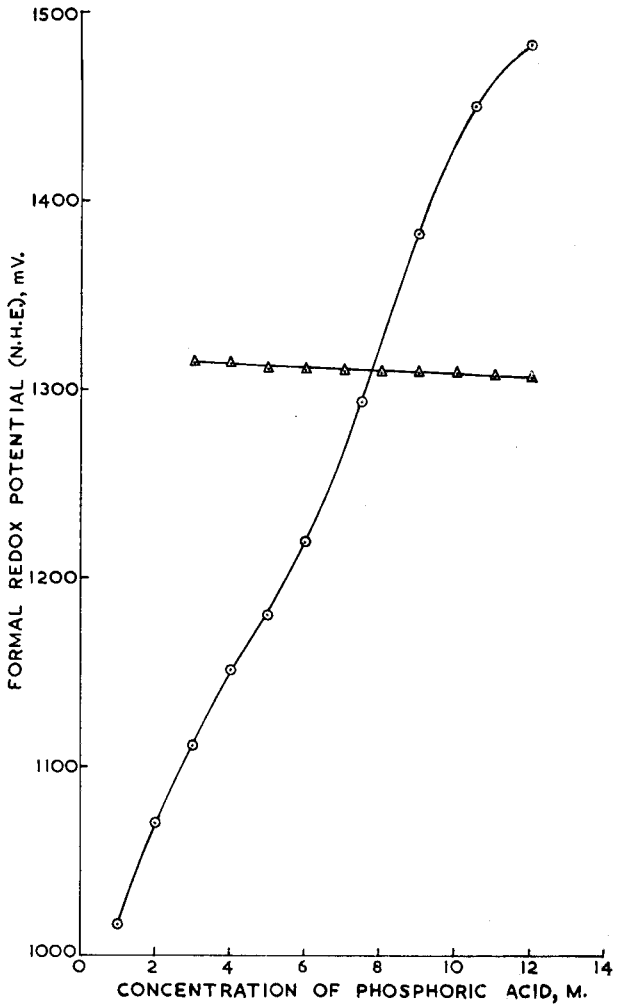

FIG. 1—Formal redox potentials of manganese^{III}/manganese^{II} and chromium^{VI} chromium^{III} couples in a medium of varying phosphoric acid concentration:
△—△ manganese^{III}/manganese^{II} couple,
⊙—⊙ chromium^{VI}/chromium^{III} couple.

potentials. We have observed that although the potential of the chromium^{VI}/chromium^{III} couple increases with an increasing concentration of sulphuric acid or perchloric acid, chromium^{VI} does not oxidise manganese^{II} in these acid media, obviously because the manganese^{III}/manganese^{II} couple has a higher potential. For instance, Vetter and Manecke[21] determined the manganese^{III}/manganese^{II} couple to have a potential of 1.488 V in 15*N* sulphuric acid at 25°; Smith and Richter[22] stated that

the formal potential of the chromium^{VI}/chromium^{III} couple is 1.350 V in 8*M* sulphuric acid. In our experiments the part played by phosphoric acid appears to be two-fold: it lowers the redox potential of the manganese^{III}/manganese^{II} couple by complexing the manganese^{III} more strongly than the manganese^{II} and it increases the potential of the chromium^{VI}/chromium^{III} couple through the usual acid effect. There is also some evidence that phosphoric acid complexes with chromium^{VI} and chromium^{III}. From a spectrophotometric study, Holloway[23] obtained evidence for two "chromate—phosphate" complexes in 1.46*M* phosphoric acid, namely $HCrPO_7^{2-}$ and $H_2CrPO_7^-$. If such complexes are formed, evidently they must be weaker than the complexes formed between phosphoric acid and chromium^{III}, because the potential of the chromium^{VI}/chromium^{III} couple would otherwise decrease and not increase as has been found experimentally.

EXPERIMENTAL

Determination of Formal Redox Potential of Manganese^{III}/Manganese^{II} Couple in a Strong Phosphoric Acid Medium

Reagents

Manganese^{II} solution: A 0.1*M* solution is prepared from AnalaR manganese^{II} sulphate (British Drug Houses Ltd., England) and standardised by the method of Lingane and Karplus.[19]

Manganese^{III} solution: 25.0 ml of the standardised 0.1*M* manganese^{II} solution is taken in a 600-ml Pyrex beaker and treated with 75 ml of syrupy phosphoric acid ("Pro Analysi" grade of E. Merck, Germany) of known strength and 12 ml of nitric acid (1:1) which has been previously boiled to expel the lower oxides of nitrogen. The mixture is heated on a hot plate until all the excess of unreacted nitric acid and oxides of nitrogen are driven off. The mixture is cooled and made up to 100 ml with double-distilled water. The strength of this solution with respect to manganese^{III} is determined by titration with standard iron^{II} sulphate solution using diphenylamine sulphonic acid as indicator. Because the original strength of manganese^{II} is known, the strength of the final solution with respect to manganese^{II} can be calculated by difference. The solution contains about 80% of manganese^{III} and 20% of manganese^{II} and is stable with respect to manganese^{III} for several days.

Syrupy phosphoric acid: "Pro Analysi" grade reagent (E. Merck, Germany) is used in this investigation. This phosphoric acid is free from impurities which can react with manganese^{III} within the time of the experiment.

Apparatus

The potentiometric assembly consists of a Cambridge potentiometer and a suspension galvanometer. A bright platinum rod (*ca.* 0.2 mm in diameter) serves as the indicator electrode and a saturated calomel electrode as the reference electrode.

Procedure

After determining the concentrations of manganese^{III} and manganese^{II} in the oxidised solution, a volume corresponding to 5.0 ml of a 0.05*M* solution of manganese^{III} is taken. Manganese^{II} solution is then added so that the total concentration with respect to manganese^{II} also corresponds to 5.0 ml of 0.05*M*. After adding enough phosphoric acid to give the required strength, the mixture is diluted to 50 ml. Because of the considerable heat of dilution of phosphoric acid the mixed manganese solutions and phosphoric acid are taken in a 50-ml measuring flask along with most of the water and phosphoric acid, then allowed to cool to room temperature before the final dilution to the mark. The mixture of manganese^{II} and manganese^{III} in phosphoric acid is kept in a 150-ml Pyrex beaker which is connected with a saturated calomel electrode through two salt bridges, one of saturated sodium perchlorate and the other of saturated sodium nitrate. This precaution has to be taken in view of the following observations. When a single salt bridge of sodium nitrate is used between the manganese^{III}/manganese^{II} half cell and the calomel electrode, it was observed that some of the nitrate ion diffusing into the half cell slowly oxidises the manganese^{II} in the concentrated phosphoric acid medium employed. A single sodium perchlorate bridge also cannot be used because the potassium ion from the saturated calomel electrode diffusing into the bridge produces a precipitate of potassium perchlorate which blocks the electrical connection. A single potassium chloride bridge is also undesirable because the chloride ion diffusing into the manganese^{III}/manganese^{II} half cell slowly reduces the manganese^{III}. The mixture in the beaker

is stirred by an electromagnetic stirrer during potential determination. The potentials attain equilibrium values within 1–2 min. Smith and Getz[24] also employed a two-bridge assembly similar to the one described here for the determination of the redox potential of the cerium^{IV}/cerium^{III} couple in a nitric or perchloric acid medium.

The potentials (uncorrected for liquid-liquid junction potential) obtained in a medium of varying phosphoric acid concentration are given in Table I.

Determination of Formal Redox Potential of Chromium^{VI}/Chromium^{III} Couple in a Strong Phosphoric Acid Medium

Reagents

0.05*M* *Chromium^{VI} solution:* Prepared from "Pro Analysi" grade chromic anhydride (E. Merck, Germany). The solution is standardised by titration with a solution of iron^{II} sulphate, which in turn has been standardised against a solution of potassium dichromate of known strength.

TABLE I—FORMAL REDOX POTENTIAL OF MANGANESE^{III}/MANGANESE^{II} COUPLE IN A MEDIUM OF VARYING PHOSPHORIC ACID CONCENTRATION (TEMPERATURE: 28°; TOTAL MANGANESE CONCENTRATION: 0.01*M*).

Concentration of phosphoric acid, *M*	Formal redox potential (N.H.E.), *V*
3.0	1.315
4.0	1.314
5.0	1.313
6.0	1.312
7.0	1.312
8.0	1.311
9.0	1.310
10.0	1.310
11.0	1.309
12.0	1.308

0.05*M* *Chromium^{III} solution:* Prepared by cathodic reduction of 0.05*M* chromium^{VI} solution in 3.0*M* phosphoric acid using a diaphragm cell. Reduction is carried out by means of four 2-V batteries connected in series, using a bright platinum gauze as the cathode and a platinum rod as the anode, for 24 hr. The completion of reduction is shown by the solution turning clear green. The solution contains mostly chromium^{III} and only a little chromium^{VI}. The concentration of chromium^{VI} in the solution is determined by taking an aliquot, diluting with 1.0*N* sulphuric acid, treating with a known excess of standard (0.05*N*) iron^{II} sulphate solution and quickly titrating with a standard solution of potassium dichromate using diphenylamine sulphonic acid as indicator. The concentration of chromium^{III} in the solution is determined by the persulphate oxidation method as described by Kolthoff and Belcher.[25]

Syrupy phosphoric acid: For the purpose of this investigation neither syrupy phosphoric acid of "Pro Analysi" grade (E. Merck, Germany) nor AnalaR grade acid (British Drug Houses, England) has been found satisfactory because of the presence of traces of reducing impurities which react rapidly when treated with an excess of chromium^{VI}, thereby decreasing its titre. We have, however, observed that the reducing impurities can be destroyed by heating on a hot plate with nitric acid (1:1) which has been previously boiled to drive off any lower oxides of nitrogen. After treatment with nitric acid, the phosphoric acid must be heated long enough to ensure the complete removal of nitric acid. The strength of the phosphoric acid is ascertained, after suitable dilution, by titration with a standard solution of sodium hydroxide using a mixture (1:1) of phenolphthalein and α-naphtholphthalein as indicator.

Procedure

After determining the concentrations of chromium^{III} and chromium^{VI} in the reduced solution, a volume corresponding to 5.0 ml of a 0.05*M* solution of chromium^{III} is taken. Enough chromium^{VI} solution is then added such that the total concentration with respect to chromium^{VI} also corresponds to 5.0 ml of 0.05*M*. After adding enough phosphoric acid to give the required strength, the mixture is diluted to 50 ml. Because of the considerable heat of dilution of phosphoric acid, the mixed chromium solutions and phosphoric acid are taken in a 50-ml measuring flask along with most of the water and phosphoric acid, then allowed to cool to room temperature before the final dilution to the mark. The potential of the mixture is measured with the apparatus already described for the determination of the manganese^{III}/manganese^{II} couple.

The potentials (uncorrected for liquid-liquid junction potential) obtained in a medium of varying phosphoric acid concentration are given in Table II. The potentials show some drift, but attain equilibrium values within 20–30 min. The time required for equilibration decreases as the concentration of phosphoric acid is increased.

Similar values are obtained by using potassium dichromate in place of chromic acid and chrome alum in place of electrolytically reduced chromium^{III}. The potentials of the two half cells as obtained from the titrations are given in Table III.

Oxidation of Manganese^{II} to Manganese^{III} with Potassium Dichromate

Ingamells[18] noticed that manganese^{II} is oxidised to manganese^{III} when treated with an excess of chromic acid in a strong phosphoric acid medium. We have observed that the reaction between

TABLE II—FORMAL REDOX POTENTIAL OF CHROMIUM^{VI}/CHROMIUM^{III} COUPLE IN A MEDIUM OF VARYING PHOSPHORIC ACID CONCENTRATION (TEMPERATURE: 28°; TOTAL CHROMIUM CONCENTRATION: 0.01*M*).

Concentration of phosphoric acid, *M*	Formal redox potential (N.H.E.), *V*
1.0	1.016
2.0	1.070
3.0	1.111
4.0	1.151
5.0	1.180
6.0	1.219
7.5	1.292
9.0	1.383
10.5	1.450
12.0	1.483

TABLE III—REDOX POTENTIALS OF MANGANESE^{III}/MANGANESE^{II} AND CHROMIUM^{VI}/CHROMIUM^{III} COUPLES COMPUTED FROM POTENTIOMETRIC TITRATION CURVES

Experiment No.	Approximate concentration of phosphoric acid, *M*	Mn^{III}/Mn^{II} couple, *V*	Approximate concentration of phosphoric acid, *M*	Cr^{VI}/Cr^{III} couple, *V*
1.	13.2	1.318	12.6	1.500
2.	12.8	1.321	12.2	1.507
3.	13.2	1.320	12.0	1.500
4.	13.0	1.308	11.7	1.496
5.	12.7	1.325	12.0	1.509

manganese^{II} and chromium^{VI} is so fast in 11.5–13.5*M* phosphoric acid that a direct titration of manganese^{II} is possible with potassium dichromate at room temperature using a potentiometric end-point. When the concentration of phosphoric acid is 11.0*M* at the end of the titration, the reaction is somewhat slow, so that in the vicinity of the equivalence point it is necessary to wait about 3 min to obtain steady potentials. Hence, we carried out the electrometric titration of manganese^{II} with potassium dichromate at room temperature maintaining the concentration of phosphoric acid to be about 12*M* at the equivalence point.

Several experiments have shown that "Pro Analysi" grade phosphoric acid (E. Merck) is quite satisfactory as a titration medium without the preliminary nitric acid treatment proposed for the redox potential determinations. Both with and without treatment with nitric acid, the potential breaks at the equivalence point are the same (*ca.* 30–35 mV/0.04 ml of 0.2*N* potassium dichromate or 50–60 mV/0.04 ml of 0.5*N* potassium dichromate). The equivalence points are also unaffected. Evidently, when the potassium dichromate is added from the burette it reacts speedily with the manganese^{II} before it can have time enough to react with traces of impurities present in the phosphoric acid. AnalaR phosphoric acid (British Drug Houses) has also been found to give correct equivalence points, but the potential breaks are about 10 mV less with untreated acid than that treated with nitric acid according to the procedure previously described. The phosphoric acid must be completely free from nitric acid, because the latter itself is capable of slowly oxidising manganese^{II}.

Procedure

About 2–10 ml of manganese^{II} solution, containing 20–150 mg of manganese^{II}, are taken in a 150-ml Pyrex beaker and treated with the requisite volume (40–50 ml) of 90% phosphoric acid. The mixture is connected to the saturated calomel electrode through a saturated sodium perchlorate bridge and a saturated sodium nitrate bridge. A bright platinum-rod electrode is used as indicator

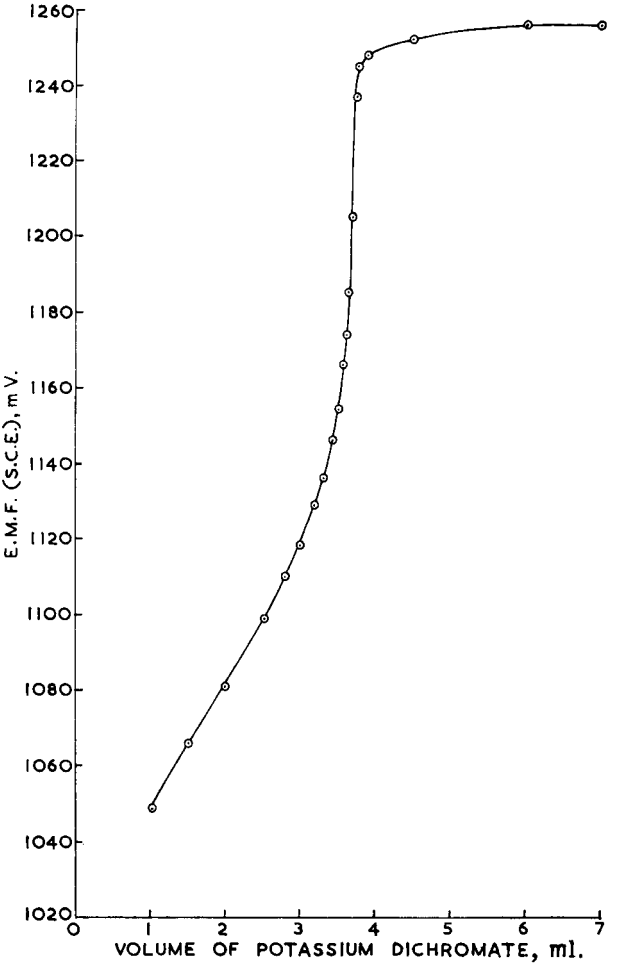

FIG. 2—Potentiometric titration of manganese^{II} (40.76 mg) with potassium dichromate in 12.0*M* phosphoric acid.

electrode. The mixture is stirred electromagnetically during the titration and the e.m.f of the cell is measured, with the help of a Cambridge potentiometer and a suspension galvanometer, 1 min after each addition of the dichromate solution. A typical potentiometric titration curve is given in Fig. 2. As the potential break at the equivalence point is not very high, the equivalence point of the titration is read from the curve obtained by plotting $\Delta E/\Delta V$ against V. A representative curve of this type is shown in Fig. 3. A large number of determinations of manganese have been carried out in this manner and some typical results are given in Table IV.

Interferences

Chloride interferes with the establishment of steady potentials and there is no break at the equivalence point. Nitrate interferes because it slowly oxidises manganese^{II} to manganese^{III} in the strong phosphoric acid medium employed here. Experiments at three different concentrations of nitric acid (0.25*N*, 0.5*N* and 1.0*N*) yielded values lower than the theoretical by 1.5, 1.5 and 1.8%, respectively. When the concentration of nitric acid was increased to 2.0*N*, the error increased to 9%.

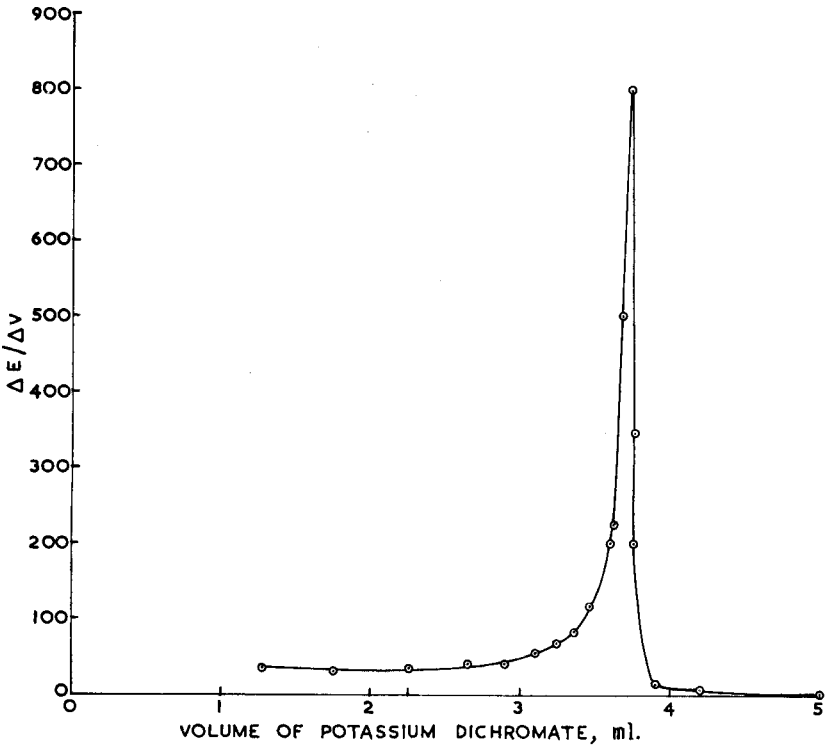

FIG. 3—Potentiometric titration of manganese^{II} (40.76 mg) with potassium dichromate in 12.0*M* phosphoric acid.

TABLE IV—POTENTIOMETRIC TITRATION OF MANGANESE^{II} WITH POTASSIUM DICHROMATE IN 12.0*M* PHOSPHORIC ACID

Manganese taken, *mg*	Manganese found, *mg*	Remarks
28.42	28.45	With 0.2*N* potassium dichromate
31.62	31.52	
40.30	40.30	
50.34	50.34	
62.53	62.75	
72.38	72.30	
108.1	108.3	With 0.5*N* potassium dichromate
116.3	116.5	
131.7	131.6	
145.0	144.7	

This shows that as the concentration of nitric acid is increased above 1.0*N*, its oxidising action rises asymptotically. Iron^{II}, uranium^{IV}, vanadium^{IV}, molybdenum^{V} and cerium^{III} are also oxidised by potassium dichromate under the conditions proposed for the titration of manganese^{II}. Iron^{II} does not interfere, however, because two widely separated breaks in the potential *vs.* volume curve are obtained, one corresponding to the oxidation of iron^{II} and the other corresponding to the oxidation of manganese^{II}. If the determination of iron^{II} is not required, a mixture of iron^{II} and manganese^{II} may be titrated with potassium dichromate in air, the volume of titrant lying between the first and second potential breaks accurately corresponding to the manganese^{II} present in the mixture. Cobalt^{II}, nickel^{II}, iron^{III}, tungsten,^{VI} molybdenum^{VI} and uranium^{VI} do not interfere as can be seen from Table V. Calcium, magnesium and aluminium also do not interfere. Sulphuric and perchloric acids do not interfere up to an over-all concentration of 1.0*N*.

