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Summaries of Papers in this Issue

The Spectrophotometric Determination of Zirconium in Mild and Low Alloy Steels

The coloured complex that is formed between zirconium, Eriochrome cyanine R and polycyclic ketoamine has been studied and applied in the spectrophotometric determination of zirconium in mild steel at concentrations ranging from 0 to 0·1 per cent. with an estimated precision of ±0·003 per cent. of zirconium. By preliminary separation of zirconium with phenylarsonic acid, other elements that form complexes with Eriochrome cyanine R and polycyclic ketoamine are partially or totally removed. Provision is also made for the removal of elements that co-precipitate or adsorb zirconium in acidic solution, thus extending the method to include more complex steels. Titanium in amounts greater than 0·02 per cent. interferes. The main advantages of the method are that residual iron can be effectively masked without any loss in stability of the complex and that the molar absorptivity of the analytical complex is high.

D. M. MATHER, F. MILLAR and A. F. POLLOCK

British Steel Corporation, General Steels Division, Scottish and Shelton Group, Dalzell Works, Motherwell, Lanarkshire.

Analyst, 1971, **96**, 393–397.

An Improved Method for the Determination of Arsenic in Steel

The steel sample is dissolved in hydrochloric and nitric acids and the arsenic reduced with hypophosphorous acid. The resulting arsenic trichloride is extracted with chloroform and then re-extracted from the organic phase with water. The aqueous solution of arsenic is allowed to react with ammonium molybdate, and the molybdoarsenate is reduced to molybdenum blue. The method is applicable to high purity iron, carbon steels, low alloy and highly alloyed steels including rustless, stainless and tool steels.

W. R. NALL

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Analyst, 1971, **96**, 398–402.

Further Polarographic Studies of Metal Complexes of Mordant Red 74: The Masking of Interfering Metals in the Determination of Beryllium or Lead

Mordant red 74 in its complexes with beryllium and lead is reduced at a potential 120 mV more negative than that at which the free dye is reduced. Either beryllium or lead can be determined in the presence of nickel, chromium(III) and iron(III) when these latter metals, which also give displaced waves with the dye, are masked with EDTA. Thorium(IV) interferes by forming a precipitate with the dye even in the presence of EDTA, and molybdate interferes by distorting the dye wave.

A. G. FOGG, J. L. KUMAR and D. THORBURN BURNS

Department of Chemistry, Loughborough University of Technology, Loughborough, Leicestershire.

Analyst, 1971, **96**, 403–406.

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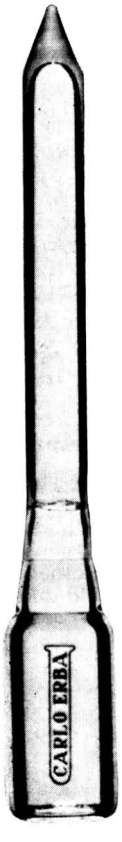
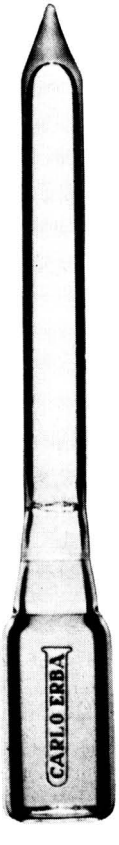


Hydrocarbons

This list includes all the normal chain paraffinic hydrocarbons from C4 to C28 and the principal isomers from C4 to C9, as well as some of the main cycloparaffinic, olefinic, diolefinic, cycloolefinic and aromatic hydrocarbons.

Fatty Acids

This list includes all the saturated fatty acids from C4 to C24, C9 pelargonic acid and the principal C16, C18, C20, C22 unsaturated acids. All these products are straight-chain compounds.



Methyl esters of Fatty Acids

This list includes the Methyl esters of the above mentioned Fatty acids, as well as the C5, C5-iso and C7.

Alcohols

This list includes the normal C4 and C5 Alcohols, together with their secondary, tertiary and branched chain isomers.

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I-20159 Milano

The Extraction and Spectrophotometric Determination of Sexavalent Uranium with Arsenazo III in Aqueous - Organic Media

The determination of uranium(VI) has been carried out by extraction - spectrophotometric methods based on the use of tributyl phosphate dissolved in isobutyl methyl ketone and trioctylphosphine oxide in benzene. Arsenazo III is used as the metallochromic reagent in a medium buffered with monochloroacetic acid - sodium monochloroacetate. The effect of many cations and anions on the procedures has been investigated, including the elimination of the important interference caused by plutonium.

The applicability of the methods evolved has been demonstrated by the comparative analysis of a series of international secondary uranium ore standards and some other low-content uranium ores that have been analysed by independent chemical methods. The trioctylphosphine oxide - benzene - arsenazo III procedure, which has been shown to be greatly superior to the other methods, permits the direct determination of uranium(VI) in the presence of plutonium when the uranium-to-plutonium ratio is greater than 0·2 per cent. The method has also been found suitable for the determination of uranium in monazitic sands, rare earth concentrates, zirconium-bearing materials and phosphoric acid solutions of the type used for the leaching of low-grade uranium ores.

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Analyst, 1971, **96**, 407–422.

Determination of Iodine and Bromine in Biological Materials by Neutron-activation Analysis

Iodine and bromine have been determined in some biological materials by neutron-activation analysis. These elements are extracted from irradiated samples with a 5 per cent. solution of trioctylamine in xylene, first the bromine being back-extracted with N sodium nitrate solution and then the iodine being back-extracted with N ammonia solution. The extraction yield is about 94 per cent. for iodine and about 86 per cent. for bromine. The limit of detection is about 0·01 μg for iodine and about 0·1 μg for bromine.

The precision of the method is about ± 6 per cent. for both elements for concentrations exceeding 0·1 p.p.m.

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Analyst, 1971, **96**, 423-426.

Loss of Cobalt and Iron from Lithium Tetraborate Fusions in Graphite Crucibles

It has been found that both iron and cobalt are lost from lithium tetraborate fusions performed at 1 200 °C in graphite crucibles. The elements segregate as tiny pieces of metal near the graphite - fusion interface owing to reduction. The same reaction could well occur with other metals of lower affinity for oxygen than carbon and so these also would disappear from the fusion.

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Analyst, 1971, **96**, 427–431.

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The Spectrophotometric Determination of Zirconium in Mild and Low Alloy Steels

By D. M. MATHER, F. MILLAR and A. F. POLLOCK

(British Steel Corporation, General Steels Division, Scottish and Shelton Group, Dalzell Works, Motherwell, Lanarkshire)

The coloured complex that is formed between zirconium, Eriochrome cyanine R and polycyclic ketoamine has been studied and applied in the spectrophotometric determination of zirconium in mild steel at concentrations ranging from 0 to 0·1 per cent. with an estimated precision of ±0·003 per cent. of zirconium. By preliminary separation of zirconium with phenylarsonic acid, other elements that form complexes with Eriochrome cyanine R and polycyclic ketoamine are partially or totally removed. Provision is also made for the removal of elements that co-precipitate or adsorb zirconium in acidic solution, thus extending the method to include more complex steels. Titanium in amounts greater than 0·02 per cent. interferes. The main advantages of the method are that residual iron can be effectively masked without any loss in stability of the complex and that the molar absorptivity of the analytical complex is high.

Probably the most popular methods published in the past for the photometric determination of small amounts of zirconium in steel have been based on the formation of the zirconium - xylenol orange complex in dilute acid.[1,2] These methods generally involve the preliminary separation of zirconium if it has to be determined in alloy steels, as at least ten elements can possibly interfere, either by forming complexes with xylenol orange or by contributing to absorbance. Rericha and Mayer[1] found that iron(III) had to be virtually completely eliminated by cathodic deposition on mercury under controlled conditions, as large amounts of residual iron, when masked by ascorbic acid, caused unstable absorbance. It is stated that molybdenum(VI) causes interference as it also forms a complex with xylenol orange, and although the authors could suppress its effect by the addition of ascorbic acid, high concentrations of molybdenum tend to retard electrolysis.[3]

As the method described by Čechová[2] for the chemical separation of zirconium with phenylarsonic acid appeared to be more suitable than electrolytic separation for batch analysis, it was decided to adopt this separation procedure and apply a new spectrophotometric finish. Hill[4] developed a method involving the use of Eriochrome cyanine R with polycyclic ketoamine for the determination of aluminium in steel and listed zirconium as one of the interfering elements. Work in this laboratory showed that the complex formed between zirconium and these reagents has a much higher molar absorptivity than the zirconium - xylenol orange complex, and it is stable enough to allow residual iron to be effectively masked by thioglycollic acid in the presence of sodium sulphite.

Experimental

Reagents—

Standard zirconium(IV) solution—Fuse 0·1351 g of Specpure zirconium dioxide with 2 g of AnalaR potassium hydrogen sulphate in a platinum crucible until a clear melt is obtained. Extract the melt into 4 M hydrochloric acid and dilute to 1 litre with the same acid.

Titanium(IV) solution—Fuse 0·0834 g of Specpure titanium dioxide with 2 g of AnalaR potassium hydrogen sulphate in a platinum crucible until a clear melt is obtained. Extract the melt into 4 M hydrochloric acid and dilute to 100 ml with the same acid.

Aluminium(III) solution—Dissolve 0·05 g of aluminium foil in 4 M hydrochloric acid and dilute to 100 ml with the same acid.

Vanadium(V) solution—Dissolve 0·1149 g of ammonium vanadate in 4 M hydrochloric acid and dilute to 100 ml with the same acid.

Thioglycollic acid, 2 per cent. v/v—Dilute 20 ml of thioglycollic acid to 1 litre with water.

Eriochrome cyanine R, 0·13 per cent. w/v—Dissolve 0·13 g of Eriochrome cyanine R (Merck) in cold boiled-out water and make up the volume to 100 ml with the same water. This solution should be freshly prepared.

Buffer solution—Mix 1 g of AnalaR anhydrous sodium sulphite, 0·75 g of polycyclic ketoamine (Amchem Products Inc., Ambler, Pa., U.S.A.) and 25·5 g of AnalaR ammonium acetate in a small beaker, dissolve the mixture in water, and make up the volume to 100 ml with water. Adjust the pH of this solution to 7·4 by adding sufficient ammonia solution (sp.gr. 0·88) or glacial acetic acid. This solution should be freshly prepared.

Mineral acids—AnalaR grade concentrated acids were used throughout and diluted as necessary.

APPARATUS—

A Beckmann D.B. spectrophotometer was used for all absorbance measurements.

DEVELOPMENT OF PROCEDURE—

Rericha and Mayer[1] found that the minimum concentration of sulphuric acid required to prevent the hydrolysis of zirconium(IV) was 0·1 M. It was therefore decided that the zirconium(IV) should be present in at least 0·1 M sulphuric acid prior to the formation of the analytical complex (*i.e.*, before the addition of the buffer solution). As the method described by Čechová[2] provided for the separation of zirconium and its subsequent fusion as zirconium dioxide, the above conditions were achieved by extracting the melt into the requisite amount of sulphuric acid to give an acid concentration of 0·9 M in the parent solution.

The optimum conditions for the formation of the analytical complex were found by transferring 2-ml aliquots of a parent solution containing 1×10^{-5} g ml^{-1} of zirconium to 50-ml calibrated flasks containing 4 ml of 2 per cent. v/v thioglycollic acid, adding a constant excess of Eriochrome cyanine R to the solutions, then buffering each in turn with buffers of constant polycyclic ketoamine and sodium sulphite composition, but with different ammonium acetate concentrations and pH values. After the most suitable ionic environment and the optimum pH value for formation of the complex had been established, the effects of various concentrations of Eriochrome cyanine R and polycyclic ketoamine were studied. These findings are reported under Results and Discussion.

PROCEDURE—

Transfer a 1-g sample (Note 1) to a 400-ml squat beaker, add 45 ml of 4 M hydrochloric acid, cover the beaker and heat it gently until the sample is dissolved. Oxidise, by dropwise additions of nitric acid (sp.gr. 1·42), and evaporate the solution to dryness; heat the residue at about 200 °C for 10 minutes. Cool, add 30 ml of 6 M hydrochloric acid, heat to dissolve the soluble salts, and adjust the volume to 50 ml with cold water. Filter the solution on a paper pad and collect the filtrate in a 250-ml squat beaker. Wash the filter with hot 0·2 M hydrochloric acid until it is free of iron and reserve the filtrate and washings. Transfer the filter to a small platinum dish, and then dry, char and finally ignite it at as low a temperature as possible until the residue is carbon-free. Allow to cool (Note 2), add 0·5 ml of 6 M sulphuric acid and 5 ml of hydrofluoric acid (40 per cent. w/w), and evaporate until all the fumes of sulphur trioxide have been expelled. Fuse the remaining residue with 1 g of potassium hydrogen sulphate, and transfer the melt to the reserved filtrate with a minimum of hot 0·2 M hydrochloric acid.

Evaporate the filtrate to 100 ml, add 1 g of phenylarsonic acid dissolved in 20 ml of hot 0·2 M hydrochloric acid, boil for 5 minutes, and allow the solution to stand overnight at room temperature (Note 3). Filter on a paper pad and wash the residue thoroughly with a hot solution containing 1 g of phenylarsonic acid in 1 litre of 0·1 M hydrochloric acid until the filtrate runs clear. Transfer the filter to a silica crucible and dry, char and ignite it under a hood at as low a temperature as possible until the residue is carbon-free. Fuse the impure zirconium dioxide with 1 g of potassium hydrogen sulphate until a clear melt is obtained (Note 4). Allow the crucible to cool, add exactly 10 ml of 4·5 M sulphuric acid, heat gently until the melt has dissolved, and transfer the extract to a 50-ml calibrated flask with hot water. Cool the solution to room temperature, dilute to the mark with cold water and mix well.

Transfer a 2-ml aliquot to a 50-ml calibrated flask containing 4 ml of 2 per cent. v/v thioglycollic acid, add 5 ml of 0·13 per cent. w/v Eriochrome cyanine R solution, rinse down the neck of the flask with a minimum amount of cold water, mix and allow the solution to stand for 5 minutes. Add 5 ml of buffer solution, immediately adjust to the mark with cold water, mix and allow to stand for 15 minutes. Measure the absorbance at 590 nm in a cell of suitable size *versus* a blank prepared by carrying 1 g of Specpure sponge iron (Johnson Matthey) through the procedure.

NOTES—

1. For high zirconium steels take a proportionately smaller sample weight.
2. If niobium, tantalum or tungsten is present, fuse the residue with 2 g of anhydrous sodium carbonate at 950 °C for 10 minutes, cool, extract with boiling water, filter on a paper pad and wash well with hot water. Discard the filtrate. Transfer the filter to the original platinum dish, and dry, char and ignite it at as low a temperature as possible until the residue is carbon-free. Fuse the residue with 1 g of potassium hydrogen sulphate and transfer the melt to the main filtrate with a minimum of hot water. Continue as under Procedure from "Evaporate the filtrate to 100 ml. . . ."
3. Alternatively, complete the precipitation by allowing the solution to stand for 2 to 3 hours at 80 °C.
4. If more than 0·02 per cent. of titanium is present, extract the melt with 30 ml of 5 M hydrochloric acid, adjust the volume to 100 ml with water and continue as under Procedure from "Evaporate the filtrate to 100 ml. . . ."

CALIBRATION WITH SYNTHETIC STANDARDS—

To six 250-ml squat beakers containing 1-g samples of sponge iron, add zirconium(IV) solution and 4 M hydrochloric acid solution according to Table I to maintain constant acidity, and then heat the mixture gently until the iron has dissolved. Oxidise it with a minimum of nitric acid (sp.gr. 1·42), boil the solution until all the nitrous fumes have been expelled, adjust the volume to 100 ml with water and continue as under Procedure from "Evaporate the filtrate to 100 ml. . . ."

TABLE I

PREPARATION OF SYNTHETIC STANDARDS

Zirconium(IV) solution*/ml	4 M Hydrochloric acid/ml	Zirconium in a 1-g steel sample, equivalent per cent.	Observed absorbance in a 1-cm cell at 590 nm
0	45·0	0	0
2·0	43·0	0·02	0·105
4·0	41·0	0·04	0·215
6·0	39·0	0·06	0·330
8·0	37·0	0·08	0·440
10·0	35·0	0·10	0·545

* 1 ml of zirconium standard solution contains 10^{-4} g of Zr.

RESULTS AND DISCUSSION

EFFECT OF pH—

Between pH 4·6 and 5·4 the colour formed did not vary a great deal, but at high pH values the absorptivity decreased rapidly. Tests showed that between pH 5·0 and 5·2 constant absorptivity was maintained.

EFFECT OF ERIOCHROME CYANINE R CONCENTRATION—

A molar ratio plot of zirconium(IV) to Eriochrome cyanine R in the presence of an excess of polycyclic ketoamine showed that the analytical complex was completely formed when a 33-fold excess of the dyestuff was present.

EFFECT OF POLYCYCLIC KETOAMINE CONCENTRATION—

Maximum enhancement of the Eriochrome cyanine R complex was obtained for the highest concentration of zirconium on the calibration graph when a minimum of 0·6 g of polycyclic ketoamine per 100 ml of buffer solution was present.

ABSORPTIVITY—

The zirconium complex had an absorbance spectrum that was virtually the same as that reported by Hill[4] for the aluminium complex, with a $\lambda_{max.}$ value between 586 and 592 nm. At 590 nm the molar absorptivity of the zirconium complex was approximately $6{\cdot}0 \times 10^4$.

STABILITY—

Colour development was complete after allowing the solution to stand for 15 minutes at room temperature. After the complex had been formed it remained stable for at least 2 hours.

ACIDITY—

Sulphuric acid (0·9 M) was used for the parent solutions to reduce hydrolysis and polymerisation of zirconium(IV) before the final colour development. Calibration graphs constructed from the same parent solutions before and after 2 months were identical and obeyed the Beer - Lambert law.

INTERFERENCE EFFECTS—

The efficiency of the separation of zirconium with phenylarsonic acid from the other common elements that form complexes with Eriochrome cyanine R and polycyclic ketoamine, namely aluminium, titanium and vanadium, was studied by preparing synthetic standards and carrying them through the procedure. The composition of these standards and the results obtained are shown in Table II.

TABLE II

EFFECTS OF POSSIBLE INTERFERING ELEMENTS AFTER ONE SEPARATION WITH PHENYLARSONIC ACID

Zirconium taken, per cent.	Aluminium present, per cent.	Titanium present, per cent.	Vanadium present, per cent.	Zirconium found, per cent.	Deviation, per cent.
0·06	0·50	—	—	0·059	−0·001
0·06	0·50	—	—	0·060	0
0·06	0·50	—	—	0·060	0
0·06	—	0·05	—	0·063	+0·003
0·06	—	0·05	—	0·062	+0·002
0·06	—	0·05	—	0·062	+0·002
0·06	—	0·50	—	0·095	+0·035
0·06	—	0·50	—	0·094	+0·034
0·06	—	0·50	—	0·097	+0·037
0·06	—	—	0·50	0·060	0
0·06	—	—	0·50	0·061	+0·001
0·06	—	—	0·50	0·060	0

It can be seen from Table II that aluminium and vanadium at concentrations up to 0·5 per cent. can be completely removed by a single separation. Titanium on the other hand tends to co-precipitate to an extent of about 25 per cent. of the total content. This effect somewhat limits the applicability of the method, but it can be partially overcome by repeated separations with phenylarsonic acid as shown in Table III.

TABLE III

REMOVAL OF TITANIUM BY REPEATED SEPARATIONS WITH PHENYLARSONIC ACID

Zirconium taken, per cent.	Titanium present, per cent.	Zirconium, per cent., present after		
		1st separation	2nd separation	3rd separation
0·06	0·50	0·095	0·069	0·060
0·06	0·50	0·094	0·069	0·060
0·06	0·50	0·097	0·070	0·061
0·06	0·10	0·069	0·061	—
0·06	0·10	0·068	0·060	—
0·06	0·10	0·070	0·060	—

If silicon, tungsten, niobium and tantalum are not removed, low results are obtained, as the oxides of these elements readily precipitate or adsorb zirconium in acidic solution. As it was exceptionally difficult to prepare synthetic standards containing these elements in solution, the efficiency of their removal (by the method described in Note 2 of Procedure) was examined by analysing a series of American steel standards containing them in various proportions. The results obtained are shown in Table IV.

TABLE IV

DETERMINATION OF ZIRCONIUM IN N.B.S. STANDARD STEEL SAMPLES

Standard No.	Interfering elements, per cent.	Number of phenylarsonic acid separations	Zirconium certificate value, per cent.	Zirconium by proposed method, per cent.
N.B.S. 1162	Al 0·020, Ti 0·037 Nb 0·096, W 0·053, V 0·058	1	0·063	0·058, 0·060, 0·061
N.B.S. 1163	Al 0·027, Ti 0·010 Nb 0·195, W 0·105, V 0·010	1	0·20	0·200, 0·196, 0·198
N.B.S. 1164	Al 0·005, Ti 0·004 Nb 0·037, W 0·022, V 0·295	1	0·010	0·009, 0·010, 0·010
N.B.S. 1167	Al 0·16, Ti 0·26 Nb 0·29, W 0·20, V 0·041	2	0·094	0·097, 0·096, 0·096
		3	0·094	0·094, 0·092, 0·093

DETERMINATION OF ZIRCONIUM IN MILD STEEL SAMPLES—

Unfortunately, no mild or plain carbon steel standards with certified zirconium contents are available in the B.C.S. range so that the accuracy of the method was tested by analysing a series of mild steels produced in a medium frequency furnace and comparing the values obtained by the proposed method with those obtained by X-ray fluorescence on N.B.S. standards to calibrate (1162–1167). The results obtained are listed in Table V.

TABLE V

COMPARISON OF THE RESULTS OBTAINED BY THE PROPOSED METHOD WITH THOSE OBTAINED BY X-RAY FLUORESCENCE

	Zirconium, per cent., by	
Sample	X-ray fluorescence	Proposed method
MF.1656	0·026	0·025, 0·024, 0·025
MF.1657	0·048	0·046, 0·044, 0·046
MF.1658	0·069	0·069, 0·069, 0·067
MF.1659	0·078	0·076, 0·079, 0·078
MF.1660	0·091	0·094, 0·092, 0·092
MF.1661	0·112	0·110, 0·112, 0·112
MF.1662	0·001	0·002, 0·003, 0·003

CONCLUSION

The proposed method is suitable for the determination of zirconium in plain and low alloy steels with zirconium contents between 0·001 and 0·1 per cent., although this range can be extended by taking a proportionately smaller sample weight. The repeatability of the method is excellent and results compare favourably with those obtained by X-ray fluorescence, as shown in Table V. As the gravimetric determination of zirconium in steel with mandelic acid is suitable only for contents exceeding 0·03 per cent.,[5] the present method provides a useful service in enabling low zirconium contents to be accurately determined. The only serious limitation to the method is the fact that titanium present in amounts greater than 0·02 per cent. causes interference. The method is not rapid but a batch of fifteen determinations can be completed by one analyst in 1½ days.

The authors thank Dr. J. M. Ottaway, Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, for his guidance, and Mr. J. Little, now of British Steel Corporation, Strip Steels Division, Ravenscraig Works, Motherwell, Lanarkshire, and Mr. W. Grierson of this laboratory for their support of this work.

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An Improved Method for the Determination of Arsenic in Steel

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The steel sample is dissolved in hydrochloric and nitric acids and the arsenic reduced with hypophosphorous acid. The resulting arsenic trichloride is extracted with chloroform and then re-extracted from the organic phase with water. The aqueous solution of arsenic is allowed to react with ammonium molybdate, and the molybdoarsenate is reduced to molybdenum blue. The method is applicable to high purity iron, carbon steels, low alloy and highly alloyed steels including rustless, stainless and tool steels.

MANY existing methods for determining arsenic are based either on reduction to elemental arsenic and subsequent iodimetric titration or distillation of arsenic trichloride to eliminate interfering elements.

The British Standard method for determining arsenic in steel[1] involves refluxing to ensure complete reduction of arsenic, followed by filtration at an elevated temperature to minimise loss of element. Special pre-treatment of the filtering media with an oxidising agent is required to reduce errors. The titration of the separated arsenic involves the use of 0·01 N solutions of iodine and sodium arsenite, both of which require to be carefully standardised, and in the final titration 1 ml of titrant is equivalent to 0·003 per cent. of arsenic on a 5-g sample.

In distillation procedures for determining arsenic, *e.g.*, in lead alloys,[2] constant supervision is required to prevent losses caused by erratic distillation and the procedure is time consuming. Submicrogram amounts of arsenic have been determined with silver diethyldithiocarbamate[3] by a method involving evolution of arsine and its absorption in pyridine. In this procedure the sample is required to dissolve readily in the solvent if a direct method is to be used or the oxidised arsenic is reduced and arsine evolved together with the hydrogen by adding metallic zinc to the sample solution. A special apparatus for the evolution is used and the amount of arsenic determined is given in the parts per million range. Arsenic has been determined also at trace levels[4] by extraction of the yellow molybdoarsenic acid with butanol and its subsequent reduction to the blue complex. Because phosphorus interfered a double extraction with isopentyl alcohol was required when the method was applied to the determination of arsenic in steel and the reduction to molybdenum blue was performed in the organic phase. In another method, based on chloroform extraction of arsenic as the chloride,[5] interference from phosphorus required only a single acid wash for complete removal and the reduction to molybdenum blue was carried out in aqueous solution under controlled temperature conditions in a water-bath.

The last method[5] appeared to offer the most advantageous procedure for accurate determinations on a routine basis, but it had been applied only to copper and copper-base alloys. The authors had indicated, however, that from the evidence of limited tests the method had a potential application to the analysis of ferrous alloys. This has been confirmed and the new method proposed gives greatly increased sensitivity (about 60 times on a weight basis) compared with the British Standard method. It is less time consuming and requires a smaller sample weight; no distillation, refluxing or filtration is required and the steps during which adventitious oxidation of arsenic can occur are eliminated.

Experimental

The method is based on the initial dissolution of the sample in an oxidising acid mixture to prevent loss of arsenic as arsine. Subsequent reduction of the quinquivalent arsenic by hypophosphorous acid has been shown to be dependent on the presence of an optimum concentration of copper ions in the solution,[5] and it was necessary to confirm that this condition applied also in the analysis of ferrous alloys. The use of copper(I) chloride dissolved in concentrated hydrochloric acid suggested itself as the most convenient way of adding the copper. The copper salt was readily soluble in concentrated hydrochloric acid, in which medium the subsequent reduction of the arsenic had to be made. The copper was then in the lower valency state and did not require further reduction. The effect of increasing copper concentration was investigated by making additions of a copper solution containing 15 g of copper(I) chloride per 100 ml of hydrochloric acid (sp.gr. 1·16) to 0·25-g portions of British Chemical Standard steel No. 218/3 containing 0·035 per cent. of arsenic and producing the molybdenum-blue colour by the method described later.

The results are shown in Table I.

Table I

Effect of increasing copper concentration on the efficiency of reduction of arsenic

Copper solution/ml	0·0	2·0	5·0	7·0	10·0
Optical density (2-cm cell) ..	0·55	0·63	0·70	0·75	0·75

From these results it can be seen that the addition of 10 ml of the copper(I) chloride solution (equivalent to about 1 g of copper) gives the maximum optical density in the final solution corresponding to the most complete reduction and extraction of arsenic under the conditions of the method.

Fig. 1 shows the transmission curve of the molybdenum-blue solution prepared from from a standard steel (B.C.S. 218/3). Maximum absorption occurs at 840 nm, which is the recommended wavelength for the determination.

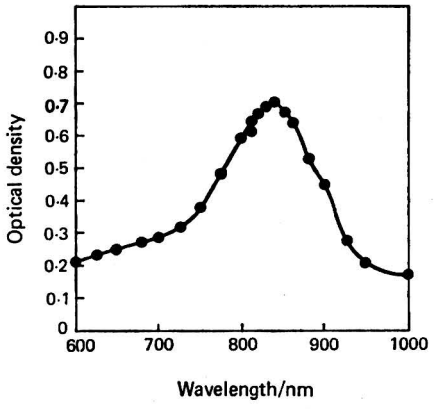

Fig. 1. Absorption curve for arsenic

Effect of other elements—

The elements present in steel that may form heteropoly-acids with molybdic acid are boron, silicon, titanium, zirconium, phosphorus, vanadium, arsenic and manganese. Under the conditions of the test those most likely to be extracted and subsequently reduced to molybdenum blue are silicon and phosphorus. Any possible interference from phosphorus is eliminated in the procedure by washing the chloroform extract with concentrated hydrochloric acid, and the results shown below (Table IV, see p. 400) for a wide variety of steels with various phosphorus contents confirm that no interference is caused by this element.

To investigate the effect of silicon on the arsenic determination, a standard solution of the element was prepared from high-purity silicon dioxide following fusion with sodium

carbonate in a platinum crucible; 1 ml of this solution contained 0·000 2 g of silicon, equivalent to 0·08 per cent. on the recommended sample weight.

B.C.S. 219/3, a low alloy steel containing 0·20 per cent. of silicon and about 0·032 per cent. of arsenic, was chosen for the silicon investigation and additions of standard silicon solution were made to separate 0·25-g portions of the alloy. The arsenic determination was then completed as described in the method. The results are shown below.

Added silicon, per cent. ..	Nil	0·4	1·2	2·0
Arsenic found, per cent. ..	0·033	0·030	0·030	0·027

British Chemical Standard alloy steels containing vanadium, titanium and manganese are not issued with certified figures for arsenic. The effect of these elements was examined by first determining the arsenic content of the standards and then making an addition of 0·030 per cent. of arsenic and re-determining the total content. In this way it was established that no interference was caused by these alloying elements, as shown in Table II.

TABLE II

EFFECT OF TITANIUM, VANADIUM AND MANGANESE ON THE MOLYBDENUM-BLUE COLOUR

B.C.S. No.		Arsenic in standard (by this method), per cent.	Arsenic added, per cent.	Arsenic recovered, per cent.
211/1	13% Cr (rustless)	0·023	0·030	0·028
220/2	7% W, 5% Mo, 2% V, 5% Cr, 0·3% Mn	0·020	0·030	0·030
235/2	18% Cr, 9% Ni, 0·3% Ti, 0·9% Mn	0·020	0·030	0·026
261/1	17% Cr, 13% Ni, 0·9% Nb, 0·8% Mn	0·015	0·030	0·028

STABILITY OF THE MOLYBDENUM-BLUE COLOUR—

Samples of six British Standard steels were treated by the proposed method and the optical density of the molybdenum-blue colour produced was measured at intervals up to 24 hours.

The results given in Table III showed that the density of the molybdenum-blue colour remained stable for at least 1 hour and only faded very slowly thereafter.

TABLE III

STABILITY OF THE MOLYBDENUM-BLUE COLOUR

British Chemical Standard	Optical density				
	Immediate	After 30 minutes	After 1 hour	After 2 hours	After 24 hours
212/1	0·505	0·505	0·50	0·49	0·485
220/1	0·775	0·775	0·775	0·76	0·76
224/1	0·78	0·78	0·78	0·77	0·77
322	0·32	0·32	0·32	0·305	0·305
295	0·60	0·60	0·60	0·59	0·58
320	0·785	0·785	0·775	0·775	0·76

TABLE IV

RESULTS ON STANDARD STEELS

Sample British Chemical Standard	Arsenic content	
	By proposed procedure, per cent.	Certified value, per cent.
212/1 (Leaded steel)	0·019	0·02
218/3 (Carbon steel)..	0·032	0·035
220/1 (High speed steel)	0·029	0·030
224/1 (Cr - V steel)	0·029	0·03
239/3 (Carbon steel)	0·029	0·032
295 (Carbon steel)	0·0235	0·024
320 (Mild steel)	0·029	0·031
321 (Mild steel)	0·002	0·003
322 (Mild steel)	0·0115	0·012
325 (Mild steel)	0·012	0·013

APPLICATION OF THE PROCEDURE—

Several British Standard steels were examined by the proposed procedure and good agreement with the standard figures was obtained. These results are shown in Table IV.

METHOD

REAGENTS—

Standard arsenic solution—Dissolve 0·132 g of arsenic trioxide, previously dried at 105 °C, in 5 ml of warm 5 per cent. w/v sodium hydroxide solution. Dilute the solution to 100 ml and acidify it with dilute sulphuric acid (1 + 1) until an acidic reaction is obtained with litmus paper. Dilute the solution to 1 litre. Dilute a 25-ml aliquot to 100 ml.

1 ml of solution ≡ 25 μg (0·025 mg) of arsenic.

Solvent acid—Add 200 ml of hydrochloric acid (sp.gr. 1·18) to 100 ml of nitric acid (sp.gr. 1·42).

Copper reagent solution—Dissolve 15 g of copper(I) chloride in 100 ml of hydrochloric acid (sp.gr. 1·18).

Ammonium molybdate solution, 1 per cent. w/v—Add, slowly, 14 ml of sulphuric acid (sp.gr. 1·84) to 60 ml of water and then dissolve 1 g of ammonium molybdate tetrahydrate in the warm solution. Cool and dilute to 100 ml. This reagent must be freshly prepared.

Hydrazinium sulphate solution, 0·15 per cent.—Dissolve 0·15 g of hydrazinium sulphate in water and dilute to 100 ml.