TABLE V

Adenda	Amount added, *mg*	Manganese found, *mg*
Nil	—	48.59
Cobalt^{II}	64.45	48.69
Cobalt^{II}	32.22	48.59
Nickel^{II}	65.73	48.59
Nickel^{II}	32.86	48.59
Iron^{III}	55.85	48.59
Iron^{III}	27.92	48.69
Molybdenum^{VI}	95.95	48.48
Molybdenum^{VI}	47.97	48.59
Tungsten^{VI}	86.80	48.59
Uranium^{VI}	59.50	48.59

Determination of Iron^{II} and Manganese^{II} in Mixtures

As already stated, when a mixture of iron^{II} and manganese^{II} is titrated potentiometrically with potassium dichromate in 12.0*M* phosphoric acid two breaks are obtained, the first corresponding to the oxidation of iron^{II} and the second to the oxidation of manganese^{II}. At the equivalence point for iron^{II} one has to wait for 5 min for the attainment of a stable potential; the potential break at the equivalence point is about 600 mV/0.04 ml of 0.2*N* potassium dichromate solution. Great precautions must be taken to expel even traces of oxygen from all solutions and to prevent oxygen leaking into the titration vessel during the titration (the use of previously boiled out solutions and a carbon dioxide atmosphere are essential). Iron^{II} is much more susceptible to aerial oxidation in 12.0*M* phosphoric acid, because under these conditions the potential of the iron^{III}/iron^{II} couple is considerably lowered (from about 0.7 V in 1*M* sulphuric acid to about 0.380 V in 12.0*M* phosphoric acid).[27] In view of this difficulty, it is best to check the result for iron^{II} on a separate aliquot of the mixture by titration with potassium dichromate in 1.0*N* sulphuric acid either potentiometrically or visually using diphenylamine sulphonic acid as indicator. The determination of manganese is not affected by the presence of oxygen, unlike that of iron. Some typical results for the determination of iron^{II} and manganese^{II} in the same aliquot of the solution are presented in Table VI and a typical potential *vs.* volume curve is shown in Fig. 4. Because the potential break for the manganese equivalence point is not high, a plot of $\Delta E/\Delta V$ *vs.* V is also drawn for the manganese part of the titration.

TABLE VI

Iron^{II}, *mg*		Manganese, *mg*	
Taken	Found	Taken	Found
45.45	45.35	46.20	46.13
53.10	52.84	22.62	22.62
61.63	61.33	32.87	32.95
79.82	79.60	12.31	12.31
85.96	85.65	27.12	27.20

Photometric Titration of Manganese^{II} with Potassium Dichromate in a Strong Phosphoric Acid Medium

Optical absorption of manganese^{III} in a strong phosphoric acid medium

Purdy and Hume[26] determined the absorption spectrum of manganese^{III} in 8*M* sulphuric acid. From the data presented in this section, it will be noticed that the general shape of the absorption curve for manganese^{III} is the same in sulphuric and phosphoric acids, showing a maximum at the same wavelength. We have now determined the absorption spectra of manganese^{III} in 9.0*M*, 10.5*M* and 12.0*M* phosphoric acid. For the purpose of this investigation, manganese^{III} is prepared by the method proposed by Ingamells,[18] namely, heating a known amount of manganese^{II} with nitric acid (1:1) in a known volume of strong phosphoric acid of known strength on a hot-plate and driving off the excess of nitric acid by continued evaporation. The liquid is then cooled and made up to a known volume. The concentration of manganese^{III} in this solution is determined by titration with

a standard iron^{II} solution. Thus the concentration of phosphoric acid and manganese^{III} in this stock solution are known. Such a solution was found to be quite stable. From this solution a 0.01*M* solution of manganese^{III} was made up with varying concentrations of phosphoric acid. The optical densities of such solutions are measured at different wavelengths, using a 1.0-cm cell in a Hilger Uvispek spectrophotometer. The absorption curves plotted from this data are shown in Fig. 5. It

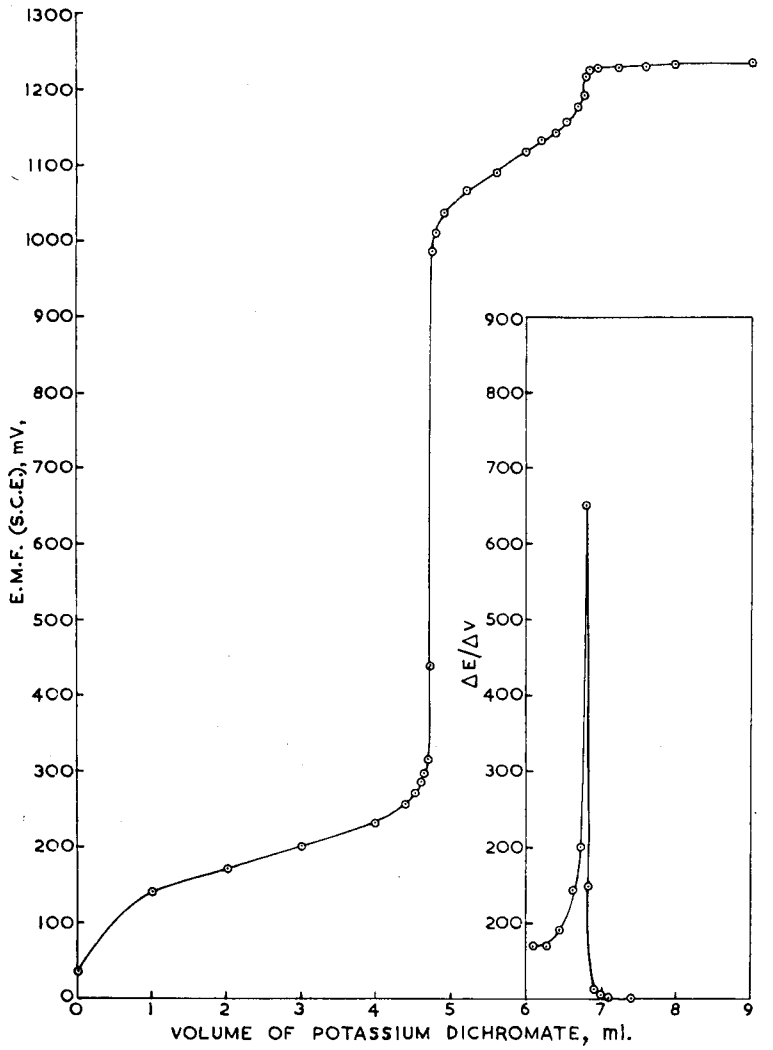

FIG. 4—Potentiometric titration of a mixture of iron^{II} (53.10 mg) and manganese^{II} (22.63 mg) in 12.0*M* phosphoric acid.

will be noted that the curves nearly overlap, showing that the absorption of manganese^{III} is about the same (at all of the wavelengths studied) in the concentration range of 9.0*M* to 12.0*M* phosphoric acid. Similar absorption curves have been obtained with manganese^{III} prepared by oxidation with potassium dichromate in a strong phosphoric acid medium using a solution of chromium^{III} of an equivalent concentration as blank. For the same concentration of phosphoric acid, the absorption curves obtained for manganese^{III} prepared by the two different methods have been found to very nearly overlap, provided the manganese^{III} concentration is the same. This overlapping of the two sets of absorption curves provides proof of the identity of the manganese species, as shown by the curves in Fig. 6. Moreover, the molecular extinction coefficients of the manganese^{III} prepared by the two different methods agree quite closely, having values of 85.0 and 84.5, respectively, at the wavelength (510 mμ) of maximum absorption. It is interesting to note that the molecular extinction

coefficient of the manganese^{III} species in 8M sulphuric acid is 142.0 as computed from the absorption curve obtained by Purdy and Hume[26] at a wavelength of 510 mμ.

The absorption curves of chromium^{III} have also been plotted in varying phosphoric acid concentrations, preparing the chromium^{III} by two different methods. In one method chromium^{III} was prepared by electrolytic reduction of chromic acid in a phosphoric acid medium as described previously. In the reduced solution chromium^{III} and any unreduced chromium^{VI} were determined as previously described. To an aliquot volume of this solution, the necessary volume of phosphoric acid is added.

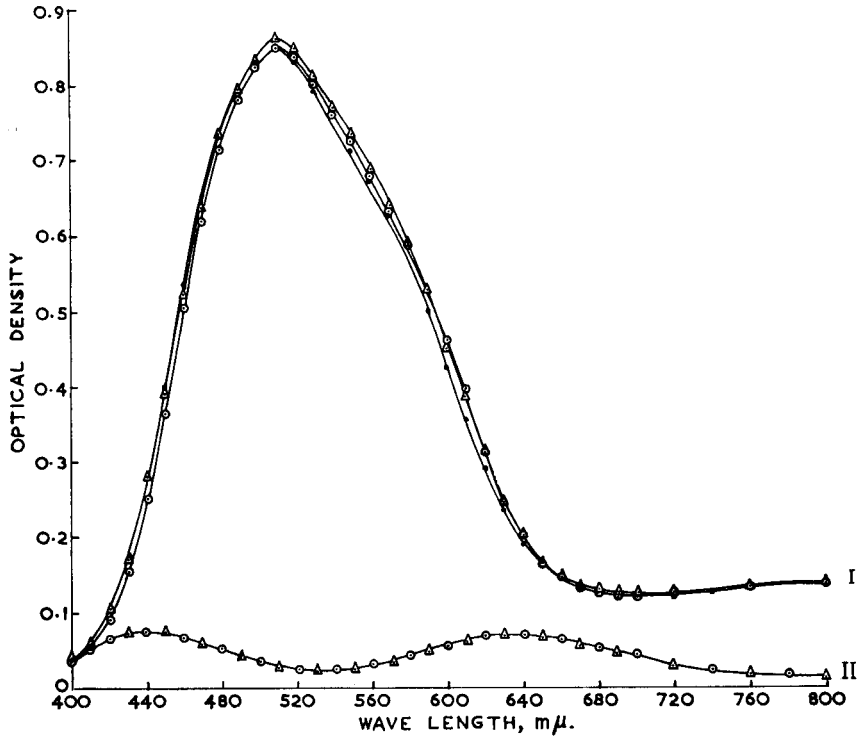

FIG. 5—Effect of phosphoric acid concentration on the absorption spectra of manganese^{III} and chromium^{III}:—

I—Manganese^{III} concentration: 0.01M;
⊙—⊙ 12.0M H_3PO_4,
△—△ 10.5M H_3PO_4,
●—● 9.0M H_3PO_4.
II—Chromium^{III} concentration: 0.003M;
⊙—⊙ 12.0M H_3PO_4,
△—△ 9.0M H_3PO_4.

In this solution the chromium^{III} exists as a phosphate complex and the optical density of the solution was measured at different wavelengths in a Hilger Uvispek spectrophotometer, using a 1.0-cm cell against a blank of phosphoric acid plus chromic acid of strength similar to that found in the electrolytically reduced chromium solution. In the second method the chromium^{III} was derived from a solution of chrome alum (Analar grade, British Drug Houses) of known strength. In this case it was found necessary to keep the solution for about 2 hr after mixing with the phosphoric acid because it was observed that the optical density changed on standing, reaching a constant value after 2 hr. Evidently the speed of formation of the chromium^{III}-phosphate complex is slow. The absorption curves of the chromium^{III}-phosphoric acid mixtures prepared by the two different methods were observed to overlap. The absorption spectrum of chromium^{III} in a medium of varying phosphoric acid concentration is shown in Fig. 5.

An examination of the absorption curves of manganese^{III} and chromium^{III} in phosphoric acid show that manganese^{III} absorbs strongly at 510 mμ where chromium^{III} has a very low absorption. In this region chromium^{VI} also has a low absorption. Hence, light of this wavelength has been

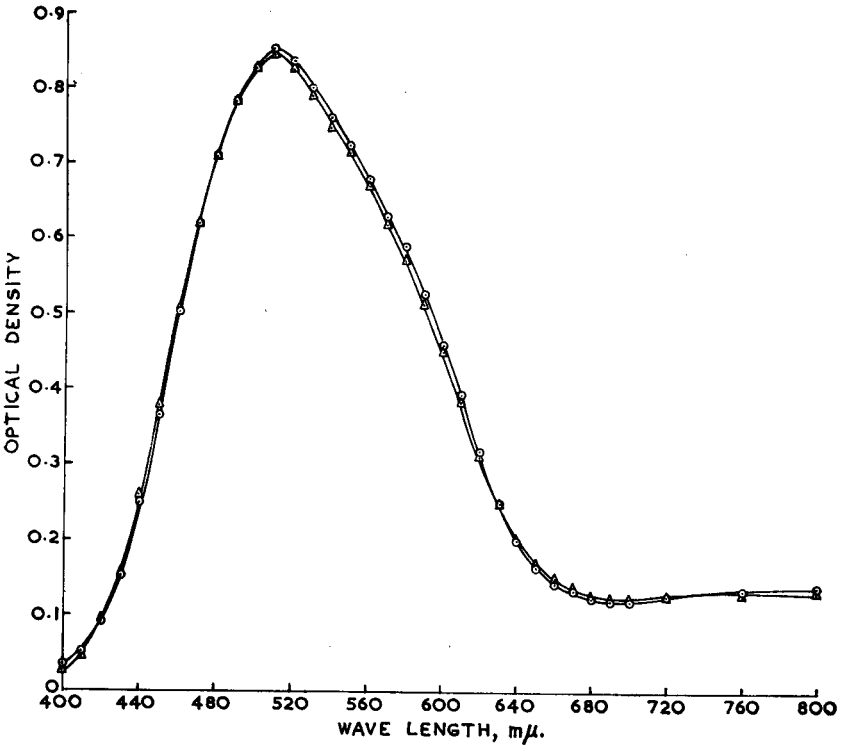

FIG. 6—Absorption spectra of manganese^{III} in 12.0M phosphoric acid (manganese^{III} concentration: 0.01M):
⊙—⊙ manganese^{III} by oxidation with nitric acid,
△—△ manganese^{III} by oxidation with chromium^{VI}.

chosen for carrying out the photometric titration of manganese^{II} with potassium dichromate in a strong phosphoric acid medium.

Speed of reaction between manganese^{II} and potassium dichromate at varying phosphoric acid concentrations

In order to ascertain the concentration of phosphoric acid at which a speedy oxidation of manganese^{II} occurs, we have carried out a photometric study of a mixture of manganese^{II} with a very slight excess of potassium dichromate at varying concentrations of phosphoric acid; the optical density of the mixture is noted at a wavelength of 510 mμ, at varying times, in a Klett-Summerson photoelectric colorimeter using optically matched tubes. The results are presented in Table VII.

TABLE VII

Time, *min*	Photometer dial reading				
	Phosphoric acid concentration, M				
	8.0	9.0	10.5	12.0	13.5
0.5		250	286	290	290
1	193	264	290	290	290
2	214	273	290	290	290
2.5	220	276	290	290	290
3	226	278	290	290	290
5	242	283	290	290	290
10	261	290	290	290	290
15	266	290	290	290	290
30	278	290	290	290	290

The data in Table VII show that the reaction between chromium^{VI} and manganese^{II} is complete within 30 sec in a medium containing phosphoric acid at a concentration of 12.0*M* and higher. Even at a concentration of 10.5*M* phosphoric acid, the reaction is complete within 1 min.

Procedure for photometric titration of manganese^{II} with potassium dichromate at room temperature

From 2.0 to 10.0 ml of manganese^{II} solution, containing 5–17.0 mg of the metal, are taken in the optical cell (2 × 4 × 8 cm) and treated with 30–33 ml of syrupy phosphoric acid of "Pro Analysi" grade (E. Merck). Enough water is then added to make up the volume to 40 ml. The cell is placed in position within the casing of a Klett-Summerson photoelectric colorimeter. An inlet tube of carbon dioxide passing through a hole in the lid of the instrument is so held that it dips into the titration mixture in a corner of the optical cell and does not obstruct the passage of light. Carbon dioxide is passed through the mixture to ensure thorough mixing of the solution. Just before the optical reading is taken, the passage of gas is stopped so that the bubbles of gas do not vitiate the passage of light. Through another hole in the lid passes the tip of a microburette. With the cell placed in

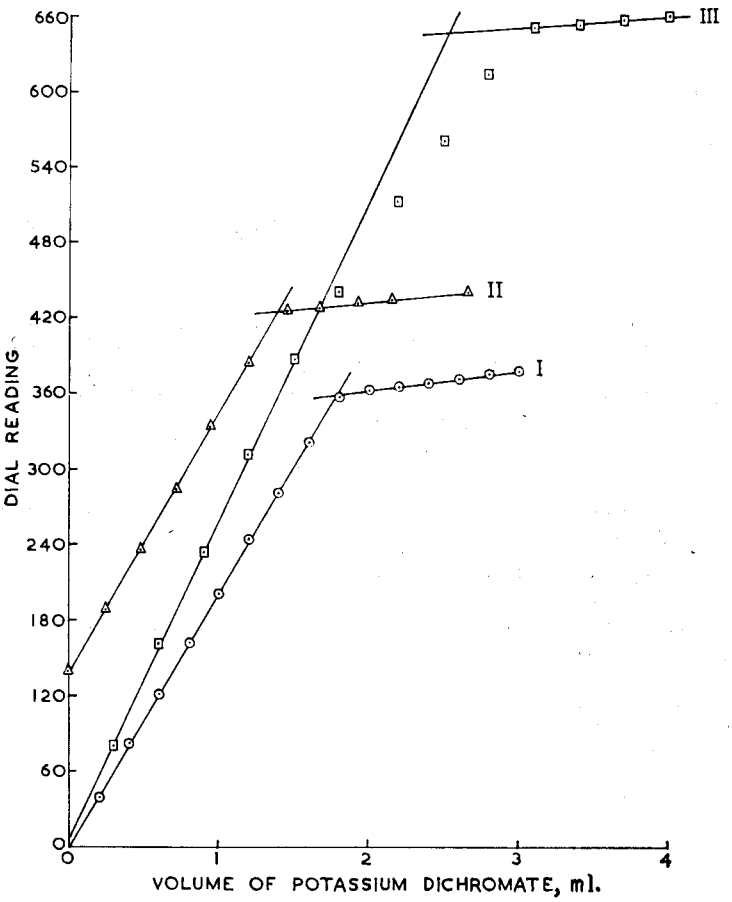

FIG. 7—Photometric titration of manganese^{II} with potassium dichromate in 10.5*M* phosphoric acid:
I—9.834 mg of manganese,
II—7.692 mg of manganese + 62 mg of cobalt,
III—14.01 mg of manganese + 6 ml of 6*N* hydrochloric acid.

position in the photoelectric colorimeter, the dial reading is adjusted to zero with a green filter (485–550 mμ) placed in the filter holder. The titration is then started, adding the 0.1*N* potassium dichromate in 0.1-ml or 0.2-ml portions according to whether the total titre is low or high. The dial readings are plotted against the volume after applying the dilution correction. The dial readings

are taken after passing carbon dioxide for 2 min following the addition of each portion of the potassium dichromate. Curve I in Fig. 7 represents a typical photometric titration curve. It will be seen from this curve that the equivalence point of the titration can be easily read. A large number of photometric titrations have been made in this manner and some typical results are given in Table VIII.