PREPARATION OF CALIBRATION GRAPH—

Place separately 1·0, 2·0, 3·0 and 4·0 ml of the standard arsenic solution (1 ml of solution is equivalent to 0·025 mg of arsenic) in each of four 125-ml conical beakers containing 0·25 g of high-purity iron; use a fifth beaker containing 0·25 g of high-purity iron for a blank determination. Continue with each beaker as described below.

Add 10 ml of solvent acid, cover the beakers with a cover-glass and allow the reaction to proceed, warming on the hot-plate as required. When the metal is dissolved, evaporate the solution carefully to dryness, avoiding undue heating. To the residue add 40 ml of hydrochloric acid (sp.gr. 1·18) to dissolve soluble salts, warming slightly if necessary, then cool, add 10 ml of copper reagent solution, adjust the temperature to about 20 °C and add 3 ml of 50 per cent. v/v hypophosphorous acid. Set aside the solution for 5 minutes.

Transfer the solution to a dry separating funnel with 10 ml of hydrochloric acid (sp.gr. 1·18), add 25 ml of chloroform and shake the mixture vigorously for 1 minute. Allow the two layers to separate and run the lower (chloroform) layer into a dry separating funnel, discarding the aqueous layer. Shake the chloroform extract with 10 ml of hydrochloric acid (sp.gr. 1·18) for 30 seconds. Allow the two layers to separate and run the lower layer into a dry separating funnel; discard the aqueous layer. Add 20 ml of water and shake the mixture vigorously for 1 minute; allow the two layers to separate, discard the lower layer and transfer the aqueous layer to a 125-ml conical beaker, washing the funnel with about 5 ml of water.

Add the reagents listed below in the order stated and mix well after each addition: 5 drops of 0·1 N iodine solution; 5·0 ml of 1 per cent. w/v ammonium molybdate solution; and 2·0 ml of freshly prepared 0·15 per cent. w/v hydrazinium sulphate solution.

Stand the beaker in a boiling water bath for 10 minutes and then cool the solution to 20 °C. Dilute the solution to 50 ml in a graduated flask.

Measure the optical density at 840 nm in 2-cm cells.

PROCEDURE—

Dissolve 0·25 g of sample in 10 ml of solvent acid and continue as described under "Preparation of Calibration Graph."

CONCLUSION

The proposed method is suitable for determining arsenic in the range 0·001 to 0·05 per cent. in most types of steel. The method allows up to twelve determinations to be completed by one analyst in a normal working day. No distillation, refluxing or filtration is involved.

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Further Polarographic Studies of Metal Complexes of Mordant Red 74: The Masking of Interfering Metals in the Determination of Beryllium or Lead

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Mordant red 74 in its complexes with beryllium and lead is reduced at a potential 120 mV more negative than that at which the free dye is reduced. Either beryllium or lead can be determined in the presence of nickel, chromium(III) and iron(III) when these latter metals, which also give displaced waves with the dye, are masked with EDTA. Thorium(IV) interferes by forming a precipitate with the dye even in the presence of EDTA, and molybdate interferes by distorting the dye wave.

A RECENT paper[1] described the basis of a method for the polarographic determination of beryllium that involves the use of the displaced ligand wave obtained with the *o*-hydroxy-*o'*-carboxyazo dye Mordant red 74 (C.I. 16315) in the presence of beryllium ions. A 10-fold excess of dye over beryllium was recommended to prevent hydrolysis of the complex. The aluminium complex of the dye is reduced at the same potential as the free dye, and an amount of aluminium five times that of beryllium could be tolerated in the determination of beryllium.

Lead(II), chromium(III), iron(III), nickel(II) and uranium(VI) also give displaced ligand waves with Mordant red 74. With the exception of lead(II), all of these metals have been shown previously to give displaced waves with *o,o'*-dihydroxyazo dyes.[2] To our knowledge this is the first report of a displaced ligand wave with lead.

The present paper describes a further investigation of the polarographic behaviour of complexes of Mordant red 74, and includes a study of the effect of interferences on the determination of beryllium or lead with Mordant red 74.

EXPERIMENTAL

The experimental techniques and conditions used were essentially those described previously; a pure sample of dye was used.[1] Polarograms were obtained for methanol - water (1 + 1 v/v) solutions that had been heated to 60 °C for 5 minutes to ensure complete complex formation, and then cooled. The most suitable apparent pH at which to buffer the solutions for polarography was determined for each metal by studying the complex formation reaction with Mordant red 74 potentiometrically; acetate buffers were used in the polarographic solutions.

Polarograms were obtained with a Cambridge Pen-recording polarograph and a Southern-Harwell, Mark II, pulse polarograph. The half-wave potential of Mordant red 74 under these conditions at a pH meter reading of 5·5 was −0·39 V *versus* S.C.E.

LEAD COMPLEX—

Potentiometric titrations of Mordant red 74 with sodium hydroxide solution both in the absence and in the presence of lead ions indicated that a 1:1 Mordant red 74 - lead complex is formed at pH 5·2 at the concentrations used for polarography. At pH values greater than 8·0 a 2:1 Mordant red 74 - lead complex is formed. A linear calibration graph for lead was obtained from polarograms with solutions buffered at a pH meter reading of 5·2 when the height of the displaced ligand wave ($\Delta E_{\frac{1}{4}} = 120$ mV) was plotted against the concentration of lead. The height of the displaced wave reached a constant maximum value at lead concentrations equivalent to that required to form a 1:1 complex. The limiting current was shown to be diffusion controlled by a study of the effect of the height of the

mercury reservoir. Typical polarograms obtained with the Cambridge polarograph showing the displaced ligand wave with lead are given in Fig. 1.

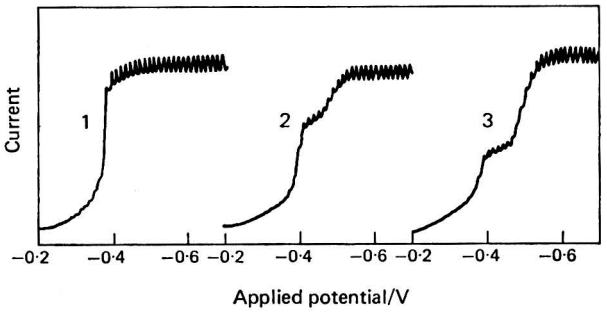

Fig. 1. Typical polarograms (Cambridge polarograph) showing the displaced ligand wave with lead. Lead concentration: 1, zero; 2, 4×10^{-4} M; and 3, 8×10^{-4} M. Mordant red 74 concentration 10^{-3} M

NICKEL COMPLEX—

Nickel ion was shown potentiometrically to form a 1:1 complex with Mordant red 74 at pH 5, and a linear calibration graph for nickel was obtained by using the height of the displaced polarographic wave ($\Delta E_{\frac{1}{2}} = 120$ mV). The displaced wave reached a constant maximum height at a nickel concentration slightly lower than the equivalent of that required for the formation of a 1:1 complex with Mordant red 74.

IRON(III) COMPLEX—

Potentiometric titrations indicated the formation of a 2:1 Mordant red 74 - iron(III) complex at pH 5·5. On polarographing solutions containing the complex at this pH value, two post-waves were obtained: the first of these is a displaced ligand wave ($\Delta E_{\frac{1}{2}} = 210$ mV), but the second wave (apparent $\Delta E_{\frac{1}{2}} = 320$ mV) was ill-defined and its origin is unknown. The calibration graph obtained for the determination of iron(III), by using the height of the displaced wave, was not linear, and precipitation of the complex occurred at iron(III) concentrations well below stoicheiometric.

URANIUM(VI) COMPLEX—

A 1:1 Mordant red 74 - uranium(VI) complex was shown by potentiometric titration to be formed at pH 5·5. The polarographic behaviour of this complex was unusual in that for solutions that were 10^{-3} M with respect to Mordant red 74 no displaced wave was observed at uranium(VI) concentrations lower than 3×10^{-4} M. Above this concentration of uranium(VI) a small displaced wave was observed ($\Delta E_{\frac{1}{2}} = 120$ mV), as shown in Fig. 2, but its height was constant and was independent of the uranium concentration. The reason for this has so far not been determined.

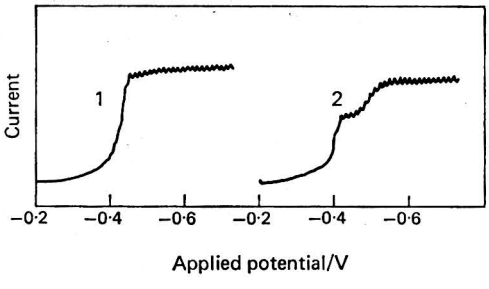

Fig. 2. The displaced ligand wave with uranium(VI). Uranium(VI) concentration: 1, 2×10^{-4} M; and 2, 4×10^{-4} M. Mordant red 74 concentration 10^{-3} M

CHROMIUM(III) COMPLEX—

Potentiometric titration of a solution of Mordant red 74 and chromium(III) nitrate with sodium hydroxide solution gave an uncertain result because of the slow rate of reaction of chromium(III) with the dye at room temperature. When the solution was heated to 65 °C and then cooled before the titration, the formation of a 2:1 Mordant red 74 - chromium(III) complex was clearly indicated at pH values as low as 3·5. The formation of the 2:1 complex at pH 5·2 was confirmed polarographically; the height of the displaced wave reached a constant value at the chromium(III) concentration equivalent to that required for the formation of a 2:1 complex.

INTERFERENCES IN THE DETERMINATION OF BERYLLIUM—

All metal ions that form complexes with Mordant red 74, even if they give no displaced wave, will interfere if present in sufficiently high concentration. The present study of interferences in the determination of beryllium was made under the conditions given in the procedure described previously,[1] *viz.*, for beryllium concentrations of up to 10^{-4} M and a dye concentration of 10^{-3} M.

No interference was observed from the following metals when present in an amount five times that of beryllium: Al, Zn, Co(II), Cu(II), Mn(II), Ca, Mg, Sr, V(V), Ba, Ce(III), La(III), Li, W(VI), Ag(I), Hg(I), Hg(II), Ti(IV), Zr(IV) and Sn(IV).

Lead(II), chromium(III), iron(III) and nickel(II), which give displaced waves with the dye, interfere when present in amounts similar to that of the beryllium. Uranium(VI) gives a displaced wave at concentrations above 3×10^{-4} M, and therefore interferes only when present in an amount at least three times that of beryllium. Cadmium and thorium interfere by forming a precipitate with the dye. Molybdenum(VI) at 10^{-4} M concentration interferes by distorting the polarographic waves.

The main interferences, therefore, are lead(II), chromium(III), iron(III), nickel(II), cadmium and thorium. The effectiveness of masking with EDTA was investigated. A 10 per cent. w/v solution of EDTA (1 ml) was added to the solutions to be polarographed before the addition of Mordant red 74. Under these conditions no displaced waves were obtained with 10^{-3} M solutions of chromium(III), iron(III) and nickel(II). Hence, amounts of these metals at least ten times that of beryllium could be tolerated provided that they were masked with EDTA. Cadmium (10^{-3} M) was also effectively masked by EDTA, but 10^{-4} M thorium and 10^{-4} M molybdenum(VI) were not.

Significant interference is restricted, therefore, to lead, thorium and molybdenum(VI).

DETERMINATION OF LEAD—

Of the displaced waves obtained with Mordant red 74, that with lead is of particular interest, as to our knowledge no displaced wave with this metal has been reported previously. Analytically, in contrast to the determination of beryllium, there is usually no difficulty in determining lead polarographically, as the hydrated lead ion gives a well defined wave at a convenient potential. Nevertheless, the displaced wave may be of value in certain systems in which another reduction process occurs at the same potential as that for the hydrated lead ion. In particular, lead can be determined with Mordant red 74 at pH 5·5 in the presence of tin(IV), which interferes in the absence of the dye unless an alkaline supporting electrolyte is used.[3]

The experimental conditions for the determination of lead are the same as those for the determination of beryllium.[1] The calibration graph for lead is linear, providing that a slight excess of Mordant red 74 is present. This is in contrast to the determination of beryllium for which a tenfold excess of dye over beryllium is necessary to ensure that a linear calibration graph is obtained. Nevertheless, the use of a 10-fold excess of the dye in determining lead may be considered advisable so that larger amounts of other metal ions can be tolerated. The use of only a slight excess of dye, in the absence of interfering metal ions, has the advantage that the free dye and complex waves are resolved more completely.

The interferences are similar to those for the determination or beryllium. If EDTA is used as a masking agent the only significant interferences are beryllium, thorium and molybdenum(VI).

Conclusions

The addition of EDTA to the supporting electrolyte used in the polarographic determination of beryllium with Mordant red 74 masks several interfering metal ions, and increases the selectivity of the procedure.

Mordant red 74 can also be used to determine lead. Lead is not normally difficult to determine polarographically by means of its own reduction wave but, in instances when this reduction wave is obscured by the reduction of another species present in the solution, the use of the displaced ligand wave with Mordant red 74 may prove advantageous.

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The Extraction and Spectrophotometric Determination of Sexavalent Uranium with Arsenazo III in Aqueous - Organic Media*

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The determination of uranium(VI) has been carried out by extraction - spectrophotometric methods based on the use of tributyl phosphate dissolved in isobutyl methyl ketone and trioctylphosphine oxide in benzene. Arsenazo III is used as the metallochromic reagent in a medium buffered with monochloroacetic acid - sodium monochloroacetate. The effect of many cations and anions on the procedures has been investigated, including the elimination of the important interference caused by plutonium.

The applicability of the methods evolved has been demonstrated by the comparative analysis of a series of international secondary uranium ore standards and some other low-content uranium ores that have been analysed by independent chemical methods. The trioctylphosphine oxide - benzene - arsenazo III procedure, which has been shown to be greatly superior to the other methods, permits the direct determination of uranium(VI) in the presence of plutonium when the uranium-to-plutonium ratio is greater than 0·2 per cent. The method has also been found suitable for the determination of uranium in monazitic sands, rare earth concentrates, zirconium-bearing materials and phosphoric acid solutions of the type used for the leaching of low-grade uranium ores.

ARSENAZO III is an organic reagent commonly used mainly for the spectrophotometric determination of many elements,[1] including thorium, uranium, zirconium, hafnium, scandium, cerium, lanthanum, rare earth elements, plutonium, neptunium, curium, protactinium, niobium, barium, strontium, sulphur, palladium[2,3] and bismuth.[4]

Generally, the colour reactions given by this reagent with various cations are very sensitive, although the main drawback to the use of arsenazo III for analytical purposes is its lack of selectivity. Consequently, most of the analytical procedures based on its use include a preliminary stage to increase the selectivity characteristics of the particular reaction considered (*e.g.*, the use of masking agents, solvent-extraction techniques and ion-exchange separations).

At present, there is general interest in suitable combined extraction - spectrophotometric methods for the rapid determination of many elements because of the numerous advantages offered by this type of method compared with older procedures that required a larger number of analytical steps. In 1957, White[5] pointed out the fundamental requirements, possibilities and some practical applications of extraction - spectrophotometric procedures based on the use of new extractants, different organic metallochromic reagents and non-aqueous media. Some of these methods have already been described for the determination of uranium in connection with the preliminary extraction of the element with trioctylphosphine oxide (TOPO),[5,6,7] tributyl phosphate (TBP),[5,8,9] tri-n-octylamine (TOA) and triisooctylamine (TiOA)[10] and 8-hydroxyquinoline[11] diluted with suitable organic solvents. Uranium has been determined so far directly in the corresponding organic extracts with different chromogenic metallochromic organic reagents such as dibenzylmethane,[5,6,8] PAN[7] and arsenazo I.[9] However, little use has been made of this type of procedure involving arsenazo III as the metallochromic reagent for uranium, the suitability of which for these purposes has already been demonstrated for thorium[12] and plutonium.[13,14]

* Paper presented at the Second SAC Conference, Nottingham, July, 1968.

As a continuation of other studies carried out in recent years in our laboratories[8,15,16,17] the purpose of the investigations described in this paper was to attempt to develop a rapid and simple extraction - spectrophotometric method for determining uranium in various secondary-type uranium ores, leached mineral residues and materials and alloys related to nuclear technology containing various amounts of the elements plutonium, thorium, aluminium, zirconium, molybdenum, iron, etc. No attempt has been made to tackle the special problems posed by the determination of uranium in α-, β- and γ-radioactive solutions. We therefore investigated the spectrophotometric determination of uranium(VI) with arsenazo III in aqueous - organic media by suitable dilution of organic extracts containing uranium with alcoholic solutions following the preliminary quantitative separation of uranium(VI) from possible contaminants by a single-stage extraction procedure based on the use of TBP - isobutyl methyl ketone and TOPO - benzene solutions.

BRIEF REVIEW OF THE REACTIONS OF ARSENAZO III WITH URANIUM

Both uranium(IV) and uranium(VI) react with arsenazo III, although the nature of the reaction (stoicheiometry of the complexes formed, molar absorptivities, acidity conditions and effects of anionic and cationic interferences), varies greatly, depending on which oxidation state is involved.

The reaction of uranium(VI) with arsenazo III can be more readily carried out because of the greater stability of uranium in the higher oxidation state. On the other hand, the use of the uranium(IV) reaction implies the need for reducing the uranium quantitatively to the quadrivalent oxidation state which, at the trace amount level, may be associated with certain practical difficulties. Conversely, the main advantage of the reaction of uranium(IV) with arsenazo III compared with that of uranium(VI) is the considerably increased analytical sensitivity and selectivity.

Reactions of uranium(VI) in moderately acidic media—

Uranium(VI) undergoes reaction with arsenazo III in moderately acidic media (pH 1 to 3) to form a 1:1 complex species[18,19,20] exhibiting maximum absorption at wavelength 655 to 660 nm and a molar absorptivity of 5·3 to 5·9 $\times$ 10^4 cm^2 mmole^{-1}.

Under these conditions the reaction exhibits poor selectivity as thorium, zirconium, iron(III), vanadium, chromium, rare earths and actinide elements interfere seriously. The selectivity can be enhanced by the use of various masking agents, *e.g.*, EDTA, phosphate, fluoride and sulphosalicylic acid.[21,22,23] Alternatively, the selectivity of the reaction can be increased by carrying out a preliminary extraction of uranium(VI) with TBP - carbon tetrachloride mixtures in the presence of salting-out agents (usually ammonium, aluminium or calcium nitrates) or masking agents (EDTA, fluoride, etc.), or with TOA or TiOA diluted with xylene,[24] and subsequently stripping the uranium(VI) from the organic phase by washing it with aqueous arsenazo III solutions[11,25,26] or with dilute hydrochloric acid.[24] The selectivity of the arsenazo III - uranium(VI) reaction at pH 1 to 3 can also be increased by extraction of the complex with a solution of guanidine in an alcohol (butanol, pentanol, etc.) in the presence of masking agents (EDTA or fluoride), the photometric measurements being carried out directly on the organic phase containing the extracted complex.[27,28,29]

Reactions of uranium(VI) in strongly acidic media—

In strongly acidic media (5 to 7 M hydrochloric, nitric and perchloric acids) and in the presence of a large excess of reagent over the stoicheiometric requirements, the reaction of uranium(VI) with arsenazo III is more sensitive and selective (the rare earth elements do not interfere) compared with the reaction that takes place at low pH values.[30,31,32,33] The formation in strongly acidic media of 1:1 metal-to-ligand complex species[20,30,31] and 1:2 species[10] with molar absorptivities for the corresponding complexes ranging from 7·1 to 8·8 $\times$ 10^4 is reported.[20,30,31,34] Even the existence of ML_3-type uranium(VI) - arsenazo III complexes has been postulated by some authors.[35,36] In strongly acidic media the number of elements that may give rise to interference in the arsenazo III - uranium(VI) reaction is considerably reduced (thorium, hafnium, zirconium and plutonium).

The arsenazo III - uranium(VI) complex species can be quantitatively extracted from strongly acidic solutions with benzyl alcohol.[33] Generally, the use of concentrated nitric acid solutions is more advantageous than that of hydrochloric acid media as the solubility of the reagent is greater in nitric acid than in hydrochloric acid of the same molarity, and the attainment of the maximum sensitivity for this type of reaction necessitates the use of nearly saturated arsenazo III solutions.[21,22] When arsenazo III is used with concentrated nitric acid solutions it is necessary to destroy any nitrous acid and oxides of nitrogen in equilibrium with the nitric acid. Russian workers[37,38] usually use urea for this purpose, but for practical reasons we prefer to use small amounts of sulphamic acid.

REACTIONS OF URANIUM(IV) IN STRONGLY ACIDIC MEDIA—

Although in 0·1 M hydrochloric acid 1:1 and 1:2 metal-to-ligand complex species are formed, in 6 to 8 M hydrochloric acid the formation of 1:2 complexes[10] and 1:3 complexes[35] has been reported, the molar absorptivities[11,34,35,39,40] of which are 1·0 to 1·27 $\times$ 10^5. The main interferences in concentrated hydrochloric acid media are caused by the thorium, titanium(III), plutonium and zirconium, but in 3 to 4 N acid zirconium can be easily masked with oxalate ions, while the interference from thorium and titanium(III) can be eliminated by carrying out the preliminary extraction of uranium(IV) with TBP[41] or with a TiOA - xylene mixture.[42] The quantitative preliminary reduction of the total uranium to uranium(IV) is usually effected with zinc in the presence of ascorbic acid and oxalic acids,[34,40,42] bismuth[39,43] or titanium(III).[40]

EXPERIMENTAL

INSTRUMENTATION AND EQUIPMENT—

Shaking machine, Chirana, Model TEIII.
Spectrophotometer, Beckman, Model B, single beam.
Recording spectrophotometer, Beckman, Model DK-2A, double beam.
Glove boxes, Technochimie and own design—These were used for all experimental work carried out with solutions containing plutonium.
*pH meter, Metrohm, Model E*388.
*Combined glass - calomel electrode, Metrohm, Model EA*120*UX.*
Graduated Pyrex tubes, 20 ml, polythene stoppered.
Calibrated flasks, 5 and 10 ml.
Calibrated glassware.

REAGENTS—

Arsenazo III—0·1 per cent. aqueous solutions were prepared from Schuchardt reagent and our own synthesised reagent.

Uranium standard solutions—Prepared from acid-free $UO_2(NO_3)_2.6H_2O$ and U_3O_8 standard samples obtained from the Spanish Atomic Commission (J.E.N.).

Plutonium solutions—Prepared from low-temperature ignited $^{239}PuO_2$ samples furnished by the French Atomic Commission (C.E.A.).

Plutonium(IV) solutions in nitric acid—Prepared by dissolving $Pu(OH)_4$ in concentrated nitric acid, to give 2 to 6 N acid, and adding an excess of sodium nitrite to ensure complete conversion of the plutonium into the quadrivalent state.

Iron(II) sulphamate solutions in 0·2 N nitric acid—Solutions containing 10 to 50 mg ml^{-1} of iron(II) were prepared as described by Eschrich.[44]

EDTA solution, 15 per cent.—Prepared by the addition of ammonia solution to an aqueous suspension of EDTA to dissolve solids and then of nitric acid until the pH was between 4 and 5.

DCTA solution, 10 per cent.—Prepared by the addition of sodium hydroxide solution to a suspension of diaminocyclohexanetetraacetic acid in water to dissolve solids and then of nitric acid until the solution was about neutral.

Chloroacetic acid - sodium chloroacetate solutions, 2·5 to 7·5 M equimolar—Prepared by addition of stoicheiometric amounts of sodium hydroxide to aqueous monochloroacetic acid (Merck Union Chimique Belge) solutions that were suitably diluted.

This type of buffer must be prepared at monthly intervals or more frequently as it decomposes relatively rapidly. However, the best results are obtained, and working is more convenient, when stock monochloroacetic acid and sodium hydroxide solutions of appropriate concentration (5 to 10 M) are mixed at the time of preparation of the samples. A detailed investigation of the ageing characteristics of this buffer system will appear elsewhere.[45]

The following solvents and extractants were used: tributylphosphate (B.D.H.), thenoyltrifluoroacetone (TTA) (Fluka), isobutyl methyl ketone (IMK) (B.D.H.), trioctylphosphine oxide (Eastman Kodak), ethanol - butanol and benzene (Merck).

PROCEDURES—

In all of the extraction studies reported 6 to 9 ml of aqueous phase were usually extracted with 2 to 4 ml of organic phase in 20-ml graduated tubes, TBP (20 per cent.) - IMK (80 per cent.) and 0·05 to 0·1 M TOPO - benzene solutions being used systematically as extractants. The influence of foreign ions and masking and reducing agents on the extraction of uranium(VI) from 0·1 to 1 N nitric acid solutions was also studied.

Solutions were shaken (usually for 10 minutes) to achieve equilibrium between the aqueous and organic phases and, after allowing a few minutes for clean phase separation, aliquots (1 to 2 ml) of the organic extracts were introduced, by pipette, into 10-ml calibrated flasks.

The aliquots were then suitably diluted with alcoholic mixtures, followed by the addition of the different reagents and finally by making up to 10 ml with water. The 10-ml aqueous - organic samples resulting from this general procedure were subsequently further investigated as described below.

STANDARD EXTRACTION - SPECTROPHOTOMETRIC PROCEDURES

EXTRACTION WITH THE TRIBUTYL PHOSPHATE (20 PER CENT.) - ISOBUTYL METHYL KETONE (80 PER CENT.) SYSTEM—

At least duplicate samples containing 15 to 300 μg of uranium(VI) are transferred, by pipette, into 20-ml graduated Pyrex glass tubes. If the samples contain plutonium, 1·5 ml of a 0·2 N nitric acid solution of iron(II) sulphamate containing 50 mg ml^{-1} of iron(II) must be added and the reductant allowed to act for 5 to 10 minutes in the cold. If no plutonium is present, 0·5 ml of each of the following solutions of masking agents should be added instead: EDTA (15 per cent.), DCTA (10 per cent.), tartaric acid (40 per cent.) and sodium fluoride (2 per cent.). Four grams of ammonium nitrate are then added followed by the necessary amount of concentrated nitric acid and water to give 0·5 to 0·75 N nitric acid in a final volume of 8 ml; 3-ml portions of TBP - IMK, previously equilibrated with 0·5 to 0·75 N nitric acid, are then added to each tube and the tubes, after being stoppered, are shaken for 10 minutes at a rate of 250 to 300 strokes minute^{-1}.

After clean phase separation, equal 1 to 2-ml portions of each organic extract (duplicated whenever possible) are transferred, by pipette, to 10-ml calibrated flasks and the following reagents are added: 0·1 ml of saturated aqueous sulphamic acid solution, 1·0 ml of 0·1 per cent. aqueous arsenazo III solution, 1 ml of 5·3 M recently prepared equimolar monochloroacetic acid - monochloroacetate buffer solution, 4 ml of absolute ethanol and 0·5 ml of 10 per cent. DCTA solution. The solution is then made up to volume with water and, after mixing thoroughly, the samples are measured after 1·5 hours at wavelength 655 nm, with 10-mm glass spectrophotometric cells. Measurements are made against the corresponding extraction - spectrophotometric blank samples prepared in exactly the same way as the samples. Measured against distilled water, the blank reference samples normally give an absorbance value of 0·065 $\pm$ 0·005.

The uranium contents of samples are calculated by interpolation of the absorbance results on a recently obtained calibration graph for the range 0·5 to 10 μg ml^{-1} of uranium(VI), adjusted by the least squares method, or by multiplying the particular absorbance values by the relevant factor obtained from the absorbance values corresponding to the average of triplicate uranium extraction "standards" carried out at two or more suitable uranium concentration levels.

EXTRACTION WITH THE 0·1 M TRIOCTYLPHOSPHINE OXIDE - BENZENE SYSTEM—

At least duplicate samples containing 15 to 300 μg of uranium(VI) are introduced into 20-ml Pyrex glass tubes. If the samples do not contain plutonium or if it is present in amounts not exceeding 150 μg, there is no need to provide special precautions and 0·5 ml of each of the following masking reagent solutions can be added: 10 per cent. DCTA, 15 per cent. EDTA, 2 per cent. sodium fluororide and 40 per cent. tartaric acid. If the samples contain plutonium in amounts between 0·15 and 5 mg, 50 mg of sodium fluoride and 0·5 ml of 10 per cent. DCTA solution should be added instead as masking agents. If, however, the plutonium content of the samples is between 5 and 20 mg after the extraction step carried out in the presence of 50 mg of sodium fluoride and 0·5 ml of 10 per cent. DCTA solution, the organic extract should be washed with an equal volume of 0·5 to 1·0 N aqueous nitric acid solution (6 to 9 ml) containing the same amount of sodium fluoride and DCTA, the tube being shaken for 10 minutes. All of the plutonium should be present as plutonium(IV). If this is not so, the samples should be treated with 40 per cent. hydrogen peroxide and 9 M sodium nitrite in 5 to 7 M nitric acid medium as described elsewhere.[46]

According to the nature of the sample solutions, after pre-treatment and addition of masking agents, concentrated nitric acid and water are added to give 0·5 to 1·0 N nitric acid in a final 6 to 9-ml volume of aqueous phase to be extracted; 3-ml portions of 0·1 M TOPO - benzene, previously equilibrated with 0·5 to 1·0 N nitric acid, are then added and, after stoppering the tubes, the samples are shaken for 10 minutes at a rate of 250 to 300 strokes minute^{-1}.

After clean phase separation, 1 to 2-ml samples (duplicated when possible) of the organic extract are introduced, by pipette, into 10-ml calibrated flasks, and the following reagents are added: 0·1 ml of saturated aqueous sulphamic acid solution, 1 ml of 0·1 per cent. aqueous arsenazo III solution, 1 ml of 5·3 M equimolar monochloroacetic acid - monochloroacetate solution, 6 ml of an ethanol (80 per cent.) - butanol (20 per cent.) mixture and 0·5 ml of 10 per cent. DCTA solution. The contents are then made up to volume (10 ml) with water, and the solution is thoroughly mixed and measured after 1·5 hours at wavelength 655 nm in 10-mm glass spectrophotometric cells against the corresponding extraction - spectrophotometric reagent blanks. The absorbance value of the reference blank solutions measured against distilled water under these conditions is normally 0·150 $\pm$ 0·010. The uranium contents of the samples should be calculated as described above for the TBP - IMK system.

When analysing plutonium-containing samples, it is convenient to reduce the scale by half by using 5-ml calibrated flasks thoroughout, the practical details otherwise remaining unchanged, with the exception that of the amounts of reagents used for the preparation of the spectrophotometric samples, one half of those described under "Standard extraction - photometric procedures" are taken. The reproducibility of the results obtained by using both alternatives (5 or 10-ml calibrated flasks) is in most instances better than $\pm$1 per cent.

RESULTS AND CONCLUSIONS

ESTABLISHMENT OF THE OPTIMUM CONDITIONS OF THE ARSENAZO III - URANIUM(VI) REACTION IN AQUEOUS - ORGANIC BUFFERED MEDIA

Following a detailed investigation[47] of the main spectrophotometric and physicochemical characteristics exhibited by the arsenazo III - uranium(VI) system in aqueous media buffered by the monochloroacetate ion, additional studies were carried out to establish the experimental optimum conditions for this reaction in aqueous - organic media. Accordingly, the influence of the following parameters was investigated: optimum pH for complex formation; nature and amount of buffer; mutual miscibility characteristics of different aqueous - organic media; kinetic features of the complex reaction; conformity with Beer's law of the arsenazo III - uranium(VI) complex; and influence of the extracted acidity on the absorbance of the coloured complex.

Generally, the main characteristics of the arsenazo III - uranium(VI) system have been shown to be similar both in aqueous and aqueous - organic (alcoholic) buffered media. As described in detail elsewhere,[47] the arsenazo III - uranium(VI) complex of analytical interest (formed with excess of ligand) exhibits two absorption maxima at wavelengths 605 and 655 nm and a maximum molar absorptivity at pH 1·5 $\pm$ 0·1 of 5·5 $\pm$ 0·3 $\times$ 10^4 at 655 nm.

Fortunately, the absorbance of the complex is insensitive towards acidity variations within the pH range 1·3 to 2·2. After testing several buffer solutions, including mono-, di- and trichloroacetic acid - salt systems, we selected the monochloroacetic acid - sodium monochloroacetate system (equimolecular proportions of acid and salt). This buffer has many advantages within the pH range 1·5 to 3·5 because of its high solubility, which gives a large buffer capacity, and its weak complexing properties towards uranium(VI).[48] Consequently, we have made extensive use of it in a range of spectrophotometric work as described elsewhere.[46,47,49]

The arsenazo III - uranium(VI) reaction has been shown[47] to exhibit good kinetic features and colour stability, both in aqueous and aqueous - organic media as the absorbance remains almost constant after 1 to 1½ hours.

The influence of the buffer concentration on the absorbance of the complex has been found[47] to be unimportant up to ionic strengths of 1·5 to 2. For this reason, systematic use has been made throughout this investigation of 2·5 to 7·5 M equimolecular monochloroacetic acid - monochloroacetate solutions (0·5 to 2 ml in a final 10-ml volume of sample). The amount of buffer was sufficient to deal with the acid present in the final volume.

A study of the miscibility of the various aqueous - organic mixtures investigated showed that clear and stable 10-ml (final volume) mixtures could be obtained from 1 to 2-ml aliquot samples of organic extract.

In the aqueous - organic systems investigated the arsenazo III - uranium(VI) complex has been shown to conform to Beer's law within the range 0·25 to 10 μg ml^{-1} of uranium(VI), provided that a sufficient excess of reagent ($C_L : C_M \geqslant 6$) over the stoicheiometric requirements is used. The stability constant[20,32,35,50] of the complex formed in different aqueous acidic media is about 10^4.