TABLE VIII—PHOTOMETRIC TITRATION OF MANGANESE^{II} WITH POTASSIUM DICHROMATE IN 10.5*M* PHOSPHORIC ACID

Manganese taken, *mg*	Manganese found, *mg*
4.998	5.054
6.735	6.782
7.650	7.643
8.678	8.624
8.802	8.843
9.716	9.730
10.09	10.15
13.31	13.34
17.14	17.12

Interferences

Iron^{III}, molybdenum^{VI}, tungsten^{VI} and uranium^{VI} do not interfere. Chromium^{III} interferes slightly if the titration is started immediately after adding phosphoric acid, because an ordinary chromium^{III} salt has some absorption in the region where manganese^{III} also absorbs; moreover, the absorption of chromium^{III} does not remain constant but changes with time because of increasing complexation with phosphoric acid. If the mixture of salts and phosphoric acid is kept for 2 hr, equilibration of phosphoric acid with chromium^{III} is almost complete and the titration with potassium dichromate can then be started without any interference from chromium^{III}. Although cobalt^{II} has some absorption in the same region as manganese^{III}, it does not interfere because its absorption remains constant throughout the determination. In the presence of cobalt, the initial dial reading of the instrument will be something positive instead of being zero, but this does not in any way interfere with the titration or with the reading of the equivalence point. Curve II in Fig. 7 is obtained with a mixture containing 62 mg of cobalt and 7.692 mg of manganese. It is evident that even a very large excess of cobalt does not interfere. Nickel does not interfere even up to 65.73 mg. Chloride (in the form of potassium chloride) does not interfere even up to 1600 mg/40 ml of the titration solution; this is an advantage of the photometric method compared with the potentiometric method. However, when 6.0 ml of 6.0*N* hydrochloric acid is added there is a bend in the photometric titration curve in the region of the equivalence point, although the equivalence point is not affected if obtained from the intersection point. This is evident from curve III in Fig. 7. Sulphate does not interfere. When the titration is performed in 0.5*N* (over-all) nitric acid, the results are lower by 1.9%.

Acknowledgement—One of us (P. K. R.) desires to thank the Council of Scientific and Industrial Research (India) for the award of a Junior Research Fellowship.

Zusammenfassung—Eine neue Methode zur direkten titrimetrischen Bestimmung von Mangan (II) wurde entwickelt. Sie beruht auf der Oxydation zu Mangan (III) mit Kaliumdichromat bei Zimmertemperatur in starker Phosphorsäure mit potentiometrischer oder photometrischer Endpunktsanzeige. Luftsauerstoff stört nicht. Die potentiometrische Methode liefert eine Genauigkeit von ±0,3% für 20–150 mg Mn in 50 ml Lösung; photometrisch können 5–17 mg Mn in 40 ml Lösung je nach der anwesenden Menge auf 0,3–1,0% genau bestimmt werden. Kaliumdichromat in 12,0 m Phosphorsäure hat ein formales Redoxpotential von etwa 1,5 V; das Reagens verspricht große Möglichkeiten in der titrimetrischen Analyse.

Résumé—Les auteurs ont développé une nouvelle méthode de dosage direct par volumétrie du manganèse II; ce dosage est basé sur son oxydation en manganèse III au moyen de bichromate de potassium, à température ordinaire et en milieu acide phosphorique fort en utilisant

un point équivalent potentiométrique ou photom/trique. L'oxygène de l'air n'est pas gênant. La méthode potentiométrique donne des résultats d'une précision de $\pm 0{,}3\%$ pour 20 à 150 mg de manganèse dans 50 ml de solution avec la méthode photométrique on peut doser de 5 à 17 mg de manganèse dans 40 ml de solution, avec une erreur de 0,3 à 1% dépendante de la quantité totale présente. Le bichromate de potassium dans une solution d'acide phosphorique à 12,0 M a un potentiel redox d'environ 1,5 V et ce réactif semble avoir des possibilités intéressantes dans le domaine de la titrimétrie.

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SPECTROPHOTOMETRIC DETERMINATION OF PALLADIUM WITH 1-THIOGLYCEROL AND A STUDY OF PALLADIUM COMPLEX FORMATION WITH FOUR SIMILAR THIO-ORGANIC COMPOUNDS

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(*Received* 31 *May* 1963. *Accepted* 22 *July* 1963)

Summary—A study of the reactivity of five thio-organic compounds with palladium^{II} ions has been made and one of them, 1-thioglycerol, selected for use as a spectrophotometric reagent for palladium. Its sensitivity is 0·01 μg of palladium/cm^2 for log $I_0/I = 0{\cdot}001$. The sensitivity of the other four compounds is about the same. Beer's law is obeyed over the palladium concentration range of 0·5 to 9 ppm. The effects of pH, order of addition of reagents, temperature and diverse ions have been investigated.

BEAMISH and McBRYDE[1,2] have published two reviews in which they summarise most of the reagents and procedures now available for the spectrophotometric determination of trace amounts of palladium. They discuss some sixteen methods, with special emphasis on optimum concentration range, effects of temperature and pH, and interferences by diverse ions. As is often the case in colorimetry and spectrophotometry, however, there is no one reagent which is best suited for the determination of palladium in all types of material and hence a variety of reagents for this metal is desirable.

In 1948, König and Crowell[3] reported that a yellow colour is formed immediately when a drop of thiomalic acid solution is added to a drop of palladium^{II} solution on a spot plate. More recently, Wagner and Yoe[4] have thoroughly investigated this colour reaction and have proposed thiomalic acid as a reagent for the spectrophotometric determination of trace amounts of palladium. As a result of their study we decided to investigate a group of thio-organic compounds somewhat similar in structure to thiomalic acid in order to determine their reactivity with palladium^{II} ions. This paper presents the results of a study of five such compounds, one of which, 1-thioglycerol, has been investigated more thoroughly than the others and used for the spectrophotometric determination of palladium. The five thio-organic compounds selected for this study are listed in Table I together with their formulae.

TABLE I

Compound	Formula
1-Thioglycerol	HS—$CH_2CH(OH)CH_2OH$
3-Mercaptopropionic acid	HS—CH_2CH_2COOH
2-Mercaptoethylamine hydrochloride	HS—$CH_2CH_2NH_2$·HCl
2-Dimethylaminoethanethiol hydrochloride	HS—$CH_2CH_2N(CH_3)_2$·HCl
2-Diethylaminoethanethiol hydrochloride	HS—$CH_2CH_2N(C_2H_5)_2$·HCl

* Present address: National Bureau of Standards, Washington, D.C., U.S.A.

EXPERIMENTAL

Apparatus

Spectrophotometers: Absorbance curves were obtained with a Beckman ratio recording spectrophotometer, Model DK-2. For measurements at a single wavelength a Beckman spectrophotometer Model DU, was used. Measurements were made in matched 1·00-cm quartz cells.

pH Meter: All pH measurements were made with a Beckman pH meter, Model G. It was checked from time to time with certified buffer solutions.

Reagents

Standard palladium solution: A standard stock solution, 1*M* in hydrochloric acid and containing 1 mg of palladium/ml, was prepared by dissolving 1·66 g of palladium^{II} chloride (American Platinum Works) in 85 ml of 12*M* hydrochloric acid and diluting to 1 litre with distilled water. The solution was standardised gravimetrically by the dimethylglyoxime method. Solutions of lower concentrations were prepared by diluting aliquots of the standard stock solution.

Organic reagent solutions: 0·1*M* Solutions of the five thio-organic compounds listed in Table I (Evans Chemetics, New York, N.Y., U.S.A.) were prepared in distilled water.

Buffer solution: A buffer solution of pH 2·5 was prepared by mixing 100 ml of 1*M* sodium acetate and 101 ml of 1*M* hydrochloric acid and diluting to 500 ml with distilled water.

Other solutions. All other solutions were prepared from analytical reagent grade compounds and were used without further purification.

RESULTS

When 1 drop of any of the five reagent solutions is added to 1 drop of palladium^{II} solution on a spot plate, an intense yellow colour is formed immediately. Absorption curves obtained for the five coloured complexes are characterised by their striking similarity (see Figs. 1 and 3-6). All five have approximately the same general shape and about the same sensitivity for palladium. The absorption maxima occur at about 270 mμ. At this wavelength the reagent solutions have only small absorbances, whereas the palladium^{II} chloride complex has a higher value. Because of the close similarity of the absorbance curves of the yellow complexes, it did not seem necessary to investigate each of the five reagents in detail. 1-Thioglycerol was selected for further study because it has neither an acidic nor a basic group and hence would probably be less influenced by changes in pH than any of the other four compounds. It was used in all of the following studies. Absorbance curves for the compound and its palladium complex are shown in Fig. 1.

Properties of the coloured complex

Effect of pH. The effect of the hydrogen-ion concentration on the colour reaction is shown in Fig. 2. Over the pH range of 2 to 7, the absorbance is practically constant and no buffer is needed. If desirable, however, a sodium acetate-hydrochloric acid buffer of pH 2·5 may be used without difficulty.

Order of addition of reagents. When no buffer is used, the reactants may be added in any order. If a buffer is used, however, it should always be added before the colour is developed in order to obtain maximum sensitivity. With the sodium acetate-hydrochloric acid buffer, an approximately 5% decrease in the absorbance was observed when the buffer was added after colour development.

Adherence to Beer's law. Beer's law is obeyed over the palladium concentration range of 0·5 to 9 ppm.

Mole ratio studies. Three methods were employed in an attempt to establish a mole ratio for the palladium complex in solution. These were the method of Yoe and Jones,[5] the slope ratio method proposed by Harvey and Manning,[6] and the continuous variations method of Job[7] as modified by Vosburgh and Cooper.[8] None of these

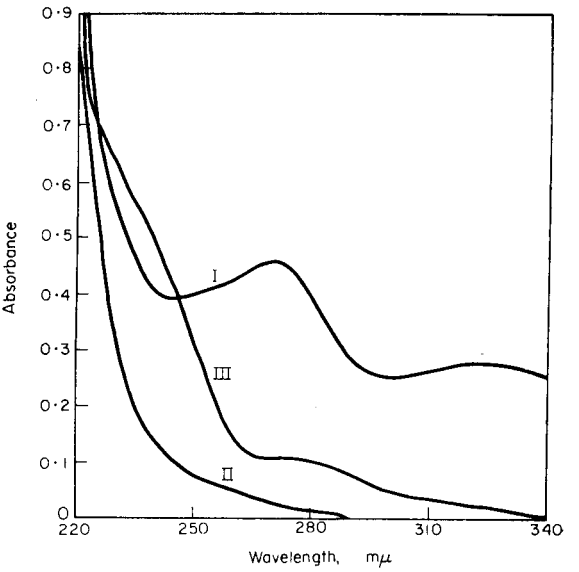

FIG. 1.—(I) 1-Thioglycerol-palladium complex (4·00 ppm of palladium),
(II) 1-Thioglycerol (0·002*M*),
(III) Palladium alone (4·00 ppm).

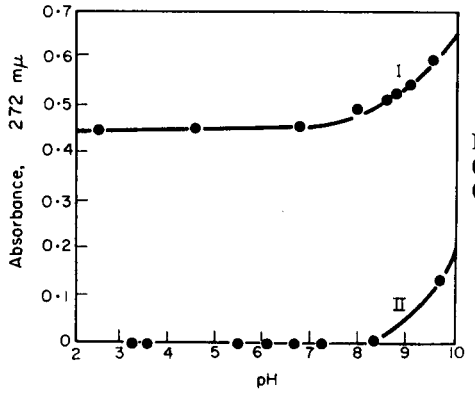

FIG. 2.—Effect of pH on the absorbance of:
(I) 1-Thioglycerol—palladium complex,
(II) 1-Thioglycerol.

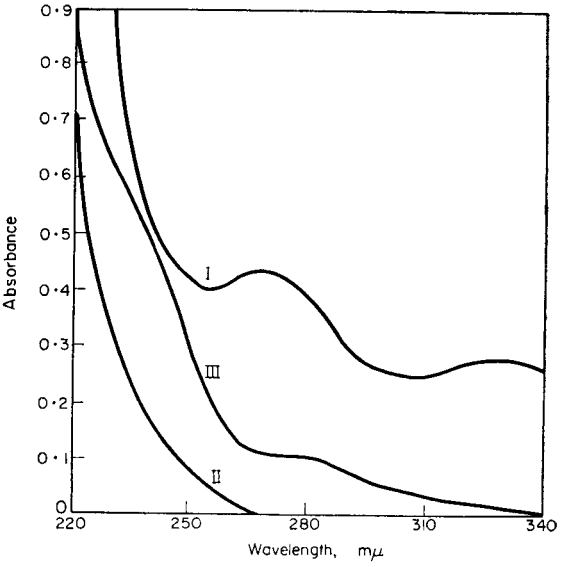

FIG. 3.—(I) 3-Mercaptopropionic acid—palladium complex (4·00 ppm of palladium),
(II) 3-Mercaptopropionic acid (0·002*M*),
(III) Palladium alone (4·00 ppm).

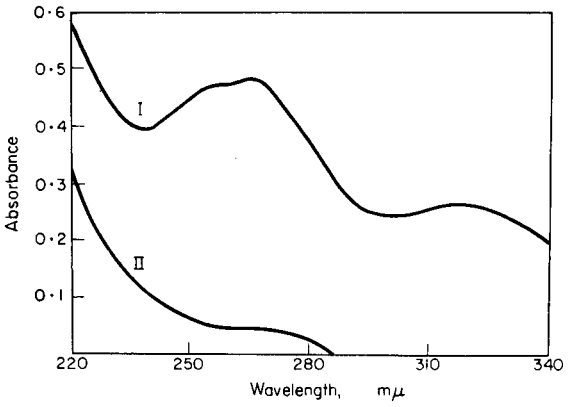

FIG. 4.—(I) 2-Mercaptoethylamine—palladium complex (4·00 ppm of palladium),
(II) 2-Mercaptoethylamine (0·002*M*).

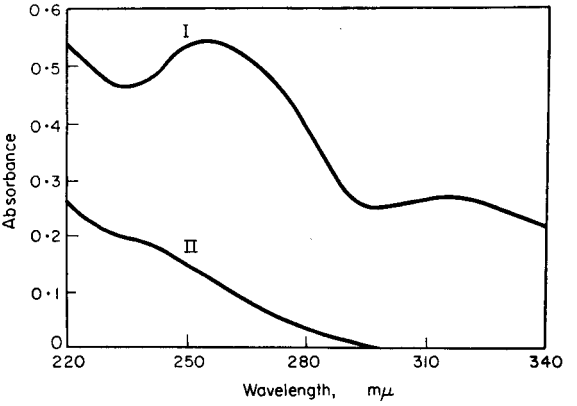

FIG. 5.—(I) 2-Dimethylaminoethanethiol—palladium complex (4·00 ppm of palladium),
(II) 2-Dimethylaminoethanethiol (0·002*M*).

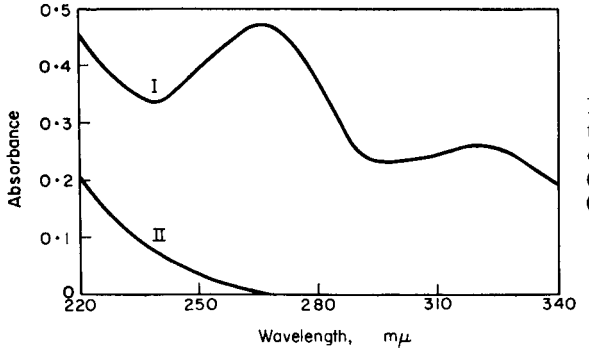

FIG. 6.—(I) 2-Diethylaminoethanethiol—palladium complex (4·00 ppm of palladium),
(II) 2-Diethylaminoethanethiol (0·002*M*).

methods gave conclusive results, indicating that the stability of the coloured complex is not high. Subsequent study, however, showed that for maximum colour development the 1-thioglycerol should be present in at least a one hundred-fold excess.

Organic solvents. Various organic solvents were used in an attempt to extract the organo-palladium complex from its aqueous solution. No visible extraction was observed with n-butanol, benzene, carbon tetrachloride, chloroform or ethyl acetate, an indication that the complex is ionic.

Effect of temperature. The colour was developed in a constant temperature bath at 15° and 35°, respectively. The absorbance values at the two temperatures were within 0·7% of that obtained at room temperature (22°).

Sensitivity. For $\log I_0/I = 0{\cdot}001$, the sensitivity is 0·01 μg of palladium/cm^2. The spot-plate sensitivity is 0·5 μg of palladium/0·05 ml of test solution and the limit of dilution is 1:100,000.

Effect of diverse ions. Two limiting values for the concentration of the diverse ions were arbitrarily chosen: (1) that concentration which caused an error of $\pm 2\%$ at a 4-ppm palladium level, and (2) that concentration which gave an error of $\pm 5\%$. The results of this study are given in Table II. The interference caused by most of the ions probably arises from their own colour, rather than to one produced by their reaction with 1-thioglycerol.

TABLE II.—TOLERANCE TO DIVERSE IONS (4·00 ppm OF PALLADIUM)

Ion	Added as	Limiting concentration, *ppm*	
		±2%	±5%
Ru^{3+}	$RuCl_3$	0·3	0·8
Rh^{3+}	$RhCl_3$	0·3	0·8
Ir^{3+}	$IrCl_3$	2·5	6·5
OsO_4^{2-}	K_2OsO_4	0·2	0·5
$PtCl_6^{2-}$	H_2PtCl_6	0·3	0·8
Au^{3+}	$AuCl_3$	1·1	2·7
Fe^{3+}	$FeCl_3$	0·1	0·3
Cu^{2+}	$CuCl_2$	0·1	0·3
VO^{2+}	$VOCl_2$	2·1	5·2
Co^{2+}	$CoCl_2$	70	>100
Ni^{2+}	$NiCl_2$	90	>100
Cr^{3+}	$CrCl_3$	10	25
Zn^{2+}	$ZnCl_2$	>100	>100
Mn^{2+}	$MnCl_2$	>100	>100
NO_3^-	$NaNO_3$	>200	>200
SO_4^{2-}	Na_2SO_4	>8000	>8000

Precision. The precision of the method was determined for one set of solutions containing 4·00 ppm of palladium and for a second set, containing in addition to 4·00 ppm of palladium, 0·2 ppm each of ruthenium, rhodium and platinum, 0·1 ppm of osmium and 1 ppm of iridium. Each set consisted of eleven samples. The standard deviation for the former was 0·011 ppm of palladium and for the latter, 0·023 ppm of palladium.

DISCUSSION

The use of any of the five compounds herein described as spectrophotometric reagents for palladium appears limited by the fact that the absorption maximum of

each coloured complex is in the ultraviolet. In this region interference of foreign ions may be caused either by their own colour (*e.g.*, Au^{3+}, Fe^{3+}, *etc.*) or by their reaction with the reagent. On the other hand, the 1-thioglycerol-palladium complex is only slightly affected by changes in pH over the range of 2 to 7. 1-Thioglycerol has a fairly good spectrophotometric sensitivity for palladium. Moreover, it can tolerate small to moderate amounts of the other platinum group metals and also gold, iron, copper, *etc.*

Zusammenfassung—Die Reaktion von 5 organischen Schwefelverbindungen mit Pd^{2+} wurde untersucht und eine davon, nämlich 1-Thioglycerin, als spektrophotometrisches Reagens für Pd ausgewählt. Die Empfindlichkeit beträgt 0,01 μg Pd in cm² für log $I_0/I = 0{,}001$, die Empfindlichkeit der anderen 4 Verbindungen ist etwa gleich. Das Beersche Gesetz gilt für Pd-Konzentrationen von 0,5 bis 9 ppm. Der Einfluß des pH, der Reihenfolge der Zugabe der Reagentien, der Temperatur und verschiedener Fremdionen wurde untersucht.

Résumé—On a étudié la réactivité de cinq composés thio-organiques avec les ions palladium (II); l'un d'entre eux, le thio-1-glycérol a pu être sélectionné comme réactif spectrophotométrique pour le palladium. La sensibilité est de 0,01 μg de Pd par cm² pour log $I_0/I = 0{,}001$. Les sensibilités des quatres autres composés sont sensiblement identiques. La loi de Beer est satisfaite pour des concentrations de palladium comprises entre 0,5 et 9 ppm. Les effets du pH, de l'ordre dans lequel sont introduits les réactifs, de la température ainsi que l'influence de divers ions ont été étudiés.

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Talanta, 1963, Vol. 10, pp. 1273 to 1276. Pergamon Press Ltd. Printed in Northern Ireland

UNTERSUCHUNG DES WIRKUNGSMECHANISMUS VOM REDOX-INDIKATOR 2-HYDROXY-4-AMINO-4'-METHOXYDIPHENYLAMIN UND SEINEANWENDUNGEN

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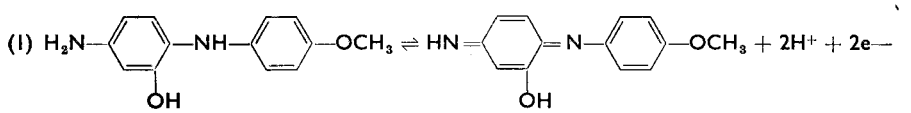
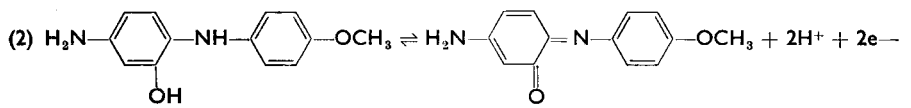
(*Eingegangen am* 11 *Juni* 1963. *Angenommen am* 30 *Juli* 1963)

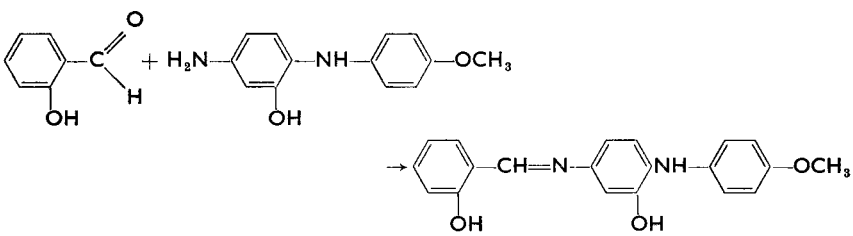
Zusammenfassung—Bei der Oxydation des 2-Hydroxy-4-amino-4'-methoxydiphenylamins (2-Oxyvariaminblaus) besteht Möglichkeit zur Ausbildung von zweierlei chinoidalen Strukturen. Es wurde festgestellt, dass tatsächlich das parachinoidale Holochinon entsteht. Im weiteren wurde die ascorbinometrische Bestimmung von einigen oxydierenden Stoffen (J_2, Br_2, JO_3^-, BrO_3^-, CrO_4^-) unter Anwendung des 2-Oxyvariaminblau-Indikators mit Hilfe eines Hexacyanoferrat(II)-Hexacyanoferrat(III) Vermittlungsystems besprochen.

VARIAMINBLAU und seine bisher untersuchten Derivate sind nur zur Endpunktsindikation von in saurer Lösung verlaufenden Redoxvorgängen geeignet.[1] Das 2-Oxyvariaminblau ist aber auch in alkalischer Lösung als Indikator brauchbar.[2,3] In einer vorhergehenden Veröffentlichung[2] haben wir erwähnt, dass sich bei der Oxydation des Indikators zur Ausbildung von zweierlei chinoidalen Strukturen Möglichkeit bietet:





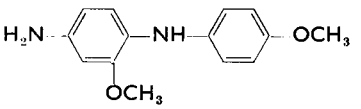
Um es nun zu entscheiden welche Reaktion tatsächlich verläuft, wurde mit Salicylaldehyd ein Kondensationsprodukt des 2-Oxyvariaminblaus erzeugt:



Bei der Oxydation dieses Derivats besteht nur zur Bildung einer orthochinoidalen Struktur Möglichkeit. In Lösung von pH 4,1 wird das obige Derivat auf Einwirkung von Jodlösung sofort ebenso blauviolett wie das ursprüngliche 2-Oxyvariaminblau. Wenn man in Lösung von pH 8,0 mit Kaliumhexacyanoferrat(III)-lösung das Kondensationsprodukt oxydiert, so erfolgt kein sofortiger Farbwechsel. Die blauviolette

oxydierte Farbe des Indikators bildet sich nur stufenweise aus. Im ersten Fall entwickelt sich die Farbe deshalb sofort, weil in saurer Lösung das mit Salicylaldehyd erzeugte Kondensationsprodukt sogleich hydrolisiert. Der Indikator wirkt also, als wäre das Salicylaldehyd überhaupt nicht zugegen. Im zweiten Fall tritt die Farbentwicklung deshalb nur langsam auf, weil das Kaliumhexacyanoferrat(III) die Doppelbindung nur langsam oxydiert, wonach erst die Oxydationsfarbe erscheint. Die Farbe lässt sich schon nach einigen Minuten bemerken, ihre volle Entwicklung bedarf aber $\frac{1}{2}$ — 1 Stunde.

Anderseits wurde dann ein Produkt erzeugt, bei welchem die Ausbildung einer orthochinoidalen Struktur verhindert ist. Dies erfolgt z.B. durch Methylieren der Hydroxygruppe, wodurch das schon bekannte 4-Amino-2-4′-dimethoxydiphenylamin entsteht[4–6]



Bei diesem besteht nur zur Ausbildung eines Holochinons von parachinoidaler Struktur Möglichkeit. Die Farbe des oxydierten Produkts dieser Verbindung ist ebenfalls blauviolett, es kann bis etwa pH 7 als Redoxindikator verwendet werden.

Diese Versuche zeigten, dass bei der Oxydation des Indikators mit hoher Wahrscheinlichkeit ein Holochinon mit parachinoidaler Struktur entsteht, für die erhebliche Erweiterung des pH-Bereichs seiner Anwendung ist vermutlich die Säure-Base Dissoziation der Hydroxylgruppe verantwortlich.

In unserer früheren Mitteilung[2] haben wir auch festgestellt, dass der 2-Oxyvariaminblau-Redoxindikator ausgezeichnet zur Endpunktsindikation der Kaliumhexacyanoferrat (III)-Ascorbinsäure Reaktion in schwach alkalischer Lösung geeignet ist. Mit Hilfe des Kaliumhexacyanoferrat(II)-Kaliumhexacyanoferrat(III) Vermittlungssystems lassen sich zahlreiche oxydierende und reduzierende Stoffe unter Anwendung dieses Indikators bestimmen. Im folgenden werden einige Beispiele derartiger Bestimmungen vorgeführt.

EXPERIMENTELLER TEIL

Unter Anwendung des Indikators wurden folgende Ionen und Substanzen bestimmt: Jod, Brom, Jodat, Bromat und Bichromat.

Reagenzien und Lösungen

Die 0,1 *n Ascorbinsäuremasslösung* wurde nach Erdey und Bodor[6,7] bereitet: 8,9 Ascorbinsäure wurden in 1000 ml in einer Glaseinrichtung destilliertem Wasser gelöst. Nach vollständigem Auflösen wurde der Titer gegen 0,1 n Kaliumjodatlösung eingestellt.

Indikator: 1 Teil festes 2-Oxyvariaminblau wurde mit 500 Teilen festen Natriumchlorids vermischt, dem Gemisch eine Messerspitze (0,3–0,9 g) zu einer Titration von 100 ml Endvolumen entnommen.

Die 0,1 *n Kaliumhexacyanoferrat*(III)-Masslösung wurde aus bei 100° getrocknetem analysenreinem Kaliumhexacyanoferrat(III) durch Einwaage bereitet und der Titer jodometrisch eingestellt.

Die Lösungen der zu bestimmenden Substanzen wurden aus den entsprechenden analysenreinen Reagenzien bereitet.

Die Wirkungswerte der Jod—und Bromlösungen wurden jodometrisch kontrolliert. Die anderen Lösungen wurden durch genau Einwaage hergestellt.

Kaliumhexacyanoferrat(II) und Kaliumbikarbonat waren ebenfalls analysenrein.

Jodbestimmung

Die Bestimmung kann auf zweierlei Weisen ausgeführt werden: Erdey und Mitarbeiter[9] stellten fest, dass die Jodlösung durch Ascorbinsäure in schwach saurer Lösung reduziert wird. Zur

Endpunktsindikation wurde Variaminblau gebraucht. Die nötige Säurigkeit stellt man mit Natriumacetatlösung ein. Statt Variaminblau bewährt sich auch das 2-Oxyvariaminblau, das sich auch in schwach saurer Lösung als Indikator anwenden lässt. Die Bestimmungen wurden übrigens nach Erdey und Mitarbeiter[9] ausgeführt, die Ergebnisse sind in der Tabelle zu sehen. Die aus 12 parallelen Bestimmungen errechnete standard Deviation betrug ±0,06% (±0,15 mg); die standard Deviation des Mittelwerts ±0,03% (±0,06 mg).

TABELLE I.—BESTIMMUNGSERGEBNISSE VON JOD, BROM, JODAT, BROMAT, PYROCHROMAT IN GEGENWART VON 2-HYDROXYVARIAMINBLAU

Sollvert, *mg*	Gefunden Mittelwert, *mg*	Zahl der parallelen Bestimmungen	Abweichung *mg*	Abweichung %
Jod direkt				
253,82	253,96	6	+0,14	+0,06
126,91	126,91	6	±0,00	±0,00
62,18	62,31	6	+0,13	+0,2
Jod indirekt				
255,9	256,0	3	+0,1	+0,05
126,9	127,0	12	+0,1	+0,1
63,45	63,32	3	−0,13	−0,2
Brom				
177,17	176,16	3	−1,01	−0,57
120,43	119,66	12	−0,77	−0,64
59,06	58,68	3	−0,38	−0,62
Jodat (JO_3^-)				
58,30	58,28	3	−0,02	−0,04
29,15	29,13	12	−0,02	−0,07
14,57	14,56	3	−0,01	−0,03
Bromat (BrO_3^-)				
42,63	42,63	12	±0,0	±0,0
21,31	21,31	3	±0,0	±0,0
10,65	10,66	3	+0,01	+0,1
Pyrochromat ($Cr_2O_7^{2-}$)				
90,0	90,07	3	+0,07	+0,07
36,00	36,02	12	+0,02	+0,05
18,00	18,05	3	+0,05	+0,3

Die Genaugikeit der Bestimmung ist also dieselbe wie wenn Variaminblau als Indikator dient.

Die Bestimmung kann auch mit Hilfe eines Vermittlungs-Redoxsystems unternommen werden. In neutraler Lösung wird Hexacyanoferrat(II) durch Jod oxydiert,[10-12] das entstehende Hexacyanoferrat(III) lässt sich mit Ascorbinsäure unter Anwendung von 2-Oxyvariaminblau als Indikator in Kaliumbikarbonat enthaltender Lösung zurücktitrieren. Die Titration wurde nach Erdey und Svehla[12] ausgeführt (nur mit dem Unterschied das 2-Oxyvariaminblau als Indikator diente). Die Ergebnisse sind in der Tabelle sichtlich, die aus 12 parallelen Bestimmungen errechnete standard Deviation betrug ±0,11% (±0,14 mg), die standard Deviation des Mittelwerts ±0,04% (±0,06 mg).

Brombestimmung

Freie Halogene so auch Bromoxydieren in neutraler Lösung Hexacyanoferrat(II) quantitativ zu Hexacyanoferrat(III),[11-14] das in bikarbonathaltiger Lösung mit Ascorbinsäuremasslösung unter Anwendung von 2-Oxyvariaminblau als Indikator bestimmt wird. Die Bestimmungen wurden nach Erdey und Svehla[12] ausgeführt, die Ergebnisse sind in der Tabelle dargestellt. Die standard Deviation betrug aus 12 parallelen Titrationen ±0,37% (±0,45 mg), die standard Deviation des Mittelwerts ±0,11% (±0,13 mg).

Jodatbestimmung

In saurer Lösung wird Hexacyanoferrat(II) durch Jodat quantitativ oxydiert.[12,15]

$$JO_3^- + 6Fe(CN)_6^{4-} + 6H^+ \rightarrow J^- + 6Fe(CN)_6^{3-} + 3H_2O$$

Da aber die Reaktion langsam vor sich geht, ist nach dem Zusatz des Hexacyanoferrat(II)-s

3–4 Minuten zu warten. (Unter geeigneten Konzentrationsverhältnissen verläuft eine der Landoltschen ähnliche Reaktion). Die Titration wurde ebenfalls nach Erdey und Svehla[12] ausgeführt, die Ergebnisse sind in der Tabelle zu sehen. Die standard Deviation ergab sich aus den Ergebnissen von 12 parallelen Bestimmungen für ±0,17% (±0,05 mg); die standard Deviation des Mittelwerts betrug ±0,07% (±0,02 mg).

Bromatbestimmung

Bromat wird durch Hexacyanoferrat(II) in saurer Lösung quantitativ reduziert.[12,16]

$$BrO_3^- + 6Fe(CN)_6^{4-} + 6H^+ \rightarrow Br^- + 6Fe(CN)_6^{3-} + 3H_2O$$

Das entstandene Hexacyanoferrat(III) wird in bikarbonatiger Lösung in Gegenwart von 2-Oxyvariaminblau als Indikator mit Ascorbinsäuremasslösung titriert. Die Ergebnsse sind in der Tabelle zu sehen. Aus 12 parallelen Bestimmungen ergab sich die standard Deviation für ±0,05% (±0,02 mg), die standard Deviation des Mittelwerts betrug ±0,02% (±0,01 mg).

Chromatbestimmung

Chromate bzw. Bichromate reagieren mit Hexacyanoferrat(II) Ionen in saurer Lösung unter Bildung von Chrom(III)-Ionen und Hexacyanoferrat(III)-Ionen.[17–20]

$$CrO_4^{2-} + 3Fe(CN)_6^{4-} + 8H^+ \rightarrow Cr^{3+} + 3Fe(CN)_6^{3-} + 4H_2O$$

Das entstehende Hexacyanoferrat(III) wird in bikarbonatiger Lösung in Anwesenheit von 2-Oxyvariaminblau als Indikator mit Ascorbinsäuremasslösung titriert. Die Titrationen wurden nach Erdey und Svehla[20] ausgeführt, die Ergebnisse sind in der Tabelle gegeben. Die standard Deviation betrug bei 12 parallelen Bestimmungen ±0,04% (±0,14 mg), die standard Deviation des Mittelwerts ±0,11% (±0,04 mg).

Summary—Either of two quinoidal structures might be formed in the oxidation of 2-hydroxy-4-amino-4′-methoxydiphenylamine (2-Oxyvariamine Blue). It is shown that it is the paraquinoidal holoquinone that is formed. The ascorbimetric determination of certain oxidising agents (I_2, Br_2, IO_3^-, BrO_3^-, CrO_4^{2-}), using 2-Oxyvariamine Blue and a hexacyanoferrate(II)hexacyanoferrate(III) system is described.

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Talanta, 1963, Vol. 10, pp. 1277 to 1285. Pergamon Press Ltd. Printed in Northern Ireland

MAGNESIUM IN BIOLOGICAL SAMPLES BY SPECTROPHOTOMETRIC MEASUREMENT OF THE 8-QUINOLINOLATE EXTRACT*

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(*Received* 17 *July* 1963. *Accepted* 26 *July* 1963)

Summary—Conditions for the simple, rapid and sensitive determination of magnesium in biological samples have been determined. Commercially available solvents and reagents are used without further purification. Magnesium is separated and converted to a form which may be measured spectrophotometrically in a single extraction as the 8-quinolinolate in 1,1,2-trichloroethane. The extraction of other 8-quinolinolates is prevented by the addition of tartrate and cyanide to the buffer solution. Adequate sensitivity is obtained (absorbance of 0·4 for 20 μg of magnesium), and even greater sensitivity is possible by reducing the volumes of both aqueous and organic phases.

A SIMPLE, sensitive and relatively rapid method for determining magnesium in serum, urine, cells and other biological samples has been lacking. The major cations present are sodium, potassium and calcium, so that the problem of interferences should not be a very great one.

The insoluble magnesium 8-quinolinolate has long been the basis for schemes to separate magnesium,[1] and its insolubility in organic solvents has been somewhat of an enigma in view of the solubility of a large number of other 8-quinolinolates. Luke and Campbell[4,5] found that they could extract magnesium with a solution of 8-quinolinol in chloroform if butoxyethanol was added to the system. On this basis they devised a method for measuring magnesium in electronic nickel in which a separation scheme was used to remove interferences. Jankowski and Freiser[3] made a more detailed study of the extraction of magnesium with 8-quinolinol in chloroform, and they studied the effect of additives other than butoxyethanol. As a result of their investigations, they described a method for the determination of magnesium by extraction of the tetra-n-butylammonium 8-quinolinolate complex with chloroform.

A spectrophotometric measurement of the organic layer would be a direct measurement of the magnesium content of the original sample. The possibility of determining the magnesium content of samples after a single extraction was very attractive, and the present work was undertaken to develop such a method for biological specimens. In order to keep the method as simple as possible for use in routine work, conditions were established so that commercially available reagents and solvents could be used without further purification. Such a procedure and the studies necessary to establish the conditions are reported in this paper.

* This paper is based on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York, U.S.A.

EXPERIMENTAL

Apparatus

A Beckman DU quartz spectrophotometer with the blue sensitive phototube was used for all analytical work. The spectra were recorded with a Beckman DK-2 spectrophotometer.

All extractions were carried out in 125-ml separatory funnels equipped with Teflon stopcocks. Mixing was provided with an electrically-driven glass stirrer with paddles and spiral tip in the direction to force the liquid downward. Proper adjustment of the height of the stirrer ensured complete movement of the bottom layer. Equilibrium was found to have been established after 1 min of stirring, but a standard time of 5 min was used.

Reagents

Unless otherwise noted, analytical-grade chemicals were used.

0·1*M Sodium thiosulphate:* Dissolve 16 g of the anhydrous salt or 25 g of its pentahydrate per litre using de-ionised water.

Buffer I: Dilute the following ingredients to 1 litre with de-ionised water: 13·3 g of sodium cyanide, 20·0 g of ammonium tartrate, 150 ml of concentrated aqueous ammonia (filtered through a coarse ashless paper) and 150 ml of 2-butoxyethanol (practical, Eastman P 2270).

Buffer II: Make up as in Buffer I with the same quantities of ingredients per litre, but omit the butoxyethanol.

8-*Quinolinol solution I* (for use with Buffer I): Make a solution containing 15 g of 8-quinolinol (Eastman 794) per litre in 1,1,2-trichloroethane (Matheson, Coleman and Bell technical grade). Filter through a coarse ashless filter paper and store in a dark bottle.

8-*Quinolinol solution II* (for use with Buffer II): Make 1 litre of solution containing 15 g of 8-quinolinol, 200 ml of butoxyethanol or 200 ml of isoamyl alcohol (Eastman P 18) in 1,1,2-trichloroethane.

Standard magnesium solution: Dissolve 0·1000 g of pure magnesium metal by warming gently with 5 ml of concentrated nitric acid. When dissolution is complete, heat to expel oxides of nitrogen and dilute to 1 litre with de-ionised water. This solution contains 100 μg of magnesium/ml. For a standard solution of 10 μg of magnesium/ml, dilute the stock solution ten-fold.

Procedure

Preparation of serum. Pipette 2 ml of fresh serum into a 10-ml volumetric flask, add 3 ml of de-ionised water, mix and dilute to the mark with 10% trichloracetic acid solution. Shake well, transfer to a 15-ml conical centrifuge tube and spin at 2000 rpm for 10 min. Pour the supernatant material through a coarse ashless filter paper and use the filtrate for the sample.

Extraction. Pipette 5 ml of the above sample into the separatory funnel, add 1 ml of sodium thiosulphate solution and stir for 2 min. Pipette 10 ml of buffer solution into the mixture and stir for another 2 min. Pipette 10 ml of 8-quinolinol solution into the mixture and stir for 5 min. Allow the layers to separate and draw the bottom layer into a stoppered flask through a dry coarse ashless filter paper. Measure the optical density at 400 mμ against a blank prepared by using the same procedure on 5 ml of de-ionised water. A standard curve should be prepared with the same volume ratios, and the unknown magnesium content may be read directly from this curve. A blank and a standard should be run every day as a check on the reagents.

Either combination of Buffer I with 8-quinolinol solution I or Buffer II with 8-quinolinol solution II may be used. In using combination I, most of the butoxyethanol ends up in the organic layer so that the absorbance will be somewhat lower than in the case of combination II in which the organic layer volume remains essentially unchanged.

Study of variables

Colour stability. Within the limits of the instrumental readings, the absorbance of the 8-quinolinol in the 1,1,2-trichloroethane extract of magnesium remained unchanged in a 6-hr period during which it was observed.

Choice of solvent. The conditions originally set out by Luke were checked on known samples of magnesium and found to be as reported. However, the volatility of chloroform was undesirable and the possibility of using a less volatile solvent was investigated. This was confined to commercially available solvents. Because a liquid with density greater than that of water was desired for ease of separation, various chlorinated solvents were tested. Fig. 1 shows standard curves for a number of solvents run under the conditions and volumes outlined by Luke. As may be seen, a number of solvents could be used instead of chloroform, but 1,1,2-trichloroethane (Matheson, Coleman and Bell technical grade) was selected because of its low blank reading as well as its favourable non-volatile character (b.p. 112–114°).