Extraction of uranium(VI) by the tributyl phosphate (20 per cent.) - isobutyl methyl ketone (80 per cent.) system

The extraction of uranium(VI) by this system requires the addition of a salting-out component to the aqueous phase to obtain a reasonably high distribution coefficient for uranium into the organic phase. Ammonium nitrate has been used as a salting-out agent (4 g of ammonium nitrate per 8 ml of aqueous phase) for the reasons given elsewhere.[9]

The most significant results obtained for the extraction of uranium(VI) by this system in the presence or absence of reducing or masking agents, or both, are reproduced in Table I, from which the following conclusions can be drawn: (*i*) the extraction of uranium(VI) in the

Table I

Extraction of 75 μg of uranium(VI) by tributyl phosphate (20 per cent.) - isobutyl methyl ketone (80 per cent.) from 0·5 ± 0·2 M nitric acid solutions saturated with ammonium nitrate with the standardised arsenazo III spectrophotometric procedure in aqueous - organic media

Aqueous phase: Reductants	Aqueous phase: Masking agent	Uranium(VI) extracted, per cent.	Minimum amount of plutonium(IV) causing interference/mg
—	—	97 ± 1	>0·05
—	a	89 ± 4	⩾0·3
$HSCH_2.COOH$ (0·5 ml of 80 per cent.)	—	0	—
$HSCH_2.COOH$ (0·5 ml of 80 per cent.)	a	0	—
Ascorbic acid (0·5 g)	—	95 ± 3	⩽0·3
Ascorbic acid (0·5 g)	a	78	—
$NH_3(OH)Cl$ (0·5 g)	—	95	—
$NH_3(OH)Cl$ (0·5 g)	a	92	—
$(SO_3H.NH)_2Fe$ [75 to 150 mg of iron(II)]	—	99 ± 1	⩾0·5 ⩽ 1*
$(SO_3H.NH)_2Fe$ [75 to 150 mg of iron(II)]	a	83	—
$(SO_3H.NH)_2Fe$ [75 to 150 mg of iron(II)]	b	(?)†	—

[a] 0·5 ml of 10 per cent. DCTA + 0·5 ml of 15 per cent. EDTA + 0·5 ml of 2 per cent. NaF + 0·5 ml of 40 per cent. tartaric acid.

[b] 50 mg of sodium fluoride.

* Poor reproducibility for the plutonium interference results.

† Most of the uranium remained co-precipitated in the aqueous phase by the PuF_3 formed.

absence of masking and reducing agents is not rigorously quantitative (about 3 per cent. negative extraction bias), while in the presence of masking agents the extraction characteristics of uranium deteriorate considerably (about 89 per cent. of the total uranium(VI) is extracted); (*ii*) in the absence of masking agents, the addition of ascorbic acid or hydroxylamine to the aqueous phase brings about a slight increase in the negative extraction bias (about 5 per cent.), while the addition of reductants in the presence of masking agents results in a considerable decrease in the percentage of uranium(VI) extracted; and (*iii*) in the absence of masking agents, the extraction of uranium(VI) is almost quantitative when iron(II) sulphamate is added to the aqueous phase.

The general conclusion is that the extraction of uranium(VI) by the TBP - IMK system is seldom quantitative in a single extraction step.

Effect of foreign ions—

The standard TBP - IMK extraction - spectrophotometric procedure given above tolerates, in the presence of masking agents, at least up to 4 mg of fluoride, 10 mg of thorium, 150 mg of iron(III), 0·5 ml of concentrated acetic acid, 0·5 ml of 10 per cent. DCTA solution, 25 mg each of vanadium(V), molybdenum(VI), cobalt(II), chromium(VI), copper(II), nickel(II), manganese(II), titanium(III), arsenic(III), bismuth(III), calcium and aluminium; various amounts between 2 and 15 mg of strontium, barium, platinum, gold, zirconium, mercury, tungsten(VI), cadmium, niobium, tin, platinum-group metals and rare earth elements; various amounts between 20 and 200 mg of sulphite, chlorate, acetate, phosphate, iodide, borate, oxalate, citrate, thiocyanate, tartrate, sulphate, EDTA, hydroxylamine, hydrochloric acid and DCTA.

Table II

Extraction of 75 μg of uranium(VI) by 0·1 M trioctylphosphine oxide - benzene from 0·5 $\pm$ 0·2 M nitric acid solutions with the standardised arsenazo III spectrophotometric procedure in aqueous - organic media

Aqueous phase: Reductants	Aqueous phase: Masking agent	Uranium(VI) extracted, per cent.	Minimum amount of plutonium(IV) causing interference/mg
—	—	100	$\geqslant$0·05
—	a	98 $\pm$ 2	$\geqslant$0·05
—	b	100	$\geqslant$2·5 $\leqslant$ 5
—	b	100	$\geqslant$15 $\leqslant$ 25*
Ascorbic acid (0·5 g)	—	100	$\leqslant$0·5
Ascorbic acid (0·5 g)	a	100	$\leqslant$0·6
Ascorbic acid (0·5 g)	b	100	$\geqslant$2·5 $\leqslant$ 5†
$NH_3(OH)Cl$ (0·5 g)	—	100	—
$NH_3(OH)Cl$ (0·5 g)	a	98 $\pm$ 2	—
$NH_3(OH)Cl$ (0·5 g)	b	100*	$\geqslant$2·5 $\leqslant$ 5†
$(SO_3H.NH)_2Fe$ [75 to 150 mg of iron(II)]	—	100	—
$(SO_3H.NH)_2Fe$ [75 to 150 mg of iron(II)]	a	92	$\geqslant$0·5 $\leqslant$ 1‡
$(SO_3H.NH)_2Fe$ [75 to 150 mg of iron(II)]	b	(?)	§
$HSCH_2.COOH$ (0·5 ml of 80 per cent.)	—	0	—
$HSCH_2.COOH$ (0·5 ml of 80 per cent.)	a	0	—

[a] 0·5 ml of 10 per cent. DCTA + 0·5 ml of 10 per cent. EDTA + 0·5 ml of 2 per cent. NaF + 0·5 ml of 40 per cent. tartaric acid.

[b] 50 mg of NaF + 0·5 ml of 10 per cent. DCTA.

* After concluding the extraction step, the organic extract was submitted to a washing cycle with a new aqueous phase of composition identical with that used for the extraction step under the same shaking conditions before aliquots of the organic extract were submitted to spectrophotometric analysis.

† When the reductants were added before the masking agents most of the uranium(VI) remained in the aqueous phase after the extraction cycle (co-precipitation of uranium(VI) on the PuF_3 precipitate).

‡ The results obtained gave very poor reproducibility when the interference of plutonium was investigated.

§ Poor reproducibility of results was obtained arising from the probable partial co-precipitation of uranium(VI) in the aqueous phase despite addition of the masking agents before the reductant.

PARTICULAR EFFECT OF PLUTONIUM—

Table I also summarises the most significant results obtained from a detailed investigation of the interference of plutonium on the extraction of uranium(VI). They indicate that in the absence of reductants and masking agents any amount of plutonium(IV) interferes in the extraction of uranium(VI) as a result of co-extraction, and that the only possible way of determining uranium(VI) in the presence of 0·5 to 1 mg of plutonium(IV) is by the use of iron(II) sulphamate provided that masking agents, especially fluoride, are absent to avoid possible complexation or precipitation of uranium(VI) in the aqueous phase.

TABLE III

EFFECT OF VARIOUS CATIONIC INTERFERENCES ON THE EXTRACTION OF 75 μg OF URANIUM(VI) BY 0·1 M TRIOCTYLPHOSPHINE OXIDE - BENZENE IN 0·5 N NITRIC ACID MEDIUM AS DETERMINED BY THE ARSENAZO III SPECTROPHOTOMETRIC METHOD IN AQUEOUS - ORGANIC MEDIA

Investigated ion	Amount/mg	Masking agent	Uranium(VI) extracted, per cent.	Remarks
Thorium(IV) ..	10 to 40	c	100	An abundant white precipitate of ThF_4 was formed in the aqueous phase
Thorium(IV) ..	⩾50	c	—	The formation of persistent emulsions did not permit pipetting of aliquots from the organic extract
Rare earths* ..	2·5 to 30	b	100	No appreciable co-precipitation of uranium(VI) could be found on the voluminous rare earth fluoride precipitates
Rare earths* ..	⩾50	b	>100†	Increasing amounts of rare earth elements are co-extracted with uranium(VI) as their concentration in the aqueous phase is increased.
Rare earths* ..	⩽5	a	100	Insufficient excess of F^- in the aqueous phase does not tolerate the presence of rare earths in amounts larger than 5 mg
Rare earths* ..	⩾7·5	a	>100†	Increasing co-extraction of the rare earths takes place as their concentration in the aqueous phase is increased
Zirconium(IV) ..	5 to 120	a	100	DCTA acts as the most effective masking agent for zirconium(IV)
Iron(III) ..	25 to 300	None	100	Regardless of the presence or absence of masking agents in the aqueous phase appreciable amounts of iron(III) are co-extracted into the organic phase. However, the co-extracted iron is masked quantitatively by addition of 0·5 ml of 10 per cent. DCTA to the aqueous - organic sample to be measured spectrophotometrically (see "Standard extraction - photometric procedures")

[a] 0·5 ml of 40 per cent. tartaric acid + 0·5 ml of 10 per cent. DCTA + 0·5 ml of 2 per cent. NaF + 0·5 ml of 15 per cent. EDTA.

[b] 0·5 ml of 40 per cent. tartaric acid + 0·5 ml of 10 per cent. DCTA + 50 mg of NaF + 0·5 ml of 15 per cent. EDTA.

[c] 50 mg of NaF + 0·5 ml of 10 per cent. DCTA.

* A synthetic rare earth solution was used containing the mixture cerium(III) - lanthanum - neodymium (2 + 1 + 1 w/w).

† Results above 100 per cent. for uranium (VI) extract do not arise from errors in the spectrophotometric method but from increased absorbance readings caused by the presence of variable amounts of co-extracted impurities that react with arsenazo III.

EXTRACTION OF URANIUM(VI) BY THE 0·1 M TRIOCTYLPHOSPHINE OXIDE - BENZENE SYSTEM FROM NITRIC ACID SOLUTIONS

From the results given in Table II, it can be readily seen that the extraction of uranium(VI) is almost quantitative in a single-extraction step, regardless of whether the extraction is carried out in the presence or absence of different reductants or masking agents, or both.

This fact, together with its additional advantage of not requiring the use of salting-out agents, causes the TOPO - benzene system to be superior to the TBP - IMK extraction system.

Further, the quantitative extraction of uranium(VI) by this sytem is insensitive to variations in the experimental conditions as shown by the results of a detailed factorial investigation involving the extraction of 60 μg of uranium(VI) (in the absence of reducing or masking agents, or both), which indicates that the extraction of the element is almost quantitative in a single-stage operation when the acidity of the aqueous phase varies within 0·5 to 7 N nitric acid (6 to 8 ml of aqueous phase), even although the extraction is carried out with 2 to 8 ml of organic phase and for any shaking period (250 to 300 strokes minute^{-1}) between 1 and 10 minutes.

EFFECT OF DIVERSE IONS—

In 0·5 M nitric acid media, and in the absence of masking agents, of the forty different cations tested (2 to 150 mg were taken depending on cases) the following were shown to interfere appreciably in the extraction of uranium(VI): bismuth(III), zirconium, lanthanum, rare earths, iron(III), thorium, tantalum, tungsten(VI), magnesium, niobium, cerium(III) and plutonium(IV).

When the extraction of uranium(VI) was carried out under the same acidity conditions and in the presence of masking agents (see "Standard extraction - spectrophotometric procedures" for details), all of these interferences could be suppressed satisfactorily with the exception of that arising from plutonium(IV). In addition under these conditions, the presence in various amounts (5 to 10 mg) of fifteen anions commonly used for masking purposes did not have an appreciable effect on the quantitative nature of the uranium(VI) extraction.

In Tables III and IV the results obtained showing the influence of some anions and cations investigated are given.

PARTICULAR EFFECT OF PLUTONIUM—

As the TOPO - benzene solution is a good extraction system, both for uranium(VI) and plutonium(IV)[51] within almost the whole acidity range between 0·5 and 10 N nitric acid, it becomes mandatory to discover a suitable way of pre-treating the aqueous phase prior to the extraction step for uranium when both elements are present. The most reasonable alternatives are: the addition to the aqueous phase of suitable masking agents for plutonium(IV) that do not complex appreciably the uranium(VI) present (*e.g.*, fluoride, DCTA, etc.); the addition of suitable reducing agents for plutonium(IV) that do not alter the sexavalent oxidation state of uranium (*e.g.*, iron(II) sulphamate, hydroxylamine, ascorbic acid, etc.); or the combined addition of both reducing and masking agents.

TABLE IV

INFLUENCE OF DIFFERENT ANIONIC INTERFERENCES ON THE EXTRACTION OF 75 μg OF URANIUM(VI) BY 0·1 M TRIOCTYLPHOSPHINE OXIDE - BENZENE IN 0·5 N NITRIC ACID MEDIUM WITH THE STANDARDISED ARSENAZO III SPECTROPHOTOMETRIC METHOD IN AQUEOUS - ORGANIC MEDIA

Investigated ion	Amount	Uranium(VI) extracted, per cent.	Remarks
SO_4^{2-}	5 to 500 mg	100	No masking agents were used in the aqueous phase
F^-	2 to 30 mg	100	No masking agents were used in the aqueous phase
F^-	40 mg	~98	When the amount of F^- in the aqueous phase was increased from 60 to 100 mg the uranium(VI) extracted decreased from 79 to 9·6 per cent.
H_3PO_4 (sp.gr. 1·71)	$\leqslant$ 0·25 ml	100	No masking agents were used in the aqueous phase
H_3PO_4 (sp.gr. 1·71)	$\leqslant$ 0·75 ml	100	$Al(NO_3)_3$ (4 g) was added to the aqueous phase
H_3PO_4 (sp.gr. 1·71)	$\leqslant$ 1·0 ml	100	$Al(NO_3)_3$ (8 g) was added to the aqueous phase. Amounts of phosphoric acid in excess of 1 ml begin to cause interference regardless of the amount of aluminium nitrate added

TABLE V

THE REDUCTION AND COMPLEXATION OF PLUTONIUM AND PERCENTAGE EXTRACTED INTO TRIOCTYLPHOSPHINE OXIDE - BENZENE AND THENOYLTRIFLUOROACETONE - BENZENE SOLUTIONS*

Composition of aqueous phase				Extraction into TOPO - benzene layer		Extraction into TTA - benzene layer	
Medium: nitric acid, N	Plutonium/ mg	Reductant used (5 to 10 minutes in the cold)	Masking agent†	Arsenazo III buffer A‡ Ethanol - butanol (675 nm)	Arsenazo I buffer B‡ Ethanol - butanol (600 nm)	Arsenazo III buffer A‡ Ethanol - butanol (675 nm)	Notes
0·5 to 1·0	0·15	—	—	99·5	—	99·2	1
0·5 to 1·0	1·0	—	a, b	2·0	—	—	1, 2, 3
0·5 to 1·0	0·15 — 0·25	$NH_3(OH)Cl$ (0·5 to 1·0 g)	—	6·1; 9·9	6·7	—	1
0·5 to 1·0	1·0 — 2·0	$NH_3(OH)Cl$ (0·5 to 1·0 g)	—	—	—	0·34; 0·20 0·25; 0·21	1
0·5 to 1·0	1·0	$NH_3(OH)Cl$ (0·5 to 1·0 g)	a	5·9	6·4	0·52; 0·05	1,3
0·5 to 1·0	1·0	$NH_3(OH)Cl$ (0·5 to 1·0 g)	a, b	2·9; 0·78	—	2·24; 2·20	1,3
0·5 to 1·0	1·0	Ascorbic acid (0·5 g)	a	>25	32·8	1·1	1,3
0·5 to 1·0	1·0	Ascorbic acid (0·5 g)	a, b	5·7	—	—	1, 3, 4
0·5 to 1·0	1·0	Iron(II) as sulphamate (100 mg)	a	11·4	9·8	—	1,3
0·5 to 1·0	1·0	Iron(II) as sulphamate (100 mg)	a, b	2·5	—	1·7	1, 2, 3
1·5 to 2·0	2·0	$NH_3(OH)Cl$ (0·5 to 1·0 g)	—	2·7	3·1	—	1
0·5 to 2·0	2·0 — 4·0	Ascorbic acid (1 g)	—	>8; 7·4	9·2	0·10	1
1·5 to 2·0	2·0	Iron(II) as sulphamate (150 mg)	a	3·1	4·2	—	1,3
1·5 to 2·0	4·0	—	a, b	0·43	—	0·81	1,2
0·5 to 1·0	4·0	$NH_3(OH)Cl$ (0·5 to 1·0 g)	—	>7	6·7	0·01	1
0·5 to 1·0	5·0	$NH_3(OH)Cl$ (0·5 to 1·0 g)	a	>5	5·3	0·01	1,3
0·5 to 1·0	5·0	Ascorbic acid (1 g)	a	>5	6·8	0·50	1,3
0·5 to 1·0	4·0 — 5·0	Iron(II) as sulphamate (150 mg)	a	>5; >7	6·1; 7·8	0·12; 0·28	1, 3, 5
1·0 to 1·5	22	—	b	21·6	—	16·5	1, 6
1·0 to 1·5	22	—	a, b	>25	—	1·4	1, 6
1·0 to 1·5	22	—	a, b	22·4	—	8·2; 7·8	1, 6
1·0 to 1·5	22	$NH_3(OH)Cl$ (0·5 to 1·0 g)	b	20·2	—	7·0; 6·6	1, 6
0·5 to 1·0	22	$NH_3(OH)Cl$ (0·5 to 1·0 g)	b	0·46	—	—	1, 3, 6
0·5 to 1·0	22	$NH_3(OH)Cl$ (0·5 to 1·0 g)	a, b	0·41	—	—	1, 3, 6
0·5 to 1·0	22	Iron(II) as sulphamate (75 mg)	b	21·4	—	—	1, 3, 6
0·5 to 1·0	22	Iron(II) as sulphamate (75 mg)	a, b	34·5	—	—	1, 3, 6
1·0 to 1·5	44	—	c	>2·2	—	0·72	1, 6

NOTES—1. No DCTA was added to the aqueous - organic solutions to be measured spectrophotometrically to avoid possible complexation of the extracted plutonium by this reagent.