Choice of wavelength. Fig. 2 shows the absorption spectra for a reagent blank 8-quinolinol solution as well as one containing magnesium. It is seen that below 400 mμ both curves rise rapidly. On the other hand, the difference curve shows a rather broad peak near 375 mμ. Luke and Campbell[4] selected 400 mμ for their method whereas Jankowski and Freiser[3] used the somewhat greater sensitivity of 380 mμ for their studies. Fig. 2 shows that the net absorbance ratio A_{400}/A_{380} is about

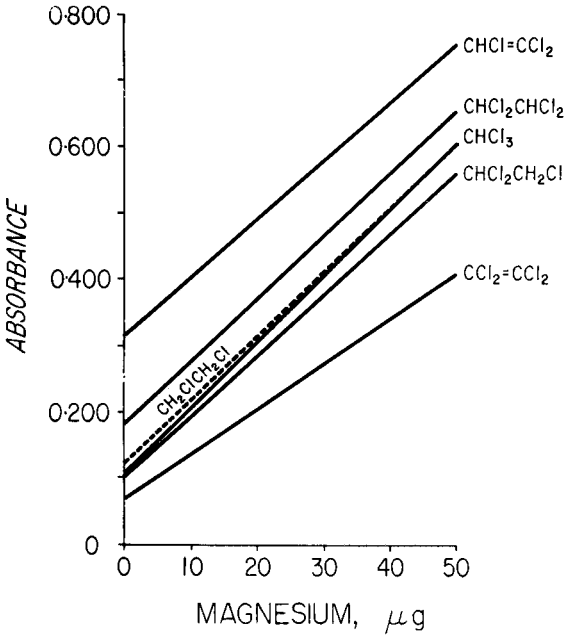

FIG. 1.—Standard curves for various solvents.

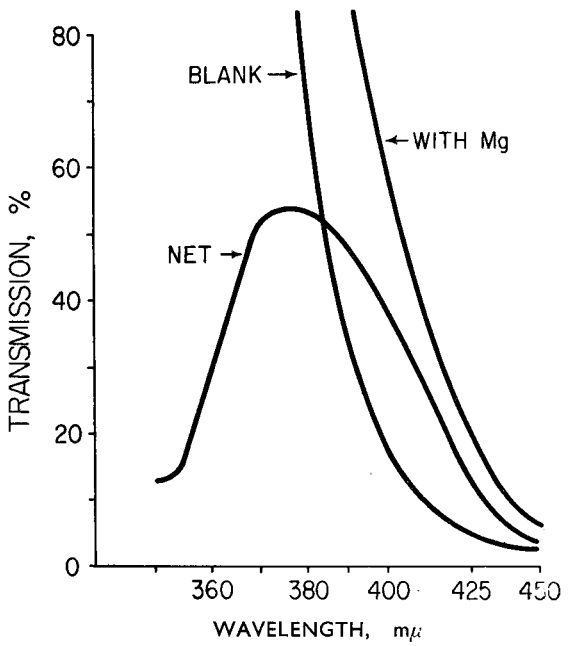

FIG. 2.—Absorption spectra for 8-quinolinol and magnesium 8-quinolinolate.

0·75. On the other hand, the ratio of the net reading to the blank is 2·2 at 400 mμ as compared to 0·77 at 380 mμ. For the latter reason, the present work was carried out at 400 mμ.

Effect of extraction aids. Two substances of equal efficacy were butoxyethanol and isoamyl alcohol (practical grade). Jankowski and Freiser[3] reported that ethanolamine was effective for the oxine-chloroform solvent, but in the present work neither ethanolamine nor triethanolamine were found to be as effective as butoxyethanol. The possible advantage of butoxyethanol over isoamyl alcohol is that the former may be mixed with either the buffer or the organic solvent while the latter must be mixed with the organic solvent because of its very limited aqueous solubility. However, even the butoxyethanol ends up almost entirely in the organic phase after equilibration with the aqueous phase. In an experiment in which an approximate change in volume was measured using graduated cylinders, quantities of butoxyethanol varying from 10 to 25 ml/100 ml of total organic phase were added to the 1,1,2-trichloroethane solution. Ten ml of the organic phase were equilibrated with an aqueous phase of 5 ml of water added to 10 ml of the buffer. A decrease in volume of the organic phase from 0·1 to 0·2 ml was noted, indicating that no appreciable differences occurred in this range.

The results shown in Table I indicate that the extraction effect has reached a plateau at 20 volume per cent. Essentially similar results were obtained when the butoxyethanol was added to the aqueous

TABLE I.—EFFECT OF BUTOXYETHANOL OR ISOAMYL ALCOHOL ON EXTRACTION

Volume per cent	Net absorbance	
	Butoxyethanol	Isoamyl alcohol
5	—	0·322
10	0·382	0·396
15	0·405	0·402
20	0·415	0·419
25	0·418	0·415

phase. In the latter case, however, the volumes at equilibrium indicated that virtually all of the butoxyethanol was in the organic phase. As long as the buffer was made up of ammonium nitrate and aqueous ammonia, even 25 volume per cent of butoxyethanol could be added to the buffer. However, when tartrate and cyanide were added as in the final buffer mixture, the aqueous phase was almost saturated with butoxyethanol at 15 volume per cent. Therefore, if larger quantities of complexing agents are necessary in the buffer solution, it would be necessary to add the extracting aid to the organic solution of oxine.

Effect of pH. Jankowski and Freiser[3] studied the extraction of magnesium with an oxine solution in chloroform containing isopentyl alcohol and reported a pH range of 10·05 to 10·28 as the optimum range. The optimum pH range was somewhat higher with ethanolamine in the aqueous phase (10·24 to 10·46).

In the present work, the pH was reduced by adding solid ammonium nitrate to the usual buffer employed in the studies. The solution for extraction contained the butoxyethanol in the organic phase initially in the concentration of 20 volume percent. The following table shows the results obtained:

TABLE II.—EFFECT OF pH ON EXTRACTION

Final pH	Absorbance
9·92	0·395
10·22	0·414
10·52[a]	0·415

[a] Usual buffer.

The buffers used were prepared and kept in polyethylene bottles with the usual screw caps, and it was surprising to find that when the buffers were used as much as 5 months after preparation they maintained exactly the same pH as originally determined. Because the buffer contains a large quantity of aqueous ammonia as the principal ingredient, any loss of this material would be expected to lower the final pH. Table II shows that an appreciable change is permissible with the same extraction efficiency (a pH change of 0·3 would mean that approximately half of the aqueous ammonia would have to volatilise).

Effect of ionic strength. Because of the striking effect of the addition of tartrate and cyanide on the solubility of butoxyethanol in the buffer, it was of interest to note the effect of inert salts on the extraction. Table III shows the effect of increasing the ionic strength with sodium nitrate.

TABLE III.—EFFECT OF IONIC STRENGTH ON EXTRACTION

Ionic strength	Absorbance
1·9[a]	0·415
2·5	0·426
3·8	0·447

[a] Usual strength.

The ionic strength in the procedure is 1·9 and the major component is the aqueous ammonia. In order to increase the ionic strength to 3·8 it was necessary to add 24 g of sodium nitrate/100 ml. Thus, the extraction may be increased by an unrealistic increase in the ionic strength. Conversely, the variations in ionic strength which may be introduced through the salt content of the usual sample will have no measureable effect on the extraction.

Elimination of interferences

Calcium. The principal bivalent ion of consequence in biological samples is calcium and Luke[5] has shown that its presence in any significant quantities interferes with the magnesium determination. Fig. 3 shows that the calcium interference is essentially a linear function of its concentration. In

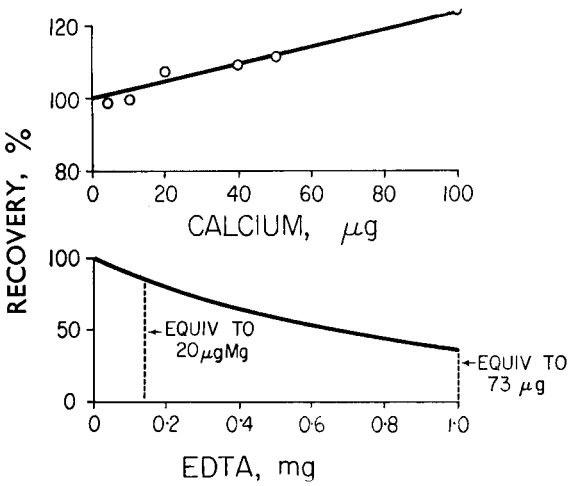

FIG. 3.—Effect of calcium and EDTA on the magnesium 8-quinolinolate recovery.

the case of serum this would amount to an increase of about 25%. EDTA with its greater affinity for calcium than magnesium eliminates the interference, but as also shown in Fig. 2, while EDTA is effective in removing the interference, its usefulness is limited because of the critical nature of the levels permissible. Thus, a previous knowledge of the calcium content of the sample would be required. Jankowski and Freiser[3] report that citrate decreased the magnesium extraction but that tartrate permitted the presence of mg-quantities of calcium in the presence of mg-quantities of magnesium. Table IV shows that a 0·1*M* solution of tartrate satisfactorily prevented the extraction of up to 400 μg of calcium in the presence of 20 μg of magnesium, but as much as 1 mg of calcium resulted in a large positive error. The limits are quite adequate for biological samples.

Iron. In ordinary serum the quantities of iron present amount to only about 2% of the magnesium content. Red cells and haemolysed blood would contain sufficient quantities to affect the magnesium determinations. Iron forms a strong complex with 8-quinolinol. Iron also forms strong complexes with cyanide as the hexacyanoferrate(II) and the hexacyanoferrate(III), depending upon the state of the iron. The hexacyanoferrate(III) is readily reduced to the hexacyanoferrate(II) in alkaline solution.[6] Attempts to use the hexacyanoferrate(III) form to prevent extraction as the 8-quinolinolate were

TABLE IV.—SUPPRESSION OF CALCIUM EXTRACTION BY 0·1*M* TARTRATE

Ca added to 20 μg of Mg, μg	Net absorbance
0	0·332
100	0·336
400	0·336
1000	0·451

unsuccessful (100 μg of iron in the presence of 20 μg of magnesium increased the absorbance by 100%). The use of the hexacyanoferrate(II) form was completely successful under certain conditions. No appreciable increase in the blank reading was obtained in the presence of 100 μg of iron if the latter was reduced to the iron^{II} form *before* the addition of the cyanide. Apparently any conversion from the hexacyanoferrate(III) to the hexacyanoferrate(II) was incomplete in the time used in the procedure. Thus, the cyanide and the thiosulphate could not be added together. Therefore the present procedure calls for addition of the thiosulphate solution to the sample, and the cyanide is added next as part of the buffer solution.

Copper. In ordinary serum, copper is present in amounts almost equivalent to the iron. Using the same procedure as for iron, it was found that 100 μg of copper caused no appreciable increase in the blank reading.

Protein removal. At the high pH used for the extraction, the presence of protein caused stable emulsions. Therefore, the standard trichloroacetic acid deproteinisation of serum was employed for the preparation of the sample. It was found that any emulsion formation reduced the extraction efficiency so that it was necessary to ensure that small bits of protein were not included in the aliquot. Passing the TCA filtrate through a coarse ashless filter paper prior to pipetting was the best procedure. The introduction of the deproteinisation step is not necessarily a disadvantage because the same filtrate may be used for calcium and inorganic phosphate analyses.

Distribution coefficient. Because the method is an extraction procedure, accurate results may be obtained if conditions are maintained constant. Luke and Campbell[4] did not report the extent of the extraction whereas Jankowski and Freiser[3] report 100% extraction based on some calculations from a molar extinction coefficient measurement. In the present work it was found difficult to obtain an accurate measurement chemically, especially in the small quantity remaining in the aqueous layer. Therefore, the distribution coefficient was determined using the radioisotope ^{28}Mg. Table V shows the results obtained in two extractions. The total counts were obtained by multiplying the counts/ml

TABLE V.—DETERMINATION OF DISTRIBUTION COEFFICIENT $D(C_{org}/C_{water})$

Sample	Total counts/ml		% Extracted	$D(C_{org}/C_{water})$
	Organic	Aqueous		
1	835,334	62,858	93·00	14·87
2	833,458	62,872	92·99	14·83

by the volume of the layer. In this experiment, the organic layer was 11·8 ml and the aqueous layer 13·2 ml as determined in a graduated cylinder. Considering the error in this measurement as well as the pipetting errors, remarkably good agreement was obtained. The total activity added to the solutions was 893,000 which gives an over-all balance of 100·58% for sample 1 and 100·36% for sample 2. The percentage extracted agrees very well, and the agreement in D is still very good although the difference is about ten times that of the recovery figure. Because the small quantity left in the aqueous layer would be difficult to measure accurately by chemical methods, it may be seen that D would be difficult to determine with any great precision.

The distribution coefficient permits us to determine the extent to which the volumes must be maintained for any desired degree of constancy in the extraction. This may be computed by the following formula:

$$\% \text{ Extracted} = 100w_0/w_t = 100D/(R + D)$$

where w_0 and w_t are, respectively, the weights of magnesium in the organic layer and in the total system, and D is the distribution coefficient and R is the ratio of aqueous volume to organic volume (v_w/v_0).

In the present experiment, the volume of sample was 5 ml, 10 ml of buffer I were added and the extraction was carried out with 10 ml of 8-hydroxyquinolinol in 1,1,2-trichloroethane (solution I). The final volumes after equilibration were 11·8 ml of organic phase and 13·2 ml of aqueous phase (a later examination showed that almost the entire butoxyethanol content originally in the aqueous phase was in the organic phase). A calculation shows that 93·0% of the magnesium would be in the organic phase. If the volume of the original sample was to increase to as much as 6 ml because of the neutralisation of a strongly acid solution or other additions, the aqueous phase would increase to 14·2 ml. With this new volume, the calculation shows that 92·5% of the magnesium would appear in the organic phase. Because the buffer and organic extractant would be introduced by pipette, an appreciable allowance for additions to the solution may be made with relatively small change in quantity extracted.

Recovery from serum. In order to show that the recovery of added magnesium was the same from serum as from standard solutions, it was necessary to first remove the magnesium from a serum sample, add a known quantity of magnesium and determine the quantity extracted. Combination II of buffer and solvent in which the butoxyethanol was added to the 1,1,2-trichloroethane solution was used. Buffer was added to samples of deproteinised serum which were then extracted twice with the 8-quinolinol solution and once with the solvent 1,1,2-trichloroethane alone. Twenty μg of magnesium were then added to the aqueous phase and the regular extraction was carried out. A similarly pre-extracted serum sample was used as the blank. For comparison a de-ionised water-buffer mixture was subjected to the same pre-extraction treatment before the addition of magnesium. Table VII shows the results obtained. The results show that the distribution of the magnesium in a serum sample is exactly the same as that obtained from standard solutions.

TABLE VII.—RECOVERY OF 20 μg OF MAGNESIUM ADDED TO SERUM

Sample	Net absorbance
Serum 1	0·435
Serum 2	0·431
De-ionised water	0·428

The addition of radioactive magnesium to a sample of serum and the subsequent removal of the protein by TCA precipitation showed that an average of 99·9% of the counts added could be accounted for in the filtrate. The extraction with the oxine solution showed that 92·5% of the activity was in the organic phase.

Reproducibility. Apparently the batches of 8-quinolinol and solvent varied considerably because the blank readings were subject to much variation. However, the net readings were very consistent as shown in Table VIII, which presents results for 20 μg of magnesium determined on separate days as a check on the reagents.

TABLE VIII.—MEASUREMENTS ON 20 μg OF MAGNESIUM

Absorbance		
Sample	Blank	Net
0·600	0·245	0·355
0·476	0·118	0·358
0·513	0·161	0·352
0·499	0·148	0·351
0·499	0·146	0·353
0·494	0·138	0·356
0·395	0·042	0·353
0·616	0·264	0·352[a]
0·513	0·161	0·352[a]
0·480	0·124	0·356[b]
0·509	0·159	0·350[c]
0·507	0·150	0·357[c]
0·496	0·140	0·356[c]
		mean 0·354
		std. deviation 0·0024

[a] No cyanide.
[b] 100 μg of copper present.
[c] 100 μg of iron present.

In serum samples, the predominant cation of significance in the magnesium determination is calcium. However, small amounts of iron and copper totalling about the equivalent of 1 μg of magnesium/ml are present.[2] Pooled serum samples were analysed using buffers with and without cyanide and a reducing agent. The results for two pools are shown in Table IX. The data for the

TABLE IX.—ANALYSIS OF POOLED SERUM UNDER VARYING CONDITIONS

Buffer	Mg, *mg/100 ml*	
	Pool 1	Pool 2
No NaCN	24·2	25·7
	24·4	25·4
	23·0	25·5
	23·3	
With NaCN	23·0	25·4
	22·9	25·3
	23·0	25·4
	23·1	
Reduce with $Na_2S_2O_3$ then add NaCN	22·9	25·4
	22·7	25·3
		25·4

two pools indicates that the use of thiosulphate made no difference, although for samples with as much as 100 μg of iron it was essential to reduce first. Pool 1 shows somewhat erratic results in the absence of cyanide although pool 2 shows no difference. The highest values of pool 1 would be in line with the extraction of the reported quantities of iron and copper. Because of the more consistent values obtained, buffers containing cyanide have been used.

DISCUSSION

The method developed simplifies the determination of magnesium in serum and other biological samples considerably. The complexing agents are all included in the buffer solution and the spectrophotometric measurement is made directly on the organic solution which isolates the magnesium. Thus a typical determination of a serum sample would be to take an aliquot of the deproteinised solution, add a measured volume of buffer and a measured volume of 8-quinolinol in 1,1,2-trichloroethane and stir. The spectrophotometric measurement of the separated organic layer, which is the lower layer, completes the determination.

If butoxyethanol is used as the extracting aid, it may be added to either the aqueous buffer solution or to the organic solvent. If isoamyl alcohol is used, it must be added to the organic solvent. Because the extraction aid ends up almost entirely in the organic phase and its origin in the aqueous phase neither increases the extraction efficiency nor shortens the equilibration time, it would seem more logical to make it up as part of the organic phase. Using the latter approach, it would be possible to increase the salt content of the buffer to any desired level whereas the incorporation of butoxyethanol in the buffer solution is limited by the nature and extent of salt concentration.

The data presented here shows adequate sensitivity (absorbance of 0·4 for 20 μg of magnesium), but the limit of sensitivity may be extended by varying conditions. Because the distribution coefficient in a constant ionic strength system is not subject to the absolute quantity of material distributed whereas the absorbance is, the sensitivity may be increased by increasing the concentration in the extractant. One way to do

this is to change the volumes of both phases. For example, if both the amounts of buffer and organic extractant were reduced to one-half and the volume of sample taken so that the same ratio of aqueous to organic volumes were maintained as before, 10 μg of magnesium should give an absorbance of 0·4. One of the investigators who is interested in determining calcium and inorganic phosphate as well as magnesium on the same TCA filtrate has been using the solutions in the ratio of 5 ml of buffer, 3 ml of TCA filtrate and 5 ml of organic extractant with excellent results. With the proper choice of volumes and micro spectrophotometric equipment, the present scheme should be easily adaptable to submicrogram quantities.

The experiments using radioactive magnesium showed that the acidification in the TCA protein precipitation procedure released all of the protein-bound magnesium. Both the radioactivity measurements and the addition of magnesium to pre-extracted serum filtrates showed that recoveries were the same from serum and standard solutions.

Zusammenfassung—Bedingungen zur einfachen, schnellen und empfindlichen Magnesiumbestimmung in biologischen Substanzen wurden aufgesucht. Es werden handelsübliche Lösungsmittel und Reagentien ohne weitere Reinigung verwendet. Mg wird als 8-Hydroxychinolat in 1,1,2-Trichloräthan in einem Extraktionsschritt abgetrennt und zur spektralphotometrischen Messung gebracht. Extraktion anderer 8-Hydroxychinolate wird durch Zusatz von Tartrat und Cyanid zur Pufferlösung verhindert. Die Empfindlichkeit ist hinreichend (Extinktion 0,4 für 20 μg), sie kann durch Verringerung der Volumina von wäßriger und organischer Phase noch gesteigert werden.

Résumé—Les conditions d'une analyse simple, rapide et sensible du magnésium contenu dans les échantillons biologiques ont été déterminées. Les solvants et les réactifs commerciaux disponibles sont utilisés sans autre purification. Le magnésium est séparé et converti en une forme susceptible de mesures spectrophotométriques par une extraction unique sous forme de 8-quinolinolate en solution dans le trichloro-1,1,2-éthane. L'extraction d'autres 8-quinolinolates est empêché par l'addition de tartrate et de cyanure à la solution tamponnée. On obtient une sensibilité convenable (absorption de 0,4 pour 20 microgrammes de magnésium) pouvant être augmentée par réduction de volume des phases aqueuses et organiques.