2. The TOPO - arsenazo I method cannot be used when fluoride is added to the aqueous phase because of the competitive complexing effect brought about by co-extracted fluoride on the extracted plutonium, which leads to inhibition of the reaction of plutonium with arsenazo I in the aqueous - organic final sample.

3. Addition of reductants prior to the masking agents. If no special remark is made, it is assumed throughout that the masking agents were added prior to the reductants.

4. The addition of ascorbic acid to the aqueous phase often leads to difficulties with the arsenazo I - plutonium reaction in the aqueous - organic medium.

5. When iron(II) is added to the aqueous solution, no co-extraction of iron has been detected when TOPO - benzene is used as the extractant. However, with the TTA - benzene system appreciable amounts of iron are co-extracted, which may lead to high plutonium results (the organic extract occasionally has a more or less intensely blood-red colour).

6. The organic extract was washed with the same reagents and under the same experimental conditions used for the extraction step, before aliquots were taken to carry out the spectrophotometric determinations in aqueous - organic media.

* Methods involving arsenazo I as the metallochromic agent and trichloroacetic acid - sodium trichloroacetate system as the buffer are similar to those described in the present paper.

† Masking agent a. Addition of 0·5 ml of 10 per cent. DCTA to the aqueous phase.
b. Addition of 50 mg of NaF to the aqueous phase.
c. Addition of 100 mg of NaF to the aqueous phase.

‡ Buffer A. Trichloroacetic acid - trichloroacetate.
B. Monochloroacetic acid - monochloroacetate.

The lack of reproducible results obtained for the extraction of plutonium(III) by the TOPO - benzene mixture in 0·5 N nitric acid media makes it unlikely that quantitative reduction of plutonium(IV) to plutonium(III) will resolve the problem. Our findings indicate that in 0·5 to 2 N nitric acid media about 7 per cent. of plutonium(III), 99 per cent. of plutonium(IV) and 90 per cent. of plutonium(VI) are extracted by the 0·1 M TOPO - benzene system.

Extraction of plutonium(IV) and plutonium(III) by the trioctylphosphine oxide - benzene system

We have carried out a detailed investigation on the reduction, masking and extraction characteristics of plutonium by determining the amount of plutonium extracted by the 0·1 M TOPO - benzene system as a function of the acidity of the medium, and of the pretreatment of the aqueous nitric acid solutions with several reductants or masking agents, or both. The results obtained are reproduced in Table V, which includes for comparative purposes results obtained by two additional extraction - spectrophotometric methods developed in recent years in our laboratories.[52] These results indicate that in all instances when reductants have been used the reduction of plutonium(IV) to plutonium(III) seems to be almost quantitative, as the extraction of plutonium by the TTA - benzene system is negligible (under the same acidity conditions the extraction of plutonium(IV) by TTA would proceed quantitatively); that the extraction of plutonium(III) by the TOPO - benzene system is important, even in the presence of a large excess of reductants, while the efficiency with which plutonium is reduced to the tervalent state has been shown to decrease according to the sequence hydroxylammonium chloride > iron(II) > ascorbic acid; and that the addition of large amounts of fluoride ions (up to 50 mg of sodium fluoride) does not bring about any substantial increase in the amount of plutonium(III) retained in the aqueous phase but, on the contrary, the larger the excess of fluoride ions the more likely a plutonium trifluoride precipitate will be formed on which uranium(VI) might largely, or even quantitatively, co-precipitate.

Extent of co-extraction of plutonium in the presence of masking or reducing agents, or both, added to the aqueous uranium-containing phase—

The main conclusions regarding the interference of plutonium on the extraction of uranium(VI) by the TOPO - benzene system can be readily drawn from consideration of the results included in Table II: in the absence of reductants and masking agents added to the aqueous phase, plutonium interferes with the uranium(VI) extraction because it is co-extracted; the use of masking agents (especially fluoride) in the absence of reductants allows the tolerance of the method for plutonium(IV) to increase considerably, as the quantitative extraction of microgram amounts of uranium can be carried out in the presence of plutonium, provided that 0·5 ml of 10 per cent. DCTA solution is added to the aqueous - organic sample (quantitative masking of about 50 μg of plutonium per 10 ml.) Further, the tolerance of the method for plutonium(IV) can be increased considerably when, after completion of the extraction step, the organic extract is washed under the same experimental conditions as those used to carry out the extraction; the sole use of reductants for plutonium(IV) does not successfully eliminate the plutonium interference, because of the appreciable co-extraction of plutonium(III) by the TOPO - benzene system; and the combined use of reductants and masking agents for plutonium in the aqueous phase, prior to the extraction of uranium(VI), gives results similar to those obtained by the use of masking agents alone (upper limit of tolerance 2·5 to 5 mg of plutonium(IV) in the presence of 50 mg of sodium fluoride).

Co-precipitation of uranium(VI) on plutonium tri- and tetrafluorides in the aqueous phase—

When the extraction of uranium(VI) is attempted in the presence of increasing amounts of plutonium (over the 2·5 to 5-mg range), by using both reductants and masking agents for plutonium in the aqueous phase, the extraction of uranium(VI) becomes increasingly less efficient with increasing amounts of plutonium because of the appreciable co-precipitation of uranium(VI) on the plutonium fluoride. It is concluded that uranium(VI) may largely co-precipitate on plutonium tetrafluoride and to a still greater extent on plutonium trifluoride. These findings explain qualitatively the greater tolerance of the method for plutonium when only masking agents (for plutonium (IV)) are added to the aqueous phase compared with the combined addition of reductants followed by masking agents (for plutoninm (III)).

TABLE VI

CORRELATION OF ANALYTICAL RESULTS OBTAINED FOR URANIUM ORES BY USING DIFFERENT METHODS

		Method (U_3O_8 per cent.)					
Description	Type of mineral	TOPO - arsenazo III*	TBP - isobutyl methyl ketone - arsenazo III†	TBP - isobutyl methyl ketone - arsenazo I‡	Dibenzoyl-methane‡	Thio-cyanate‡	OIEA standard values
"S–I" (Australia)	Torbernite	0·480	0·487	0·483	0·481	0·481	0·471
"S–II" (Spain)	Torbernite	0·313	0·309	0·313	0·311	0·318	0·313
"S–III" (U.S.A.)	Carnotite	0·425	0·415	0·415	0·419	0·426	0·418
"S–IV" (Australia)	Uraninite	0·370	0·371	0·372	0·374	0·381	0·375
"La Virgen" (Spain)	Torbernite, autunite, pitchblende	0·057 2	0·058 8	0·059 5	—	0·057 5	—
"Carretona" (Spain)	Autunite, pitchblende, phosphuranylite	0·089 8	0·093 9	0·092 0	—	0·094 1	—
"Alameda de Gordón" (Spain)	Autunite, renardite	0·234	0·238	0·239	—	0·259	—
"Fraga" (Spain)	Lignite	0·018 7	—	0·020 2	0·019 7	—	—
"Calaf" (Spain)	Lignite	0·017 8	—	0·018 3	0·018 1	—	—
"Cardeña" (Spain)	Autunite, torbernite	—	0·103	—	0·111	0·108	—

* Average of twelve determinations.
† Average of four determinations.
‡ Average of fifty-six determinations.

Our findings further indicate that when the amount of plutonium(IV) exceeds the 2·5-mg level, the formation of a dark grey precipitate of plutonium tetrafluoride can be easily seen when sufficient fluoride is present, on which uranium(VI) does not co-precipitate appre ciably until the limit of about 20 mg of plutonium(IV) is exceeded. This value constitutes about the upper compatibility limit for the quantitative extraction of uranium(VI) in the presence of plutonium(IV) when the extraction is carried out in the presence of fluoride. According to our results, the co-precipitation of 15 to 150 μg of uranium(VI) on plutonium tetrafluoride becomes quantitative when about 50 mg of plutonium(IV) are present in the aqueous phase. On the other hand, when plutonium(IV) is first reduced to plutonium(III), the co-precipitation of uranium(VI) on the pink precipitate of plutonium trifluoride becomes appreciable for amounts of plutonium larger than 2·5 mg.

Limits of applicability of the extraction - spectrophotometric method for uranium in the presence of plutonium without their previous separation—

From Table II it is concluded that under suitable experimental conditions (absence of reductants; addition of 50 mg of sodium fluoride and 0·5 ml of 10 per cent. DCTA to the aqueous phase; washing of the organic extract following the extraction cycle; and use of DCTA in the aqueous - organic spectrophotometric sample) the TOPO - benzene - arsenazo III extraction - spectrophotometric method allows the uranium to be quantitatively separated and determined in samples, provided that the uranium-to-plutonium ratio is greater than 0·1 to 0·2 per cent. at the lower limit of the method (*i.e.*, when the total amount of uranium originally present in the sample does not exceed 30 μg) or greater than 1 to 2 per cent. at the upper limit (total amount of uranium about 300 μg per sample).

If the uranium content is smaller than the values stated, the method could still be applied, by suitably effecting the preliminary separation of uranium from plutonium; separation by anion exchange may be suitable for this purpose.[53,54]

Comparative study of the methods when applied to the analysis of samples of uranium ores

The practical applicability of the two methods for the determination of uranium described in the present paper has been tested on several samples of secondary uranium ores, low grade uranium minerals and sterile leached residues, the mineralogical and chemical characteristics of which were well known.[55] The results obtained from the study of ten such materials are given in Table VI. The method selected for the attack of the samples consisted simply in leaching uranium from the different materials by boiling them under reflux with dilute nitric acid (1 + 1) for prolonged periods (6 to 8 hours), as described elsewhere.[55]

Most of the uranium values included in Table VI, as determined by the arsenazo I, dibenzoylmethane and thiocyanate methods have been taken from an earlier publication[54] for comparative purposes.

From the results given in Table VI it is apparent that the two methods described in this paper furnish results that agree well with those found for the same samples by other workers who used different methods.

The two methods considered here have also been used for the determination of uranium in monazitic sands, "New Brunswick Laboratories" uranium - thorium standards and rare earth concentrates. The results obtained (not included here) indicate that both methods can be applied to the determination of very small amounts of uranium in a large variety of natural mineral samples.

Discussion

Despite the good agreement shown by the two extraction - spectrophotometric methods for the determination of uranium in different natural and synthetic samples and standards, the TOPO - benzene method exhibits so many practical advantages compared with the TBP - IMK method that only the former is used in our laboratories in routine and research problems. Consequently, only a few specific questions relating to the TOPO - benzene - arsenazo III method will be dealt with briefly in this section.

Our experimental findings on the extent of plutonium(III) co-extraction by the TOPO - benzene system and on that of co-precipitation of uranium(VI) on the plutonium trifluoride and plutonium tetrafluoride precipitates have been shown to differ considerably from those

included in the scarce information available on these aspects. Co-precipitation of uranium(VI) on plutonium trifluoride has been reported by Maria,[56] although he found tolerance limits much greater than ours by a factor of 10 to 100. Analogously we found the amount of plutonium(III) co-extracted by the TOPO - benzene system to be 0·3 to 4 per cent., depending on the experimental conditions (Table V) compared with 0·04 and 0·1 per cent. reported by Maria[56] and Baltisberger,[7] respectively. The characteristic co-precipitation of uranium(VI) on plutonium trifluoride so clearly shown by us to occur below the 20-mg plutonium level has not been reported by any of the authors mentioned. Further, the co-precipitation problem is not even mentioned in Baltisberger's paper. On the other hand, the widely differing characteristics shown by the plutonium trifluoride and plutonium tetrafluoride precipitates regarding the extent of uranium(VI) co-precipitation on them do not seem to have been correctly differentiated by previous workers.

Contrary to Baltisberger's findings, our experimental results indicate (Tables II and V) that the combined use of iron(II) sulphamate and fluoride to prevent the plutonium interference in the aqueous phase is unsatisfactory because of the poor reproducibility of the results obtained. Of course, the order of addition of reagents followed in this procedure might give rise to widely varying results, depending on which of the plutonium fluorides is formed as the trifluoride is about ten times more effective as a co-precipitant for uranium(VI) than the tetrafluoride, according to our results.

As the main problem encountered in the application of the TOPO - benzene - arsenazo III extraction - spectrophotometric method for the determination of uranium(VI) was the direct elimination of the important interference caused by the presence of plutonium, it seems worthwhile to sum up as follows the main facts established experimentally in this connection.

(*i*) The preliminary complexation of plutonium(IV), followed by the addition of reductants, furnishes much more satisfactory results than the reverse order of addition. This procedure largely reduces the risk of losing partially, or even entirely, the uranium(VI) that may be co-precipitated on the plutonium trifluoride and plutonium tetrafluoride precipitates.

(*ii*) When reductants were added, following the use of fluoride *plus* DCTA as masking agents, no appreciable plutonium trifluoride precipitate formation could be observed when 5-mg amounts of plutonium(IV) were initially present, even after 24 hours had elapsed.

(*iii*) It is concluded that the use of reductants in the aqueous phase to convert plutonium(IV) into plutonium(III) becomes unnecessary because if they are used alone, the plutonium(III) is appreciably co-extracted; if they are used prior to the addition of masking agents (especially fluoride) plutonium trifluoride is formed on which uranium(VI) may largely co-precipitate; and, finally, if they are used after preliminary treatment of the plutonium(IV) with a large excess of fluoride, no additional improvement seems to result from their addition.

From this, it becomes obvious that the best experimental conditions to suppress the plutonium interference are also about the worst for the quantitative extraction of uranium(VI) into the organic phase. In fact, the order of addition of the reagents (Table V) to the aqueous phase has been shown to be unexpectedly critical in this connection.

Finally, there are the following fortunate circumstances concerning the minimisation of the plutonium interference in the uranium(VI) determination when using the proposed extraction - spectrophotometric method: uranium(VI) forms with arsenazo III in aqueous - organic monochloroacetic medium a complex compound exhibiting a much higher molar absorptivity than the corresponding plutonium(IV) complex species; the uranium(VI) - arsenazo III complex exhibits a very sharp absorption band, the maximum of which appears at 655 nm while that of the equally sharp corresponding plutonium(IV) complex is located at 670 nm; and 0·5 ml of 10 per cent. DCTA solution masks effectively up to 50 μg of plutonium per 10 ml of aqueous - organic phase and has no appreciable effect on the uranium(VI) - arsenazo III complex.

Since this work was completed it has been found that the use of xylene instead of benzene as a solvent for TOPO in the extraction of uranium(VI) from aqueous nitric acid gives more quantitative results and is to be preferred, even though the mutual solubility of the components of the aqueous - organic system becomes slightly reduced and may result in somewhat more critical conditions for the stability of the final sample from the point of view of emulsion formation.

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Determination of Iodine and Bromine in Biological Materials by Neutron-activation Analysis

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Iodine and bromine have been determined in some biological materials by neutron activation analysis. These elements are extracted from irradiated samples with a 5 per cent. solution of trioctylamine in xylene, first the bromine being back-extracted with N sodium nitrate solution and then the iodine being back-extracted with N ammonia solution. The extraction yield is about 94 per cent. for iodine and about 86 per cent. for bromine. The limit of detection is about 0·01 μg for iodine and about 0·1 μg for bromine.