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Talanta, 1963, Vol. 10, pp. 1287 to 1289. Pergamon Press Ltd. Printed in Northern Ireland

CONTRIBUTIONS TO THE BASIC PROBLEMS OF COMPLEXOMETRY—XIII*

DETERMINATION OF ALUMINIUM AND TERVALENT CHROMIUM IN THE PRESENCE OF CHROMATE

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(*Received* 24 *July* 1963. *Accepted* 18 *August* 1963)

Summary—Aluminium can be determined in the presence of tervalent chromium and chromate using 1,2-diaminocyclohexanetetra-acetic acid (DCTA), which forms a complex with aluminium even in the cold. This phenomenon enables a successive determination to be made of aluminium (iron) and tervalent chromium in the presence of hexavalent chromium. This procedure cannot be carried out with the commonly used ethylenediaminetetra-acetic acid (EDTA).

IN a previous paper[1] it was mentioned that aluminium forms a complex with 1,2-diaminocyclohexanetetra-acetic acid (DCTA) almost instantaneously, even at room temperature, and in the presence of a large excess of neutral salts, such as sodium chloride, potassium nitrate, *etc.* This behaviour of DCTA is different from that of the normally used ethylenediaminetetra-acetic acid (EDTA) and it enables aluminium to be determined by back-titration of an added excess of DCTA with lead nitrate or zinc sulphate, using Xylenol Orange as indicator, in the presence of tervalent chromium. The determination of tervalent chromium can be carried out in the same sample after addition of a further excess of DCTA and boiling the mixture for the quantitative formation of the chromium–DCTA complex. It has been further found, in accordance with previous findings,[2] that DCTA is not oxidised in a slightly acidic medium on boiling with chromate. This enables the determination of aluminium (iron) and tervalent chromium to be made in the presence of a large excess of chromate, without the need for a time-consuming separation. Only by using DCTA is it possible to determine the above-mentioned elements in the same sample of ore, concentrate, mineral, alloy, ferrite, *etc.*

EXPERIMENTAL

Reagents

0·05*M* DCTA, 0·05*M* $Al(NO_3)_3$, 0·05*M* $KCr(SO_4)_2$, 0·05*M* $K_2Cr_2O_7$ and 0·05*M* $ZnSO_4$ were prepared from analytical-grade chemicals. Other reagents include aqueous ammonia (1:10), solid sodium acetate, solid hexamethylenetetramine and 0·2% Xylenol Orange.

Successive determination of aluminium and tervalent chromium

Procedure: Take an aliquot containing not more than 25 mg of chromium in a 600 to 800-ml tall-form beaker, add a sufficient excess of 0·05*M* DCTA solution to complex all aluminium, adjust the pH to 5–5·5 with solid hexamethylenetetramine (indicator paper), dilute to 250 ml, add some drops of Xylenol Orange solution and titrate the slightly yellow-green solution with 0·05*M* lead nitrate solution to intense violet. Add a further amount of 0·05*M* DCTA solution, more than sufficient to complex all chromium, boil 10 min, cool and dilute to 500–600 ml. Adjust the pH to

* Part XII: see *Talanta*, 1963, **10**, 899.

5–5·5 (indicator paper), if necessary, and titrate once more with 0·05*M* lead nitrate solution from orange-red to intense blue-violet. This colour change, like that for aluminium, is very sharp.

Some results are given in Table I.

Remarks: With the first back-titration there is almost no practical limit to the amount of aluminium that can be determined. Although concentrations of chromium higher than 25 mg/150 ml do not disturb this titration by their coloration, a limit of 25 mg applies for the second titration, where an intensely violet coloured chromium-DCTA complex is formed. If the original solution contains a large amount of chromium it is better to determine the two elements in separate aliquots: in the first aliquot only to determine aluminium, and in the second one, adequately smaller, to

TABLE I.—SUCCESSIVE DETERMINATION OF ALUMINIUM AND TERVALENT CHROMIUM

Taken, *ml*		0·05*M* DCTA,	Back-titn. 0·05*M* Pb,	0·05*M* DCTA,	Back-titn. 0·05*M* Pb,	Found, *ml*	
0·05*M* Al	0·05*M* Cr	*ml*	*ml*	*ml*	*ml*	0·05*M* Al	0·05*M* Cr
3·39	10·71[a]	8·00	4·58	15·00	4·34	3·42	10·66
1·13	10·71[a]	2·00	0·88	20·00	9·30	1·12	10·70
16·94	3·21	20·00	3·11	6·00	2·82	16·82	3·18
22·58	2·14	23·00	0·49	4·00	1·84	22·51	2·16
1·69	9·85	7·00	5·31	12·00	2·19	1·69	9·81
13·55	0·54	20·00	6·44	5·00	4·52	13·56	0·48

[a] Maximum of Cr in 600 ml.

determine the sum of aluminium and chromium. Although this second titration can be carried out even with EDTA (after boiling the solution), it is better to use only one reagent (DCTA) which simplifies the calculation.

Determination of aluminium and tervalent chromium in the presence of chromate

Chromate in a slightly acidic medium does not oxidise DCTA even on long boiling.[2] The yellow coloration of chromate does not affect the colour change of Xylenol Orange. Therefore the successive determination of aluminium and chromium can be carried out by the above procedure with the exception that for both back-titrations of DCTA a solution of zinc sulphate is used. Lead nitrate is less suitable because of the formation of lead chromate which dissolves slowly in an excess of DCTA.

Procedure: To an acidic solution containing aluminium, tervalent chromium and chromate, add an excess of DCTA and determine aluminium as described above, using 0·05*M* zinc sulphate as titrant and Xylenol Orange as indicator. After further adding of DCTA and boiling the solution for 10 min, determine tervalent chromium also by back-titration with zinc sulphate. The colour changes at the end-points of these two titrations are very sharp.

By this procedure one can determine aluminium and chromium in the presence of 75 mg of hexavalent chromium. Some results are given in Table II.

TABLE II.—DETERMINATION OF ALUMINIUM AND TERVALENT CHROMIUM IN THE PRESENCE OF CHROMATE

Taken, *ml*			0.05*M* DCTA,	Back-titn. 0·05*M* Zn,	0·05*M* DCTA,	Back-titn. 0·05*M* Zn,	Found, *ml*	
0·05*M* Al	0·05*M* Cr^{III}	0·05*M* Cr^{VI}	*ml*	*ml*	*ml*	*ml*	0·05*M* Al	0·05*M* Cr^{III}
0·50	0·45	5·00	3·00	2·52	3·00	2·52	0·48	0·48
0·50	9·01	10·00	2·00	1·47	15·00	5·99	0·53	9·01
3·03	0·90	1·00	6·00	2·96	3·00	2·04	3·04	0·96
5·04	0·90	20·00	10·00	4·94	5·00	4·11	5·06	0·89
5·04	9·01	15·00	6·00	0·97	18·00	9·00	5·03	9·00
5·04	0·45	10·00	10·00	4·91	5·00	4·46	5·09	0·54
10·09	1·80	30·00[a]	20·00	9·90	5·00	3·08	10·10	1·92
10·09	0·90	20·00	12·00	1·91	5·00	3·99	10·09	1·01
25·24	9·01	10·00	26·00	0·75	12·00	2·96	25·25	9·04
—	—	10·00	—	—	5·00	4·98	—	0·02
—	4·50	10·00	—	—	10·00	5·46	—	4·54

[a] Maximum of Cr^{VI} in 500 ml—colour change not sharp.

Zusammenfassung—Aluminium kann neben dreiwertigem Chrom und Chromat mit 1,2-Diaminocyclohexantetraessigsäure (DCTA) bestimmt werden. Das Reagens bildet auch in der Kälte einen Aluminiumkomplex. Diese Erscheinung erlaubt die aufeinanderfolgende Bestimmung von Al (Fe) und dreiwertigem Chrom neben Chromat. Diese Bestimmung ist mit der in der Komplexometrie üblichen Äthylendiamintetraessigsäure (EDTA) nicht möglich.

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Talanta, 1963, Vol. 10, pp. 1291 to 1295. Pergamon Press Ltd. Printed in Northern Ireland

POLAROGRAPHIC DETERMINATION OF COPPER^{II}, ARSENIC^{III} AND ARSENIC^{V} IN COPPER ARSENITE

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(*Received* 13 *May* 1963. *Accepted* 10 *June* 1963)

Summary—A method is proposed in which Cu^{II}, As^{III} and As^{V} can be determined in copper arsenite without prior separation. It is based on the fact that Cu^{II} and As^{III} yield prominent, distinguishable, widely-separated cathodic polarographic waves in a 0·1*M* LiCl—0·01*M* EDTA—0·001*M* LiOH solution using a dropping mercury electrode, whereas As^{V} does not give a wave in this medium. The As^{V} is determined by difference after reduction with sulphurous acid.

PRESENT methods for the determination of copper and arsenic are time-consuming, the arsenic usually having to be separated by distillation, which is not suitable for the individual determination of arsenic^{III} and arsenic^{V}.

Swann, McNabb and Hazel[1] reported a well-defined cathodic wave for arsenic^{III} in 0·1*M* lithium chloride–0·01*M* lithium hydroxide solution. Arsenic^{V} does not give a wave under these conditions.

R. L. Pecsok[2] described the polarographic wave obtained for copper^{II} in alkaline ethylenediaminetetra-acetate (EDTA) solution.

A rapid polarographic method has been developed in this laboratory for the determination of copper^{II}, arsenic^{III} and arsenic^{V} without prior separation. A procedure has been developed for the determination of these elements in copper arsenite, which is present in Paris Green. The method is based on the fact that copper^{II} and arsenic^{III} give single, widely separated cathodic waves in 0·1*M* lithium chloride–0·01*M* EDTA–0·001*M* lithium hydroxide solution using a dropping mercury electrode. Arsenic^{V} does not give a wave under these conditions and is determined by difference after reduction with sulphurous acid. The half-wave potentials are −0·46 v for copper and −1·86 v for arsenic^{III} at pH = 11·0.

EXPERIMENTAL

Apparatus

A Sargent Model XXI automatic recording polarograph and a Leeds and Northrup pH-meter were used in the study.

The polarographic cell assembly was the same as that described by Swann *et al.*[3] It was equipped with a constant temperature bath which maintained the temperature within ±0·1°. A silver-silver chloride reference electrode and a dropping mercury electrode were employed.

Reagents

Standard arsenic solution (0·250 mg/ml of arsenic): Dissolve 33·0 mg of pure dry arsenic trioxide (As_2O_3) in 1·0 ml of 2·0*M* lithium hydroxide and 5 ml of water. Neutralise with hydrochloric acid and dilute to 100 ml.

Standard copper solution (0·25 mg/ml of copper): Dissolve 25·0 mg of pure copper wire in 2·0 ml of dilute sulphuric acid and 2·0 ml of concentrated nitric acid. Evaporate to dense white fumes to remove nitric acid and dilute to 100 ml.

EDTA (0·25M): Add 75·1 g of EDTA (free acid), 20·8 g of lithium hydroxide monohydrate and 800 ml of water to a 1-litre beaker. Stir until dissolved and dilute to 1 litre.

Lithium chloride-lithium hydroxide supporting electrolyte: Dissolve 42·4 g (1·0*M*) of lithium chloride and 0·42 g (0·01*M*) of lithium hydroxide monohydrate in water and dilute to 1 litre.

Sulphurous acid: Saturated solution of sulphur dioxide in water.

Phenolphthalein indicator: Dissolve 1 g of indicator in 50 ml of ethyl alcohol and add 50 ml of water.

Preparation of calibration curves

To a series of 25-ml volumetric flasks add 2, 4, 6 and 8 ml of standard arsenic^{III} solution and the same amounts of standard copper^{II} solution. Add 1 drop of phenolphthalein indicator and 1·0 ml of 0·25*M* EDTA. Neutralise with 0·1*M* lithium hydroxide to the red colour of the indicator. Add 2·5 ml of the supporting electrolyte to each flask and dilute to the mark. Pipette 20 ml into the polarographic cell, and adjust the pH to 11·0 $\pm$ 0·1 with 0·1*M* lithium hydroxide or 0·1*M* hydrochloric acid. Pass oxygen-free nitrogen through the solution for 5 min. Carry out the polarographic measurements between $-0·2$ and $-0·6$ v for copper^{II} and between $-1·4$ and $-2·1$ v for arsenic^{III}. Plot the wave height of each curve against the concentration of the respective constituent, and calculate the slope of the straight line obtained.

Prepare a series of arsenic solutions as described adding 1·0 ml of saturated sulphurous acid solution to each flask. Treat as in the preparation of the calibration curve. The slope obtained here will be slightly less than in the above case.

Procedure

Weigh out a sample of the material containing from 5 to 100 mg of arsenic, dissolve in 1·0 ml of concentrated hydrochloric acid (sulphuric acid of the same normality may be used), and dilute to 100 ml.

Pipette a 5-ml aliquot into a 125-ml glass-stoppered Erlenmeyer flask. Add an equal volume of saturated sulphurous acid solution and enough hydrochloric acid to bring the acid concentration to 0·13–0·2*M*. Stopper the flask and place on a water-bath.

After 10 min remove from the water bath and boil for 3–5 min to expel excess sulphurous acid. Treat as in the preparation of the calibration curves and calculate the total concentration of arsenic from the slope of the curve prepared with sulphurous acid.

Pipette a second 5-ml aliquot into a 25-ml volumetric flask. Treat as in the preparation of the first calibration curves and calculate the concentrations of arsenic^{III} and copper^{II} from the slope of their respective curves. Subtract this value found for arsenic^{III} from that for total arsenic to give the amount of arsenic^{V} in the sample. Copper can be determined in either aliquot.

RESULTS AND DISCUSSION

The method was tested on an analysed sample of Paris Green containing 24·0% of copper and 42·8% of arsenic^{III} but no arsenic^{V}. A solution of arsenic^{V} was prepared from arsenic pentoxide and standardised iodimetrically. Portions of this solution were added to the sample. The results of the analysis are given in Table I.

TABLE I.—RESULTS OF PARIS GREEN ANALYSIS

As^{III}, *mg/litre*		As^{V}, *mg/litre*		Cu^{II}, *mg/litre*	
Present	Found	Present	Found	Present	Found
19·6	19·3	28·8	29·1	21·8	22·2
34·2	34·1	30·1	30·3	19·6	19·6
33·4	33·2	18·6	18·4	18·9	18·7
51·1	51·3	41·3	40·8	29·0	29·8
17·1	17·9	25·7	25·1	97·3	97·2
51·6	51·5	40·6	41·0	29·2	30·0
35·4	35·2	19·1	19·4	20·1	20·2
9·93	9·93	8·56	8·52	5·60	5·54

Copper^{II}: The characteristics of the copper wave have been fully described by R. L. Pecsok.[2] The wave is not affected by sulphite or changes in EDTA concentration above that necessary to complex copper.

Arsenic^{III}: The arsenic^{III} wave is well defined, and the wave height is directly proportional to the arsenic^{III} concentration. It is not affected by EDTA.

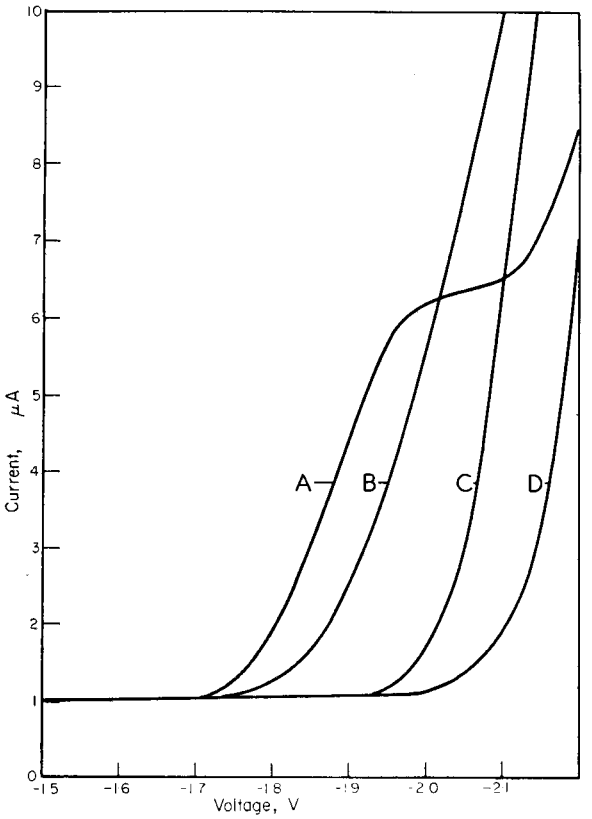

FIG. 1.—Effect of gelatin on arsenic^{III} wave
Supporting electrolyte: 0·1*M* lithium chloride—0·001*M* lithium hydroxide.
A—Arsenic^{III} in supporting electrolyte.
B—Arsenic^{III}, gelatin 0·01% and supporting electrolyte.
C—Gelatin and supporting electrolyte.
D—Supporting electrolyte alone.

The wave height decreased and the half-wave potential became more negative with increasing pH as described by Swann *et al.*[1] The method may be used at any pH value between 9 and 12. Below pH 9, two waves were formed. Above pH 12, the half wave potential was too close to that of lithium to allow its full development.

Lingane[4] reported that no definite limiting current is found for the arsenic^{III} wave in 0·5*M* sodium tartrate–0·01% gelatin at pH 4·5 and that no wave is formed in the same solution at pH 8·8. This has been shown to be a result of the early reduction of sodium;[1] gelatin also interferes, as shown in Fig. 1. To form a complete cathodic wave for arsenic^{III} in alkaline media, the concentration of sodium ions must not be greater than $1 \times 10^{-3}M$ and gelatin must be absent.

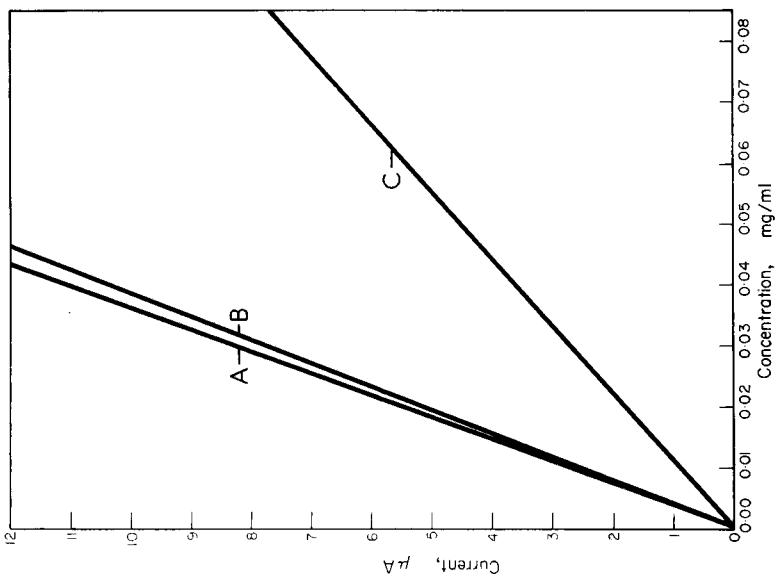

FIG. 3.—Calibration curves for arsenic and copper.
A—Arsenic^{III} in 0·1*M* lithium chloride—0·01*M* EDTA—0·001*M* lithium hydroxide (pH = 11·0).
B—Arsenic^{III} in 0·1*M* lithium chloride—0·01*M* EDTA—0·001*M* lithium hydroxide—0·044*M* lithium sulphite.
C—Copper^{II} in 0·1*M* lithium chloride—0·01*M* EDTA—0·001*M* lithium hydroxide (with and without sulphite).

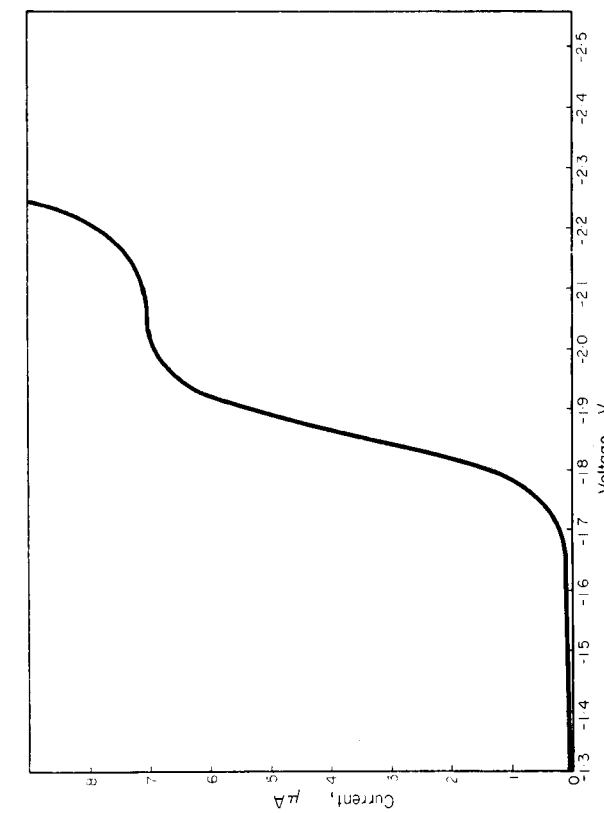

FIG. 2.—Polarogram of arsenic^{III} in 0·1*M* lithium chloride—0·01*M* EDTA—0·001*M* lithium hydroxide.

In 0·5*M* lithium tartrate, between pH values of 9 and 12, the wave has no definite limiting current. Tartrate is not used in the procedure because the copper^{II} wave is not as well defined as in EDTA at this low concentration.

The arsenic^{III} wave is shown in Fig. 2.

Arsenic^{V}: Since arsenic^{V} is not reduced at the dropping mercury electrode, it must be reduced to arsenic^{III} with sulphurous acid before making the polarographic measurements.

Kurtenaker and Fürstenau[5] made a complete study on the kinetics of the reduction of arsenic^{V} to arsenic^{III} with sulphurous acid. They found that the maximum rate of reaction occurs when the acid concentration is between 0·13 and 0·2*N*. The acid can be either hydrochloric or sulphuric acid. The reaction is accelerated by heating and, when heated on a water bath, is completed within 10 min.

Sulphite ion suppresses the arsenic^{III} wave with increasing sulphite concentration, in the absence of EDTA. The extent of suppression varies with both the arsenic^{III} and sulphite ion concentrations. When EDTA and sulphite are present, the arsenic^{III} wave is suppressed to a constant value, after which it is dependent only on the arsenic^{III} concentration. This effect can be seen in the calibration curves shown in Fig. 3. The sulphite is not reduced in this medium and does not affect the half-wave potential of either the copper or the arsenic wave.

Although it has not been tested, the method should be useful for the determination of arsenic and copper in a variety of materials, with the one provision that the amount of copper is not greater than 10 times that of arsenic. The method is applicable to copper arsenite containing calcium and magnesium arsenite.

Zusammenfassung—Eine Methode zur Bestimmung von Cu(II), As(III) und As(V) in Kupferarsenit ohne Trennung wird vorgeschlagen. Sie beruht auf den gut unterscheidbaren kathodischen polarographischen Stufen von Cu(II) und As(III) in einer Lösung mit 0,1m LiCl, 0,01m EDTA und 0,001m LiOH an einer Hg-Tropfelektrode. As(V) gibt in dieser Lösung keine Stufe; es wird nach Reduktion mit schwefliger Säure aus der Differenz bestimmt.

Résumé—On décrit une méthode de dosage de Cu (II), As (III) et As (V) dans l'arsenite de cuivre sans séparation préalable. Cette méthode est basée sur le fait que Cu (II) et As (III) fournissent, dans une solution à 0,1 M LiCl/0,01 M EDTA/0,001 M LiOH et avec une électrode à goutte de mercure, des courbes cathodiques polarographiques importantes, nettes et séparées, alors que As (V) n'en fournit pas dans ces conditions. L'arsenic (V) est déterminé par différence après réduction par l'acide sulfureux.

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Talanta, 1963, Vol. 10, pp. 1297 to 1298. Pergamon Press Ltd. Printed in Northern Ireland

PRELIMINARY COMMUNICATION

Determination of submilligram amounts of cobalt by ferricyanide titration with photometric end-point detection

(*Received* 25 *September* 1963. *Accepted* 28 *September* 1963)

THE well-known potentiometric titration of Co^{II} in ammoniacal solution with ferricyanide is a selective and fairly accurate determination of 2–100 mg of cobalt.[1–4] Of the more common metals only Mn^{II} and Cr^{III} interfere in small amounts.

The determination of smaller amounts of cobalt by this method proves to be impossible, because of poor response of the Pt-electrode, especially when the ions of other heavy metals are present.

The titration reaction can be represented formally by

$$Fe(CN)_6^{3-} + Co^{2+} \rightarrow Fe(CN)_6^{4-} + Co^{3+}$$

The product formed in the course of this reaction has a much greater colour intensity than would be expected from a mixture of ferrocyanide and either $Co(NH_3)_6^{3+}$ or any other ammonia complex of Co^{III}. The nature of the product is not yet clear. We presume that it may be a mixed complex of the two reaction products, *viz.*, $Co^{III}(NH_3)_5$-N-C-$Fe^{II}(CN)_5$. In the literature[5] a complex of the composition Co^{III}-EDTA-N-C-$Fe^{II}(CN)_5$ has been described as an intermediate in the oxidation of Co^{II}-EDTA by ferricyanide. In the mixed complex EDTA should act as a five-co-ordinate ligand.

The stability and the reproducibility of the colour of the mixed complex of Co^{III} with ammonia and ferrocyanide do not permit its use for the accurate quantitative spectrophotometric determination of cobalt. However, it can be used for the photometric end-point detection in the titration. In this way the amount of cobalt to be determined in the titrimetric determination can be considerably smaller than in the case of potentiometric end-point detection, because:

(*a*) the titration is possible in the concentration region of $10^{-4}M$, where the potentiometric titration fails. A light path length of 4 cm has to be used in these dilute solutions:

(*b*) the minimum volume required for a photometric titration is smaller than that for a potentiometric titration, using normal techniques; a minimum volume of 10 ml is readily attainable, so that an amount of 10^{-3} mmole or 0·06 mg of Co could be determined in this way.

We titrated a number of samples, each containing 0·6 mg of cobalt. The sample was treated with 4 ml of 50% ammonium citrate solution and 10 ml of 25% ammonia. The errors proved to be not larger than about 1%, which is a little higher than the errors in the potentiometric determination. Amounts of 120 mg of iron, 90 mg of nickel, 60 mg of copper, 30 mg of Cr^{III} or a mixture of 15 mg of Cr^{III}, 15 mg of Fe^{III}, 15 mg of Ni and 15 mg of Cu did not interfere. Thus tervalent chromium appears to be harmless, as contrasted with the interference of this substance in the potentiometric method. Manganese, even in small amounts, interferes, as in the case of the potentiometric determination.

We would suggest that this method may be useful for the rapid determination of small amounts of cobalt and this investigation is continuing.

In recent publications[6,7] the application of amino acids instead of ammonia is described in this kind of titration. We therefore propose also to examine the use of these complexing agents for the titration with photometric end-point detection.

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Summary—The titrimetric determination of $cobalt^{II}$ by oxidation with hexacyanoferrate(III) in ammoniacal solution, ordinarily carried out potentiometrically, can be extended to smaller amounts of cobalt by means of photometric end-point indication.

Résumé—Le dosage titrimétrique du cobalt(II) avec le hexacyanoferrate(III) dans un milieu ammoniacal est plus sensitif quand le point d'équivalence est déterminé par photométrie au lieu d'avec potentiométrie.

Zusammenfassung—Die volumetrische Bestimmung von Kobalt(II) mit Hexacyanoferrat(III) in ammoniakalischer Lösung ist bei photometrischer Endpunktsbestimmung erheblich empfindlicher als bei potentiometrischer Endpunktsindikation.

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Talanta, 1963, Vol. 10, pp. 1299 to 1305. Pergamon Press Ltd. Printed in Northern Ireland

SHORT COMMUNICATIONS

Titrimetric determination of tin^{II} with potassium hexacyanoferrate(III) in the presence of redox indicators

(*Received* 28 *May* 1963. *Accepted* 30 *August* 1963)

In connection with our investigations into the application of hexacyanoferrate(II) and hexacyanoferrate(III) in titrimetric analysis,[1] a direct determination of tin^{II} has now been developed. The method consists of the oxidimetric titration of tin^{II} in strong hydrochloric acid solution with standard potassium hexacyanoferrate(III) in the presence of a suitable redox indicator. In strong hydrochloric acid solution tin^{II} is present in the form of a chloro complex. The oxidation process may thus be formulated:

$$SnCl_4^{2-} + 2Cl^- + 2Fe(CN)_6^{3-} \rightarrow SnCl_6^{2-} + 2Fe(CN)_6^{4-}.$$

EXPERIMENTAL

Reagents

Stock solution of tin^{II}: This was *ca.* 0·3*M* in tin^{II} chloride and prepared by dissolving the appropriate quantity of $SnCl_2 \cdot 2H_2O$ ("Pro Analysi") in water acidified to a concentration of 1·2*M* in hydrochloric acid. The concentration of tin^{II} was determined by titration with standard iodine solution using starch as indicator.[2] More dilute solutions of tin^{II} were prepared from the stock solution by suitable dilution.

0·1*M Potassium hexacyanoferrate(III) solution:* Prepared by dissolving the chemically pure salt in water. The concentration was determined iodometrically.[3]

3,3′-*Dimethylnaphthidine solution:* Dissolve 0·2 g of indicator, with warming, in 100 ml of glacial acetic acid.[4]

o-Dianisidine solution: Dissolve 0·2 g of indicator in 100 ml of 2·5% acetic acid.[5]

Redistilled water was used throughout the work.

Procedure

Solutions of tin^{II} strongly acidified with hydrochloric acid were found to be only slightly susceptible to the action of atmospheric oxygen. Even after standing for several months, tin^{II} solutions in 1·2*M* hydrochloric acid did not show any appreciable change in concentration of tin^{II}. Therefore the complication of saturating the solution with carbon dioxide as in other methods for the titrimetric determination of tin^{II} was eliminated.

The effects of changing the concentration of tin^{II} and of hydrochloric acid and of using different redox indicators were studied. During the titration the solution normally remained clear. Only when the concentration of tin^{II} was comparatively high (>0·1*M* tin^{II} chloride) and the concentration of acid relatively low (2*M* hydrochloric acid) was a white precipitate formed. This precipitate had no perceptible influence on the actual titre. The method gave good results for amounts of tin from 0·01 to 0·80 g; the colour change was sharpest for amounts of tin from 0·01 to 0·30 g and it also became sharper on increasing the degree of acidification. The error of the determination was found to be identical with the error of the volume reading of solution in the burette (*ca.* 0·002 ml; 5-ml burette, graduated to 0·01 ml).

A very important condition for accurate titration is the need for sufficient acidification of the solution. It was found that for an amount of tin from 0·01 to 0·09 g the concentration of hydrochloric acid had to be at least 6*M*. If the amount of tin exceeded 0·1 g, the concentration must be increased to *ca.* 9*M*. A deficiency of acid resulted in a less distinct end-point and even in a different change of colour. When the concentration of hydrochloric acid was sufficient, after exceeding the end-point by 1 drop of titrant, the originally colourless solution became intensely coloured, purple-red in the presence of 3,3′-dimethylnaphthidine and red in the presence of *o*-dianisidine. In the case of a deficiency of acid the solution became green in the case of both indicators. The titre in the presence of a slight deficiency of hydrochloric acid was the same as in the presence of a sufficient quantity of the acid, but the colour change was less distinct.

TABLE I.—TITRATION OF TIN^{II} CHLORIDE SOLUTION WITH STANDARD POTASSIUM HEXACYANOFERRATE(III) USING 3,3′-DIMETHYLNAPHTHIDINE AS INDICATOR.

$SnCl_2$ taken, *ml*	0·1024*M* $K_3Fe(CN)_6$ required, *ml*	Sn found		
		Present method, *g*	Iodometric method, *g*	Difference, %
5·00[a]	2·79, 2·79, 2·80 — 2·79(3)	0·01697	0·01696	+0·06
10·00[a]	5·58, 5·57, 5·58 — 5·57(7)	0·03389	0·03392	−0·09
15·00[a]	8·37, 8·37, 8·38 — 8·37(3)	0·05088	0·05088	0·00
20·00[a]	11·14, 11·16, 11·16 — 11·15(3)	0·06777	0·06784	−0·10
25·00[a]	13·95, 13·95, 13·95 — 13·95(0)	0·08477	0·08480	−0·04
15·00[b]	16·73, 16·74, 16·74 — 16·73(7)	0·10171	0·10176	−0·05
20·00[b]	22·31, 22·31, 22·31 — 22·31(0)	0·13558	0·13568	−0·07
25·00[b]	27·88, 27·88, 27·87 — 27·87(7)	0·16941	0·16960	−0·11

[a] *Ca.* 0·03*M* tin^{II} chloride.
[b] *Ca.* 0·06*M* tin^{II} chloride.

TABLE II.—TITRATION OF TIN^{II} CHLORIDE SOLUTION WITH STANDARD POTASSIUM HEXACYANOFERRATE(III) SOLUTION USING *o*-DIANISIDINE AS INDICATOR.

Ca. 0·14*M* $SnCl_2$ taken, *ml*	0·1024*M* $K_3Fe(CN)_6$ required, *ml*	Sn found		
		Present method, *g*	Iodometric method, *g*	Difference, %
5·00	13·94, 13·95, 13·94 — 13·94(3)	0·08473	0·08480	−0·08
10·00	27·88, 27·87, 27·89 — 27·88(0)	0·16943	0·16960	−0·10
15·00	41·82, 41·81, 41·84 — 41·82(3)	0·25415	0·25440	−0·10
20·00	55·72, 55·78, 55·74 — 55·74(7)	0·33877	0·33920	−0·12
25·00	69·79, 69·71, 69·84 — 69·78(0)	0·42405	0·42400	+0·01

The following indicators were examined: 3,3′-dimethylnaphthidine, *o*-dianisidine, Lauth's violet, dimethylamine, safranine, methylene blue, ferroïn and erio green. Positive results were obtained only with the first four indicators, the best results being with 3,3′-dimethylnaphthidine and *o*-dianisidine. With these two indicators the colour change was unusually sharp, more so than in the iodometric determination of tin^{II}. The difference when tin^{II} was determined iodometrically and by the present method did not exceed 0·2% (see Tables I and II). The analysis required only a few min which was a great advantage.

It was found that the presence of lead, silver, zinc or aluminium did not interfere with the determination of tin. However, the method cannot be applied in the presence of substances oxidised by potassium hexacyanoferrate(III).

For examining the applicability of the method a tin-lead alloy was analysed as follows. Suitable amounts of alloy were weighed and dissolved in concentrated hydrochloric acid under an atmosphere of carbon dioxide, and the resulting solution diluted to the mark in a 250-ml volumetric flask. The concentration of tin was determined by direct titration of a suitable aliquot (5 or 10 ml) of this solution with standard potassium hexacyanoferrate(III) in the presence of 3,3′-dimethylnaphthidine as indicator. As one can see from Table III, the error of the analysis did not exceed *ca.* 0·2%, assuming that the gravimetric results were correct.

TABLE III.—DETERMINATION OF TIN IN TIN-LEAD ALLOYS.[a]

Alloy	Weight, *g*	Sn found		
		Present method, *g*	Gravimetric method,[b] *g*	Difference, %
I	0·7170	0·6445	0·6453	−0·12
	0·3698	0·3320	0·3328	−0·24
II	1·1482	0·3439	0·3444	−0·15
	1·3694	0·4113	0·4108	+0·12

[a] The amount of tin in alloy No. I is *ca.* 90% and in alloy No. II *ca.* 30%.

[b] Alloy treated with concentrated nitric acid and the resulting precipitate of β-metastannic acid converted to tin^{IV} oxide.

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Summary—A titrimetric determination of tin^{II} in strong hydrochloric acid solution with standard potassium hexacyanoferrate(III) solution using 3,3′-dimethylnaphthidine or *o*-dianisidine as indicator is described.

Zusammenfassung—Es wird eine titrimetrische Bestimmung von $Zinn^{II}$ in starker Salzsäure mit eingestellter Kaliumhexacyanoferrat (III)-Lösung und 3,3′-Dimethylnaphthidin oder o-Dianisidin als Indikator beschrieben.

Résumé—On décrit un dosage volumétrique de l'étain stanneux en milieu chlorhydrique fort par une solution standard d'hexacyanoferrate(III) de potassium, en titilisant la diméthyl-3-3′-naphtidine ou l'O-dianisidine comme indicateur.

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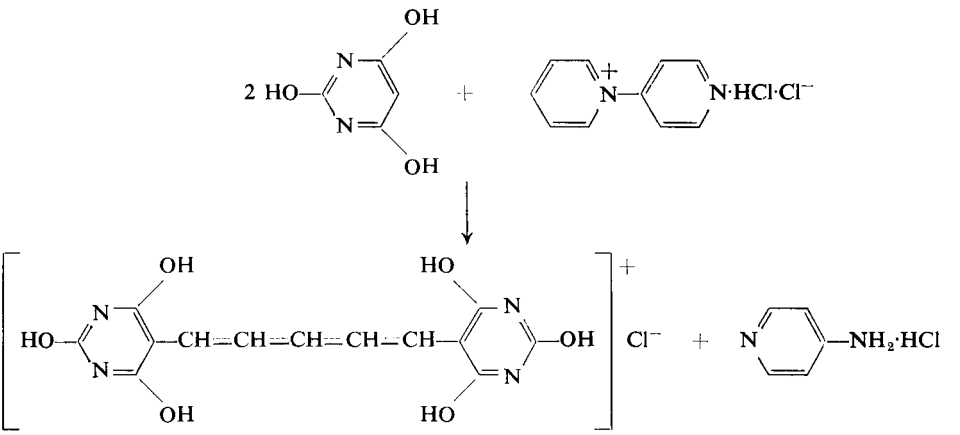
New specific spot tests for barbituric and thiobarbituric acids, malonic acid and urea

(*Received* 11 *June* 1963. *Accepted* 25 *July* 1963)

LIKE many other organic compounds with active methylene groups, barbituric acid forms a polymethine dye with glutaconic aldehyde. The colour is blue but it appears reddish, probably because of its intense fluorescence. The formation of this polymethine dye has been used analytically both for the detection and determination of pyridine.[1]

In the case of barbituric or thiobarbituric acid, the polymethine dye can be formed directly by heating with pyridyl pyridinium dichloride in dimethylformamide solution. This reaction is specific because other substances react with pyridyl pyridinium dichloride only after glutaconic aldehyde has been formed by the action of alkali. The reaction seems to proceed as follows:



and it serves as a selective and specific test for barbituric acid and thiobarbituric acid, using pyridyl pyridinium dichloride as reagent.

Malonic acid was found to react with urea to form barbituric acid, even though the yield seems to be low; therefore this reaction was further extended as a test for malonic acid. When thiourea was substituted for urea (with the intention of forming thiobarbituric acid), no dye was formed, probably because of the very low yield of the reaction with thiourea.

It is interesting to note that diethyl malonic ester, malonic diamide and even malonic ester monoamide do not react similarly with urea and pyridyl pyridinium dichloride. The test seems, therefore, to be specific for malonic acid.

The reaction was reversed and tried as a test for urea, through heating with malonic acid and pyridyl pyridinium dichloride. This test was found to have a limit of identification of 200 μg, far above other tests for urea.

EXPERIMENTAL

Test for barbituric and thiobarbituric acid

Procedure: Take a little of the test solid or the residue from 1 drop of test solution in a micro tube. Add 1 drop of the reagent, then heat the mixture in a glycerol bath at 120° for 2–3 min. In the

presence of barbituric acid a reddish-blue colour develops on cooling, which fluoresces under ultraviolet light. In the case of thiobarbituric acid the colour is blue and there is no fluorescence.

Reagent: A 1% solution of pyridyl pyridinium dichloride in dimethylformamide.

Limit of identification: 0.5 μg of barbituric acid;
0.5 μg of thiobarbituric acid.

Test for malonic acid

Procedure: Dissolve in a micro test tube a little of the test solid or the residue from 1 drop of test solution in Reagent I. Evaporate off the alcohol, then heat the mixture in a glycerol bath at 130° for a few min. Add Reagent II and reheat the mixture in the glycerol bath at 120° for a few min. In the presence of malonic acid, a reddish-blue colour develops on cooling, which fluoresces under ultraviolet light.

Reagents: I. A saturated solution of urea in methyl alcohol.
II. A 1% solution of pyridyl pyridinium dichloride in dimethylformamide.

Limit of identification: 50 μg of malonic acid.