The precision of the method is about ±6 per cent. for both elements for concentrations exceeding 0·1 p.p.m.

MANY investigations have been carried out on iodine and bromine in biological and geological fields,[1,2,3,4,5,6] because both elements have important essential functions in biochemistry.

By using the radioactivation technique, Cosgrove, Bastain and Morrison[7] determined mixed halides in zinc sulphide phosphors and Dimitriadou, Turner and Fraser[8] analysed paper chromatograms of human serum to determine iodine.[9] Other workers have determined the concentration of both elements in many materials by this technique.

On the other hand, radiochemical separation of radioiodine and radiobromine in irradiated samples has been carried out by solvent extraction and an anion-exchange method. Iodine and bromine have generally been extracted with either carbon tetrachloride or chloroform,[10] and also distillation methods have been used. However, Moore[11,12] mentioned that the application of the technique for the extraction of radioactive simple acids (*e.g.*, hydrochloric acid, sulphuric acid, etc.) would be a potentially useful field of investigation for the radiochemist because it has not yet been reported by other workers.

In this work, iodine and bromine in some irradiated samples are extracted with a solution of trioctylamine in xylene and are determined by neutron-activation analysis.

Experimental

Reagents—

All reagents used were of analytical-reagent grade.

Trioctylamine - xylene solution—Dissolve 25 g of trioctylamine in 475 g of xylene.

Sodium nitrate solution, N.

Ammonia solution, N.

Potassium iodide standard solution—Prepare an aqueous solution containing 10 μg ml^{-1} of iodine.

Potassium bromide standard solution—Prepare an aqueous solution containing 100 μg ml^{-1} of bromine.

Potassium iodide carrier solution—Prepare an aqueous solution containing 10 mg ml^{-1} of iodine.

Potassium bromide carrier solution—Prepare an aqueous solution containing 10 mg ml^{-1} of bromine.

Preparation of sample—

Vegetable sample—Weigh 100 g of fresh vegetable sample, dry it in an oven at 110 °C for 24 hours, and then grind it to a powder. Transfer a portion of the powdered sample into a nickel crucible and fuse it with 5 g of sodium hydroxide and 1 g of sodium peroxide powder

in a muffle furnace at 450 °C for 30 minutes. After cooling, dissolve the fused cake in 50 ml of distilled water and warm the solution on a water-bath. After centrifuging it transfer the solution into a 100-ml measuring flask. With a pipette, place 0·5 ml of this solution on a 3 × 3-cm polythene sheet, evaporate it carefully to dryness with an infrared lamp and then heat-seal it.

Urine sample—Prepare 1 g of Dowex 1X8 resin (50 to 100 mesh) in a glass column 5 cm long and 1·0 cm in diameter. Wash the column with 100 ml of distilled water followed by 50 ml of N hydrochloric acid solution at the rate of about 2 ml minute^{-1}. Pass 100 ml of urine through the column. Wash the resin with 50 ml of distilled water and then dry it in an oven at 110 °C for 5 hours. After transferring the dried resin into a nickel crucible, fuse it with about 5 g of sodium hydroxide and 1 g of potassium nitrate in a muffle furnace at 450 °C for 5 hours and continue as for the vegetable sample described above.

IRRADIATION—

The vegetable and urine samples and two standard specimens were packed together in a polythene bag and the irradiation was then carried out in a thermal neutron flux of about 8×10^{13} neutrons cm^{-2} s^{-1} for 20 minutes in the JRR-2 reactor of the Japan Atomic Energy Research Institute.

ACTIVITY MEASUREMENT—

A TMC 400-channel pulse height analyser was used for the quantitative measurement of the photopeaks, mainly the 0·45-MeV peak for iodine-128, and 0·55-MeV or 0·78-MeV peak for bromine-82.

The photopeak area was evaluated according to Covell's method,[13] and iodine-128 and bromine-82 were identified from their half-lives as determined by following their decay and from the γ-ray energy associated with their radioactive decay.

RADIOCHEMICAL SEPARATION OF IODINE AND BROMINE—

Open the irradiated sample into a 50-ml glass beaker and add to it the equivalent of 10 mg each of iodine and bromine carrier. Dissolve the sample in 10 ml of distilled water and warm the solution on a water-bath. Then transfer the solution into a separating funnel, wash the beaker with 5 ml of distilled water, and add the washings to the funnel. Adjust the solution to a pH between 4 and 5 with a few drops of 0·1 N nitric acid solution and add 2 ml of 30 per cent. hydrogen peroxide solution. Extract the iodine and bromine with 15 ml of 5 per cent. solution of trioctylamine in xylene, shaking the mixture for 1 minute. Repeat this step twice. Discard the aqueous phase, then back-extract the bromine with 15 ml of N sodium nitrate solution, shaking the mixture for 1 minute. Precipitate the bromine from this solution as silver bromide. After back-extracting the bromine, add 15 ml of N ammonia solution to the separating funnel containing the organic phase and then back-extract the iodine, shaking the mixture for 1 minute. Precipitate the iodine as silver iodide. After filtering the precipitates, count the radioactivity of iodine-128 by γ-ray spectrometry and then, after allowing the precipitate containing bromine-82 to stand for 24 hours, measure

TABLE I

RESULTS OBTAINED FOR THE DETERMINATION OF IODINE AND BROMINE IN SOME BIOLOGICAL SAMPLES

	Iodine, p.p.m.	Bromine, p.p.m.
Dried vegetable—		
Daucus carota Linn, var. *sativa* DC ..	0·032, 0·030, 0·031	0·221, 0·215, 0·200
Brassica pekinensis Rupr.	0·016, 0·017, 0·015	0·157, 0·163, 0·160
Spinaci oleracea Linn.	0·013, 0·014, 0·013	0·118, 0·118, 0·110
Raphanus sativus Linn.	0·009, 0·011, 0·009	0·134, 0·141, 0·138
Allium cepa Linn.	0·040, 0·041, 0·037	0·455, 0·433, 0·463
Urine (human)—		
No.		
1	0·169, 0·199, 0·181	2·541, 2·688, 2·760
2	0·161, 0·157, 0·163	2·368, 2·476, 2·558
3	0·109, 0·098, 0·093	2·251, 2·347, 2·238
4	0·199, 0·207, 0·213	4·100, 4·125, 4·199
5	0·357, 0·342, 0·311	5·307, 5·321, 5·219

the radioactivity of bromine-82 by γ-ray spectrometry. The time required for the separation, including that from the end of irradiation to counting, is less than 30 minutes for five samples. As iodine-128 is a short-lived radionuclide, counting of its radioactivity must be carried out without delay.

Results and discussion

The amounts of iodine and bromine contained in some biological materials have been determined by thermal-neutron activation analysis. The results obtained are shown in Table I. A systematic addition method for the determination of these elements has also been examined. Several samples were prepared and spiked with various amounts of standard. The results obtained by the addition method are shown in Table II.

Table II

Determination of iodine and bromine in urine and vegetable samples by standard method

Sample	Amounts of sample	Amounts of standard added/μg		Activity in the precipitate/counts minute^{-1}		Iodine found/μg	Bromine found/μg
		Iodine	Bromine	Iodine	Bromine		
Vegetable ..	1 g	—	—	7 019·5	2 560·8	—	—
		0·01	0·1	9 171·5	3 691·7	0·032	0·226
		0·02	0·2	11 125·5	4 646·8	0·034	0·241
		0·05	1·0	17 609·8	7 529·0	0·031	0·257
Urine ..	1 ml	—	—	35 088·0	19 581·8	—	—
		0·01	1·0	37 351·7	27 447·0	0·155	2·489
		0·02	2·0	39 265·1	35 249·0	0·168	2·501
		0·05	5·0	46 121·9	58 243·8	0·159	2·531

By using this solvent-extraction technique, it appears from the results obtained that the proposed method is simple and rapid, particularly for the determination of iodine. The radiochemical purities of the separated iodine-128 and bromine-82 were checked from their γ-ray spectra and decay curves (Fig. 1).

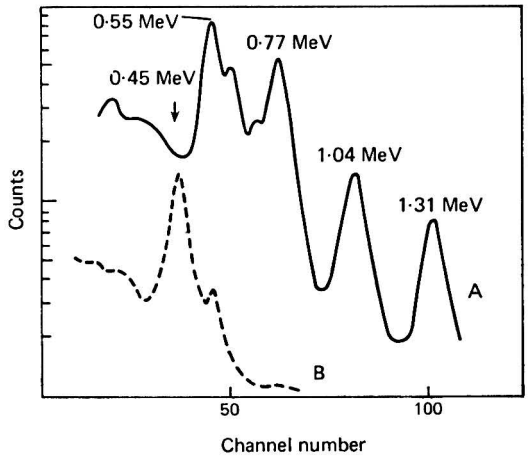

Fig. 1. γ-Ray spectra of A, bromine-82 and B, iodine-128 from the irradiated sample after solvent extraction

During dry ashing or alkali fusion of biological samples in a muffle furnace it is known that some elements are partially lost; therefore, to determine the loss of iodine and bromine when the samples were prepared for irradiation, the urine and vegetable samples were spiked

with known amounts of iodine-131 and bromine-82. These spiked samples, prepared for alkali fusion, were then heated in a furnace at selected temperatures for periods ranging from 5 to 10 hours. After the fused samples had been dissolved in distilled water to give a clear solution, the loss of both elements and the chemical yield were determined by the subsequent procedure as described above. The results obtained are given in Table III, in which losses after alkali fusion are expressed as percentages. The results show that iodine and bromine are largely retained when the alkali fusion of some biological materials is carried out at temperatures below 450 °C.

TABLE III

LOSS OF IODINE AND BROMINE ON ALKALI FUSION OF URINE AND VEGETABLE SAMPLES IN A MUFFLE FURNACE

Ashing time/hours ..	5	10	5	10	5	10	5	10
	Loss from urine sample, per cent.				Loss from vegetable sample, per cent.			
Heating temperature/°C	Iodine		Bromine		Iodine		Bromine	
300	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·1
400	1·8	2·1	1·0	1·5	1·4	3·1	1·1	1·7
450	6·2	6·9	5·2	5·5	6·6	7·3	5·0	6·9
500	7·0	7·9	6·1	6·5	7·8	9·1	6·0	6·9
550	8·3	8·5	8·0	8·1	8·6	8·8	8·0	8·4
600	12·5	11·9	10·0	10·6	11·8	12·1	9·8	13·2

The sensitivity of detection was calculated on the basis of the minimum detectable photopeak area for iodine-128 and for bromine-82. The sensitivity limits of this method were determined and found to be 0·01 μg for iodine and 0·1 μg for bromine, and the precision was about ± 6 per cent. for both elements for contents exceeding 0·1 p.p.m.

Extraction with carbon tetrachloride will separate radioiodine from the other radionuclides, and the same solvent can also be used subsequently to extract the radiobromine. However, use in this work of an amine of high molecular weight, which is a liquid anion exchanger, enabled the iodine and bromine to be extracted and separated with ease. Moreover, the radiochemical yield for both elements, particularly that for bromine, is higher than that reported by other workers.[2]

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Loss of Cobalt and Iron from Lithium Tetraborate Fusions in Graphite Crucibles

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It has been found that both iron and cobalt are lost from lithium tetraborate fusions performed at 1 200 °C in graphite crucibles. The elements segregate as tiny pieces of metal near the graphite - fusion interface owing to reduction. The same reaction could well occur with other metals of lower affinity for oxygen than carbon and so these also would disappear from the fusion.

In the course of work to develop a spectrographic method for the analysis of high silica materials (sands, etc.) in the solid form, cobalt and manganese oxides were found to be good internal standards. In this method the sample, suitably powdered, is mixed in one of two possible proportions, 1 + 6 or 1 + 9, with a lithium tetraborate flux containing the internal standards. The mixture is fused at 1 200 °C in a graphite crucible for 15 minutes, shattered in water, ground, mixed with graphite, pressed into disc electrodes and then excited by an a.c. arc on a direct-reading spectrometer. The graphite crucibles were produced from 24-inch long, 1½ inch diameter Morganite EY9 graphite rods.

Preliminary experiments

Originally the internal standards were mixed without fusion with the lithium borate flux. In an experiment to determine optimum fusion time for samples, it was found that melts after 20 minutes' fusion appeared appreciably less blue than those after 10 and 15 minutes. On arc-ing the discs prepared from the fusions, not only had the cobalt internal standard intensity decreased for the 20-minute fusion but also the intensity of iron contained in the sample. The variation in the cobalt intensity in the fusions was thought at first to be caused by possible bad mixing. A fresh batch of flux was prepared containing the same amounts of tricobalt tetroxide (Co_3O_4) (about 1 per cent.) and trimanganese tetroxide (Mn_3O_4) (about 0·5 per cent.) as the original, but on this occasion the flux was pre-fused in platinum after mixing, and then re-ground.

In similar fusion-time experiments with the new flux (5, 10, 15 and 20 minutes) it was found that melts still became progressively paler blue in colour. Intensities of iron and cobalt again decreased with time of fusion, the decrease being appreciable after 15 minutes, although that of manganese appeared to increase slightly (see Fig. 1).

As there was an apparent loss of two components, one an internal standard and the other an element to be determined in analysis, it was decided to investigate the phenomena more closely.

From visual observations of successive fusions in the same crucible, it appeared that loss of blue colour increased with repeated use of the crucible. Crucibles were therefore pre-treated by carrying out in them several fusions of about 2 g of a mixture of pure silica and pure lithium borate, 1 + 9. After four of these fusions it was found that any fusion of 0·2 g of high silica material and 1·8 g of lithium borate containing 1 per cent. of cobalt oxide would consistently yield a pale blue bead. The beads were much paler in colour than the same mixture fused, say, in a platinum crucible or in a previously unused graphite crucible.

It was thought that the iron and cobalt might migrate into the crucibles, so several fusions involving iron and cobalt were performed to test this hypothesis. It was found that, in three crucibles tested, after five successive fusions there was a small amount of movement of iron and cobalt to the graphite but not enough to account for the large losses in colour.

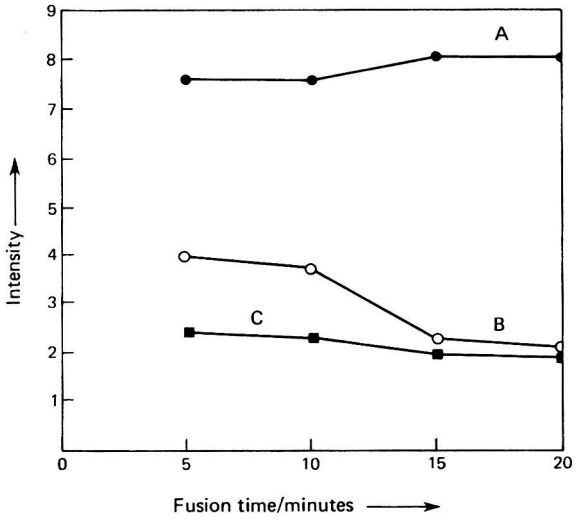

Fig. 1. Change in iron, manganese and cobalt with length of fusion time: A, Mn 2576 Å; B, Co 3405 Å; and C, Fe 2599 Å

Investigation

In addition to the fact that the loss of cobalt blue colour from fusions became more apparent when the crucible had been used once or twice previously, another phenomenon noted was that the molten fusion product increasingly tended to stick to the crucible. It was decided to "pre-treat" a crucible as described earlier, and then fuse in it a mixture containing cobalt and iron. Each part of the system was then analysed to determine the distribution of cobalt and iron. Initially all of the cobalt is contained in the mixture to be fused and none in the crucible or its lid. Most of the iron is in the mixture to be fused but a small amount is present as an impurity in the graphite (0·48 mg of iron oxide (Fe_2O_3) per 10 g of graphite, *i.e.*, 0·0048 per cent.).

The components investigated were as follows: (*i*) lid of crucible; (*ii*) crucible; (*iii*) ash produced from burning of the crucible and lid during fusion; (*iv*) melts that can be poured from the crucible; and (*v*) melts that stick to the crucible.

A mixture of 0·2 g of B.C.S. 267 silica brick (0·79 per cent. of Fe_2O_3) and 1·8 g of flux (0·86 per cent. of Co_3O_4) was fused at 1200 °C for 30 minutes. Table I shows how the cobalt and iron were redistributed after fusion.

Table I

Migration of iron and cobalt in system

Masses of Fe_2O_3 and Co_3O_4 in fractions

	Unfused mixture	Free melt	Sticking melt
Fusions—			
Fe_2O_3/mg	1·6	0·6	1·1
Co_3O_4/mg	16	1	16
Percentage of total mass	100	75	24
Concentration of Fe_2O_3 in cooled melt, per cent.	0·079	0·036	0·23
Concentration of Co_3O_4 in cooled melt, per cent.	0·79	0·067	3·32

Graphite—	Migrated Fe_2O_3/mg	Migrated Co_3O_4/mg
Lid	None	None
Crucible	None	0·11
Ash	None	0·03

Co_3O_4 and Fe_2O_3 were determined photometrically by standard techniques, the former with nitroso-R-salt and the latter with *o*-phenanthroline.