Test for urea

Procedure: Dissolve in a micro test tube a little of the test solid or the residue from 1 drop of test solution in Reagent I. Evaporate off the alcohol, then heat the mixture in a glycerol bath at 120° for a few min. Add Reagent II and reheat the mixture in the glycerol bath at 120° for a few min. In the presence of urea a reddish-blue colour develops on cooling, which fluoresces under ultraviolet light.

Reagents: I. A saturated solution of malonic acid in methyl alcohol.
II. A 1% solution of pyridyl pyridinium dichloride in dimethylformamide.

Limit of identification: 200 μg of urea.

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Summary—Barbituric and thiobarbituric acids may be detected with pyridyl pyridinium dichloride, giving, respectively, a reddish-blue and blue colour. The limit of identification is 0·5 μg in each case. Malonic acid can be converted to barbituric acid by heating with urea and is then detected in a similar manner. The limit of identification is 50 μg of malonic acid. Urea may be detected with a limit of identification of 200 μg by reversing the test for malonic acid. All four tests are specific.

Zusammenfassung—Barbitursäure und Thiobarbitursäure lassen sich mit Pyridyl-pyridiniumdichlorid nachweisen; sie geben eine rötlichblaue bzw. blaue Färbung. Die Nachweisgrenze ist in beiden Fällen 0,5 μg. Malonsäure kann durch Erhitzen mit Harnstoff in Barbitursäure übergeführt und ebenso nachgewiesen werden. Die Nachweisgrenze beträgt 50 μg Malonsäure. Harnstoff kann mit einer Nachweisgrenze von 200 μg durch Umkehr der Probe auf Malonsäure gefunden werden. Alle vier Proben sind spezifisch.

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The purification of Calcein

(*Received* 8 *August* 1963. *Accepted* 27 *September* 1963)

INTRODUCTION

THE introduction of ethylenediaminetetra-acetic acid (EDTA) is almost certainly the outstanding development of recent years in titrimetric analysis. Subsequent to the introduction of this reagent, many papers appeared describing suitable indicators for use in the titration of certain cations or groups of cations. One such publication was by Diehl and Ellingboe,[1] who described the preparation

of fluorescein-complexone (Calcein) and recommended its use as an indicator for the titration of calcium in alkaline solution with EDTA. The end-point is signified either by a colour change from yellow-green to brown or, if the titration is carried out under ultraviolet illumination, by the disappearance of a green fluorescence.

However, in the titration of small quantities of calcium, some difficulty is experienced in end-point detection because of the persistence of a residual fluorescence[2,3] which is attributed to the presence of impurities in commercially available Calcein.[4] The difficulty in end-point detection has been confirmed in the author's laboratory, and this paper describes purification techniques which produce an indicator that can be successfully used in titrations with very dilute solutions of calcium and EDTA.

EXPERIMENTAL

Purification of commercial Calcein

As a result of some preliminary experiments, it appeared that chromatography offered the best chance of purifying commercial Calcein and the following methods were developed. The adsorbent used throughout these experiments was γ-alumina (B.D.H. for chromatographic adsorption analysis).

1. *Column technique.* Three different batches of Calcein from Supply House A and one batch from Supply House B were investigated. Small quantities of the four samples were dissolved in warm aqueous ethanol (1 + 2) and passed through columns of γ-alumina, 10 mm in diam. and 200 mm long. It soon became apparent that the major portion of each sample was adsorbed on the alumina; consequently the columns were eluted with aqueous ethanol (1 + 2) until the eluate exhibited little or no fluorescence.

The three samples from Supply House A each gave an intense brown-coloured band at the top of the column little more than 10 mm long, whereas the sample from Supply House B gave three bands—a short pink band at the top of the column, a diffuse central band which was orange in colour, and a short yellow band towards the bottom of the column. The bands were carefully isolated and indicator solutions prepared by trituration with 0·2% w/v potassium hydroxide solution. Their behaviour as indicators under ultraviolet illumination was assessed by their efficiency in the titration of 0·00025*M* calcium solution with 0·00025*M* EDTA at a pH above 12. Five ml of 20% v/v triethanolamine were added before titration, to complex any aluminium which may have been derived from the chromatographic alumina. The results are summarised in Table I.

It would appear from these results that the samples from Supply House A lend themselves more readily to this purification technique.

TABLE I—BEHAVIOUR OF PURIFIED CALCEIN AS AN INDICATOR

Source	Description	Comments
Supply House A, Batch 1.	Brown band	Strongly fluorescent. Very faint residual fluorescence. Good end-point
Supply House A, Batch 2.	Brown band	Weakly fluorescent. Very faint residual fluorescence. Good end-point.
Supply House A, Batch 3.	Brown band	Strongly fluorescent, Very faint residual fluorescence. Good end-point.
Supply House B.	Pink band	Weakly fluorescent. Faint residual fluorescence. Fair end-point.
Supply House B.	Orange band	Strongly fluorescent. Very faint residual fluorescence. Good end-point.
Supply House B.	Yellow band	Strongly fluorescent. Considerable residual fluorescence. Poor end-point.

2. *Batch technique*. Three different batches of Calcein from Supply House A and one batch from Supply House B were investigated.

Small quantities of each sample were finely ground, dissolved in warm aqueous ethanol (1 + 2), then shaken for 1 hr with a large excess (100–200 fold) of γ-alumina. The suspensions were subsequently filtered, washed with aqueous ethanol (1 + 2) until the washings were no longer fluorescent and the residues triturated with 0·2% potassium hydroxide solution. Their efficiency as indicators under ultraviolet illumination was assessed as described previously.

In the case of all three samples from Supply House A, a strong residual fluorescence was observed and the end-point was very poor. However, the sample from Supply House B gave a very good end-point with only a very faint residual fluorescence.

CONCLUSIONS

Using chromatographic γ-alumina in either a column or a batch technique, depending on the source of supply, it is possible to purify commercial Calcein from two major suppliers in Great Britain to such a degree that it can be used as an indicator in titrating calcium solutions with EDTA to give reproducible results at concentrations down to 0·00025*M*.

Acknowledgments—The author wishes to thank John Laing Research and Development Ltd. for permission to publish this paper and Mr. G. E. D. Brown for experimental assistance. Grateful thanks are also recorded to Mr. E. J. Newman, Hopkin and Williams Ltd., Chadwell Heath, Essex, Great Britain, for gifts of several batches of Calcein and for helpful discussion.

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Summary—Chromatographic techniques are described for purifying commercial Calcein. The purified product can be used successfully for titrating calcium solutions with EDTA, giving reproducible results at concentrations down to 0·00025*M*.

Zusammenfassung—Chromatografische Methoden für die Verreinigung von technisch reinem Calcein sind beschrieben. Das verreinigte Produkt kann mit Erfolg, als Kalzium Lösungen gegen ÄDTA titriert werden, und ergibt reproduktionsfühige Resultate bis zu einer Konzentration von 0·00025*M*.

Résumé—On décrit les méthodes chromatographiques pour purifier le Calcein industriel. On peut utiliser avec succès le produit purifié pour titrer les solutions de calcium avec EDTA et on obtient les résultats reproduiçables aux concentrations jusqu'à 0·00025*M*.

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[3] A. J. Barnard, H. Flaschka and W. C. Broad, *Chemist-Analyst*, 1957, **46**, 18.
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Talanta, 1963, Vol. 10, pp. i to iii. Pergamon Press Ltd. Printed in Northern Ireland

NOTICES

(Material for this section should be sent directly to the Associate Editor)

The **Applied Chemistry Section of I.U.P.A.C.** has issued a report (*Pure and Applied Chemistry*, 1963, 7, No. 1) on the evaluation of dried yeast, for which a definition and standards are given, together with lists of sources of reference methods of analysis and other recommended methods.

DEUTSCHE DEMOKRATISCHE REPUBLIK

Der Fachverband Analytische Chemie der Chemischen Gesellschaft in der Deutschen Demokratischen Republik veranstaltet gemeinsam mit der Arbeitsgruppe für physikalische Methoden der analytischen Chemie der Deutschen Akademie der Wissenschaften zu Berlin vom 14. bis 17. April 1964 in Eisenach eine Tagung

Moderne elektrochemische Analysenmethoden

unter der wissenschaftlichen Leitung von Herrn Prof. Dr.-Ing. K. Schwabe, TU Dresden.

Auf der Tagung sollen neuere methodische Erkenntnisse vorgetragen und diskutiert werden.

Plenarvorträge haben bereits zugesagt: H. Berg, K. Cruse, L. Erdey, V. Gutmann, Mitarbeiter von J. Heyrovsky, L. Holleck, W. Kemula, E. Pungor.

Anfragen bitten wir zu richten an Herrn Dr. H. Kriegsmann, Leiter der Arbeitsgruppe für physikalische Methoden der analytischen Chemie der Deutschen Akademie der Wissenschaften zu Berlin, Berlin-Adlershof, Rodower Chaussee 116–125, DDR.

UNITED KINGDOM

Friday 10 *January* 1964: **Annual General Meeting:** *Society for Analytical Chemistry, Western Section*: Cardiff.

Wednesday 15 *January* 1964: **Coulometry—Principles and Progress:** E. BISHOP: *Society for Analytical Chemistry, Midlands Section*: Wolverhampton and Staffordshire College of Technology: 6.30 p.m.

Wednesday 22 *January* 1964: **Discussion Meeting:** *Society for Analytical Chemistry, Microchemistry Group*: *The Feathers*, Tudor Street, London E.C.4: 6.30 p.m.

Friday 24 *January* 1964: **Annual General Meeting:** *Society for Analytical Chemistry, Scottish Section*: *More's Hotel*, Glasgow: 1.45 p.m.

Saturday 25 *January* 1964: **Annual General Meeting** followed by **Thin-Layer Chromatography:** G. B. CRUMP: *Old Nags Head Hotel*, Lloyd Street, Manchester 2: 2.30 p.m.

Wednesday 29 *January* 1963: Discussion Meeting on **Assay of Penicillins:** *Society for Analytical Chemistry*: Royal Society of Medicine, 1 Wimpole Street, London W.1.

Tuesday–Thursday 14–16 *April* 1964: **Conference on Automatic Control in Chemical Processes and Allied Industries:** *Society of Chemical Industry*: Donnan Laboratories, University of Liverpool.

The object of the conference will be to review the present state of equipment and technique for automatic control in the chemical industry; to discover the gaps which prevent maximum profitability in the industry; and to indicate future lines of advance.

Provisional arrangements provide for papers to be presented under three main headings: the measuring problem as it concerns progress in process-system design and operation; the control problem as it concerns progress in process-system design and operation; economics of process control.

Those interested in obtaining further particulars should write to the General Secretary, Society of Chemical Industry, 14 Belgrave Square. London S.W.1.

British Standards Institution has announced the following *Special Issue: Handbook No.* 11*:* 1963*: Methods of test for textiles.* This third edition of the handbook reproduces a comprehensive range of tests derived from authoritative sources. It does not include tests for colour fastness because these are too numerous and are available in other publications of BSI and the Society of Dyers and Colourists.

Section One deals with humidity, conditioning, moisture content *etc.* Section Two gives methods of sampling and testing fibres. Section Three, relating to yarns, includes counting or numbering

systems, tests for physical properties and guidance on the designation of yarn structure. Section Four, dealing with fabrics, provides methods for measuring length, width, weight and thickness; for cloth analysis; and for the assessment of 'performance' properties. A group of tests for carpets is also given. Section Five gives chemical tests and describes procedures for determining ash content, alkalinity, fluidity, *etc.* Several procedures are also given for analysing fabric mixtures. The question of abrasion testing of fabrics is treated in an appendix. (*Price* £3.)

The following *New British Standards* have also been announced: *Addendum No.* 1: 1963 *to B.S.* 1016: *Part* 13: 1961 (*PD* 5035): *Methods for the analysis and testing of coal and coke. Part* 13: *Tests special to coke:* 13.11: *The micum test (using the half-micum drum).* This describes the procedure for determining the micum indices for coke using the half-micum drum. (*Price* 3*s.*)

B.S. 3670: 1963: *Methods of sieve analysis of woodflour.* This specifies apparatus and procedure for, and method of reporting results of sieve analysis of woodflour. (*Price* 3*s.*)

B.S. 3671: 1963: *Ponceau MX for use in foodstuffs.* This specifies limits for matter volatile at 135°, matter insoluble in water, di-isopropyl ether extract, subsidiary dyes, primary aromatic amine, chloride and sulphate, total dye content, copper, arsenic and lead. The dye is also required to satisfy a limit test for heavy metals (as sulphides). (*Price* 3*s.*)

The following *Revised British Standards* have also been announced:

B.S. 770: 1963: *Methods for the chemical analysis of cheese.* This describes the preparation of the sample for analysis and gives methods for the determination of moisture, fat, salt, nitrogen and hydrogen-ion concentration. (*Price 4s.6d.*)

B.S. 1741: 1963: *The chemical analysis of liquid milk and cream.* This provides methods of analysis of milk and cream for determination of total solids, fat, total nitrogen, lactose, chloride, ash and titratable acidity. (*Price 6s.*)

UNITED STATES OF AMERICA

Tuesday 7 *January* 1964: **Application of Laser Light Sources in Instrumentation:** R. T. DALY: *Society for Applied Spectroscopy, New York Section*: Hotel Manhattan, 8th Avenue and 44th Street, N.Y.: 8.00 p.m.

Tuesday–Thursday 7–9 *January* 1964: **Tenth National Symposium on Reliability and Quality Control:** Statler Hilton Hotel, Washington, D.C.

Amongst the **Winter Gordon Research Conferences,** the following may be of interest to analytical chemists. Further information may be obtained from Dr. W. GEORGE PARKS, Director of Gordon Research Conferences, University of Rhode Island, Kingston, Rhode Island.

Conference on Polymers

Monday–Friday 27–31 *January* 1964: Miramar Hotel, Santa Barbara, California.

The programme includes:

Organochemical and analytical studies of polymers. — W. KERN

Conference on Electrochemistry: Electrode Reactions

Monday–Friday 3–7 *February* 1964: Miramar Hotel, Santa Barbara, California.

The programme is as follows:

Monday, 3 *February*

Double layer and electrode kinetics. — P. DELAHAY
Cation effects on electrode kinetics. — L. GIERST
Rotated disc electrodes. — A. C. RIDDIFORD

Tuesday, 4 *February*

Anion effects on potential distribution at a germanium electrode. — P. J. BODDY
Electrochemical studies of rapid homogeneous reactions employing ac techniques. — D. SMITH

Wednesday, 5 *February*

Coulometry applied to electrode mechanism studies. — A. J. BARD
Electron exchange reactions. — H. TAUBE
Photo currents at mercury electrodes. — G. C. PARKER

Thursday, 6 *February*

Kinetics of hydrogen and deuterium discharge on platinum. — M. BREITER
Chemical reactions of solvated electrons. — L. DORFMAN

Friday, 7 *February*

Electron exchange reactions. — N. SUTIN

Wednesday–Saturday 22–25 *April* 1964: **Fourth Rare Earth Research Conference:** Camelback Inn, Phoenix, Arizona.

The meeting will be devoted to the physics, chemistry, metallurgy and ceramics of the rare earths and related elements, their compounds and alloys. Attendance is restricted to persons actively engaged in the areas of research falling within the scope of the meeting.

Further information may be obtained from the Conference Chairman, Dr. LEROY EYRING, Department of Chemistry, Arizona State University, Tempe, Arizona.

The **American Society for Testing and Materials** has announced that the following publication is now available:

Compilation of Gas Chromatographic Data, STP 343. The volume comprises nine comprehensive tables of gas chromatographic data to simplify the techniques of analysing chemicals and materials by gas chromatography. Single substances, as well as mixtures of two or more substances, can be identified with the tables in this publication. The tables are organised for rapid retrieval of data. (*Prices if prepaid: $20.00; $16.00 to ASTM members.*)

NETHERLANDS

Wednesday–Saturday 20–23 *May* 1964: Symposium on **Modern Methods of Analysis of Organic Compounds:** "*Fachgruppe Analytische Chemie*" of *Gesellschaft Deutscher Chemiker* and *Sectie voor Analytische Chemie* of *Koninklijke Nederlandse Chemishe Vereniging:* Eindhoven.

The Symposium represents a continuation of the meeting on the same subject at Munich in 1960 and covers the following items:

1. *Elemental analysis.*
2. *Constitution determination via molspectroscopy.*
3. *Separation techniques.*
4. *Analysis of high polymers.*
5. *Analysis of natural products, clinical analysis.*

The following plenary lectures (tentative titles) will be given:

Die Bedeutung der Analyse für die Organische Chemie.	J. F. ARENS
Stereochemische Analyse.	W. HÜCKEL
New Developments in Clinical Analysis.	E. J. VAN KAMPEN
Spektrochemische Verfahren zur Konstitutionsermittlung.	G. KRESZE
Derzeitiger Stand der organischen Mikroelementaranalyse.	W. SCHÖNIGER
Analytical Separation Techniques.	A. J. P. MARTIN
Gruppenreaktionen in der organischen Analyse.	S. VEIBEL
Analysis in Polymer Research.	P. W. O. WIJGA
Aminosäuren und Peptide.	F. WEYGAND

Requests to contribute a paper to the Section Meetings must be submitted not later than 31 December, 1963, on a form which can be obtained from the GDCh-Geschäftsstelle, 6000 Frankfurt (Main), Postfach 9075. Lectures can be read (15–20 min) in English, French or German.

Talanta, 1963, Vol. 10, p. iv. Pergamon Press Ltd. Printed in Northern Ireland

PAPERS RECEIVED

Studies in the relation between molecular structure and chromatographic behaviour—I: Chromatographic behaviour of some nitrophenols chromatographed on alumina-impregnated surfaces: L. S. BARK and R. J. T. GRAHAM. (30 *September* 1963)

A proposed method for the colorimetric determination of trace amounts of cyanide ion in waters: L. S. BARK and H. G. HIGSON. (30 *September* 1963)

Precipitation of zinc 8-hydroxyquinaldate from homogeneous solution: S. HIKIME and L. GORDON. (30 *September* 1963)

Determination of mercury in rocks by neutron-activation analysis: D. F. C. MORRIS and R. A. KILLICK. (1 *October* 1963)

Thermoanalytical properties of analytical-grade reagents: I—Ammonium salts—I: L. ERDEY, S. GAL and G. LIPTAY. 7 *October* 1963)

Thermoanalytical properties of analytical-grade reagents: II—Ammonium salts—II: L. ERDEY, S. GAL and G. LIPTAY. (7 *October* 1963)

A simple method of analysis of carbon in uranium and plutonium carbides: H. D. SHARMA and M. S. SUBRAMANIAN. (8 *October* 1963)

A simple separation of large quantities of 8-hydroxyquinoline from trace gallium: ALAN TOWNSHEND and LOUIS GORDON. (11 *October* 1963)

A spectrophotometric study of the complex formed between the acid chloranilate and molybdate ions in water solutions: WALTER F. LEE, NIRMAL K. SHASTRI and EDWARD S. AMIS. (11 *October* 1963)

Wet oxidation of organic compositions: Mixed nitric acid and hydrochloric acids with ammonium perchlorate as oxygen donors: G. FREDERICK SMITH. (14 *October* 1963)

Absorptiometric determination of indium with Thorin: HORACIO A. MOTTOLA. (17 *October* 1963)

Some favourable aspects of potentiometric EDTA titrations: I—Selectivity: R. C. SCHONEBAUM and E. BREEKLAND. (23 *October* 1963)

Some favourable aspects of potentiometric EDTA titrations: II—Possibility of automation: R. C. SCHONEBAUM and E. BREEKLAND. (23 *October* 1963)

5-(8-Hydroxy-5-quinolymethyl)-8-hydroxy-1-methylquinolinium: J. W. FALLER and J. P. PHILLIPS. (25 *October* 1963)

Reaction of copper$^{(II)}$ and copper$^{(I)}$ with 8-mercaptoquinoline: ALFIO CORSINI, QUINTUS FERNANDO and HENRY FREISER. (28 *October* 1963)

Magnesium-Murexide complex and its application in complexometric determinations of magnesium: B. K. HANDA. (28 *October* 1963)

Use of oxycellulose for the collection of traces of metals: II—Application of oxycellulose in solvents and in solutions of different organic components: E. SCHULEK, Zs. REMPORT-HÓRVATH and A. LÁSZTITY. (28 *October* 1963)

Coulometric titration of weak acids in non-aqueous solvents: GILLIS JOHANSSON. (28 *October* 1963)

Isotopic dilution analysis by ion-exchange: II—Substoichiometric determination of traces of indium: J. RŮŽIČKA and J. STARÝ. (16 *October* 1963)

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