As can be seen from Table I, no iron has migrated into any part of the crucible or lid and a very small amount of cobalt has been picked up by the crucible.

Most of the iron and cobalt has migrated into the sticking part of the melt, which is only a small fraction of the total melt. There is, therefore, a large build-up of these elements in this part of the melt, as is shown by the comparison of concentrations in Table I.

Visual examination of the sticking melts revealed that even these had a very pale blue colour. There was, however, a black layer, which was harder than graphite, adhering to the edge of this part of the melt. Further experiments were therefore performed to examine this region.

Crucibles were pre-treated as before and the mixtures of 0·2 g of B.C.S. 267 *plus* 1·8 g of flux were fused in each of them for 50 minutes at 1 200 °C, to allow the effect to develop fully. The melts were allowed to cool in the crucibles and were then removed; they had the appearance shown in Fig. 2.

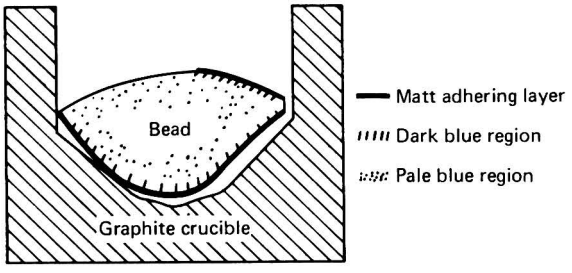

Fig. 2. Appearance of fusions

The dark blue regions of cobalt colour were noticeable only where the graphite crucible, or floating graphite powder, had been in contact with the melt. This layer was examined in four ways: by chemical analysis, X-ray diffraction, microscopy and electron probe microanalyser.

CHEMICAL ANALYSIS—

The adhering layer was removed with a diamond drill; however, it was impossible not to remove with the layer some of the bead produced from the fusion. The amount of the layer removed was about one half of the deposit on the bead. The results of analysis are shown for comparison in Table II.

TABLE II

COMPARISON OF ANALYSIS OF SURFACE LAYER WITH ORIGINAL UNFUSED MIXTURE

	Oxide in surface layer (about one half of total layer)	Oxide mix before fusion
Mass of Co_3O_4/mg	6·54	15·8
Mass of Mn_3O_4/mg	0·65	≃10
Mass of Fe_2O_3/mg	0·45	1·58
Concentration of Co_3O_4, per cent. ..	6·75	0·79
Concentration of Mn_3O_4, per cent. ..	0·67	≃0·5
Concentration of Fe_2O_3, per cent. ..	0·46	0·079

Co_3O_4, Mn_3O_4 and Fe_2O_3 were determined photometrically by standard techniques, Co_3O_4 with nitroso-R-salt, Mn_3O_4 as permanganate by oxidation with periodate and Fe_2O_3 with *o*-phenanthroline.

It is clear from Table II that there is a build-up of cobalt and iron in this layer, which indicates considerable migration to the graphite - fusion interface. It can be calculated that nearly all of the iron and cobalt have appeared in the matt layer.

X-RAY EXAMINATION—

An X-ray powder diffraction photograph revealed the presence of cobalt metal, graphite and a little silica in the surface layer.

EXAMINATION BY ELECTRON PROBE MICROANALYSER—

Measurements were made of iron Kα and cobalt Kα intensities along a line moving inwards from the edge of the bead (Figs. 3 and 4). It appears that there is a build-up of cobalt and iron into a layer about 10 μm thick at the surface of the bead.

This layer consists of metallic particles close to the surface. No manganese was detected in these metallic particles, and the composition of the particles estimated from the intensities was shown to be about 10 per cent. of iron and 90 per cent. of cobalt, which would suggest the formation of metal regions by reduction of the oxides from the bulk of the melt.

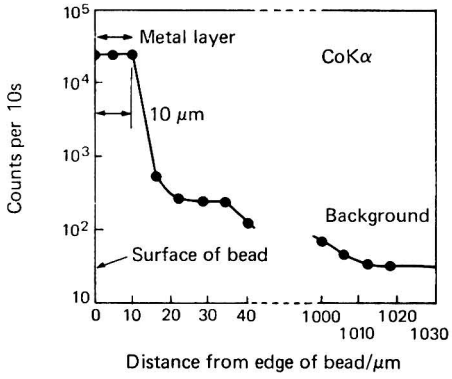

Fig. 3. Logarithmic graph of cobalt Kα intensity against distance from edge of bead

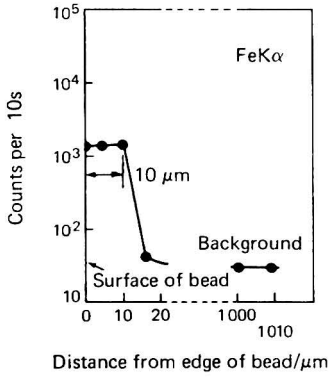

Fig. 4. Logarithmic graph of iron Kα intensity against distance from edge of bead

EXAMINATION BY MICROSCOPY—

Examination under a microscope revealed the presence of metal particles at and near the surface of that part of the bead which had been in contact with either the graphite crucible or the graphite powder floating on the surface. The metal appears as a fern-like structure, which seems to grow inwards from the outside of the bead. Fig. 5 shows a section through the bead. Fern-like structures appear at the bottom of the melt and also, to a lesser extent, on the top surface. Fig. 6 shows a magnification of the lower edge of the bead and brings out the fern-like metal structure. Fig. 7 shows part of the top edge of the bead and displays not only the fern-like metal structure but also the bluer area of the bead, which shows as a darker region in the photograph.

Fig. 8 shows the lower region of the bead viewed by reflected light. The white regions are metal particles showing through the surface.

DISCUSSION

From the chemical, microscopic, X-ray and electron probe microanalyser evidence it is clear that the iron and cobalt metals are being produced by reduction with graphite in areas where the flux and graphite are in contact. Manganese does not appear to be reduced noticeably.

If a study is made of graphical representations of ΔG° of formation against temperature for the oxides of iron, cobalt, manganese and carbon, such as those given by Ellingham[1] and other workers,[2] it is clear that at 1200 °C carbon has a greater affinity than iron or cobalt for oxygen, as the $-\Delta G^\circ$ value for the formation of carbon monoxide is greater than those for iron oxides (Fe_2O_3, FeO and Fe_3O_4) and cobalt oxide (CoO).

Study of similar diagrams for other oxides such as lead oxide (PbO), nickel oxide (NiO), zinc oxide (ZnO) and copper oxides (CuO and Cu_2O) reveals that these too can be reduced by carbon at 1200 °C. This would suggest that the fusion process intended as a means of homogenising the sample serves only to segregate several important metallic components contained in it.

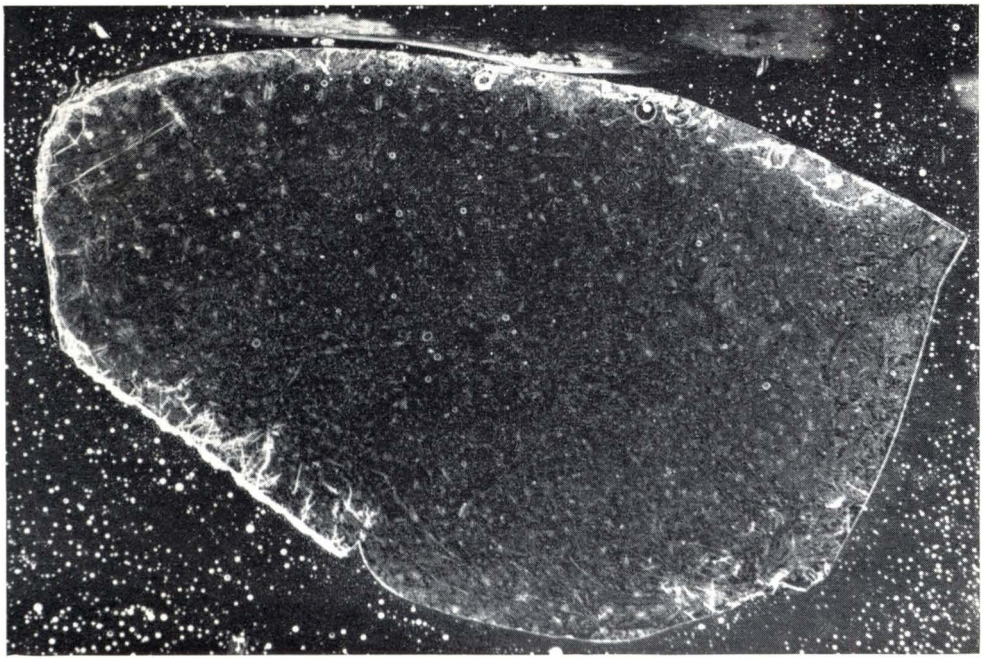

Fig. 5. Section through bead

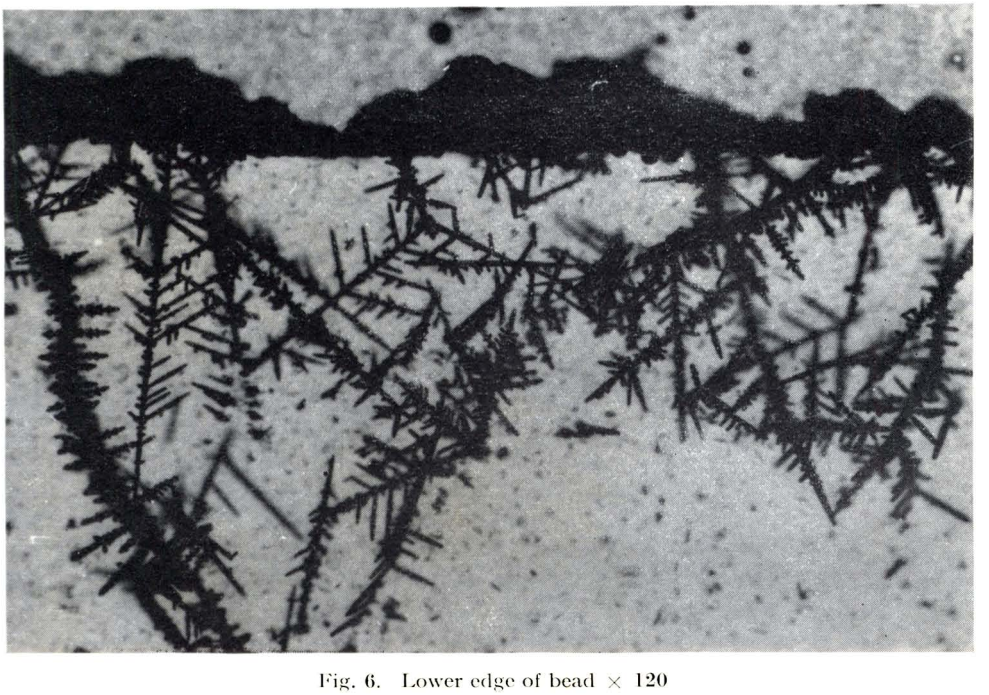

Fig. 6. Lower edge of bead × 120

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The mechanism seems to comprise reduction of the dissolved oxides whenever they come into contact with the graphite. The surface must then become depleted of dissolved oxides, and migration of these oxides to this region must occur to maintain the uniformity of their concentrations throughout the fusion. The reaction must ultimately continue to some equilibrium point at which almost all of the iron and cobalt have been reduced.

Conclusions

When preparing lithium tetraborate fusions of high-silica materials in graphite crucibles at 1 200 °C cobalt oxide added as internal standard and iron oxide contained in the sample are lost from the melt. The mechanism of this loss comprises reduction of these metals from their combined state by the graphite of the crucible. The reaction appears to occur more readily if the crucible has been used previously and if the fusion time exceeds 10 minutes. If fusions at 1 200 °C are required for an analysis technique it would be advisable not to use graphite crucibles, but to make use of those made of a non-reducing material such as platinum or platinum alloys.

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A Sensitive, Specific Method for Determining the Riboflavin Content of Children's Urine

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A four-part method based on Wahba's technique is presented for the specific determination of the riboflavin content of children's urine. First, the interfering urinary constituents are precipitated by treating the urine sample with zinc acetate and formalin solutions; they are then removed by centrifugation. The riboflavin in the resultant supernatant liquid is next separated from the other soluble molecular constituents of urine by elution chromatography, by using a talc column. The riboflavin eluate is then subjected to one-dimensional chromatography on silica gel plates to confirm the identity of the fluorescent spot before the determination. Finally, the thin-layer chromatographic plate is dried and the riboflavin content of the plate is measured fluorimetrically by using the technique of reflectance densitometry.

This investigation originated in an observation made by Mr. F. G. Warburton, Chief Biochemist, Hope Hospital, Salford, based on paper-chromatographic experiments, that the urine of certain mentally retarded children contained abnormally large amounts of riboflavin (or a substance that has similar fluorescent and chromatographic properties). To determine the significance or otherwise of this observation, it was necessary to devise a rapid, specific and sensitive method for determining riboflavin in children's urine.

A survey of the reported methods for determining this vitamin revealed that currently the most widely used are microbiological[1,2] and fluorimetric.[3,4] The former, which have superseded procedures involving animal growth rates, are still time consuming, while the fluorimetric methods are not entirely specific.[5] Various techniques have been adopted in an attempt to estimate fluorescent substances that may interfere in fluorimetric measurements. These include chemical treatment of the urine with potassium permanganate and hydrogen peroxide followed by reduction with sodium hydrosulphite,[4,6,7] or selective adsorption of the vitamin from the urine on lead sulphide[3] or Florisil.[8] Recently, Wahba[9] reported a procedure for the selective adsorption of riboflavin from adults' urine involving the use of a talc column, followed by an absorption spectrophotometric determination of the vitamin in the eluted solution. This method is not specific for any particular flavin and is not sensitive enough for use with small volumes of urine.

In this paper we describe a method for the specific determination of flavins in small volumes of human urine based on direct fluorimetric densitometry of thin-layer chromatograms of solutions eluted from a talc column.

Method

Apparatus—

The equipment used for the thin-layer chromatographic experiments was supplied by Shandon Scientific Co. Ltd. The thin-layer chromatograms were made quantitative by using a "Chromoscan" automatic recording and integrating double-beam reflectance densitometer (J. Loebl and Co. Ltd., Gateshead-on-Tyne). Electronic absorption spectra were recorded on a Perkin-Elmer 137 spectrophotometer.

Reagents and materials—

Zinc acetate.
Toluene.
Methanol.

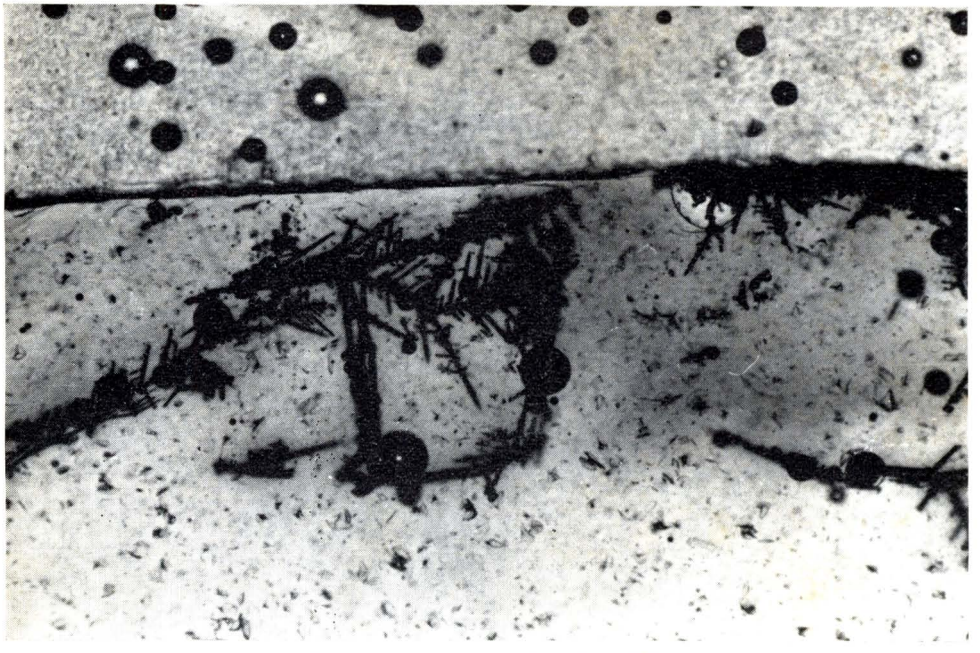

Fig. 7. Upper edge of bead × 120

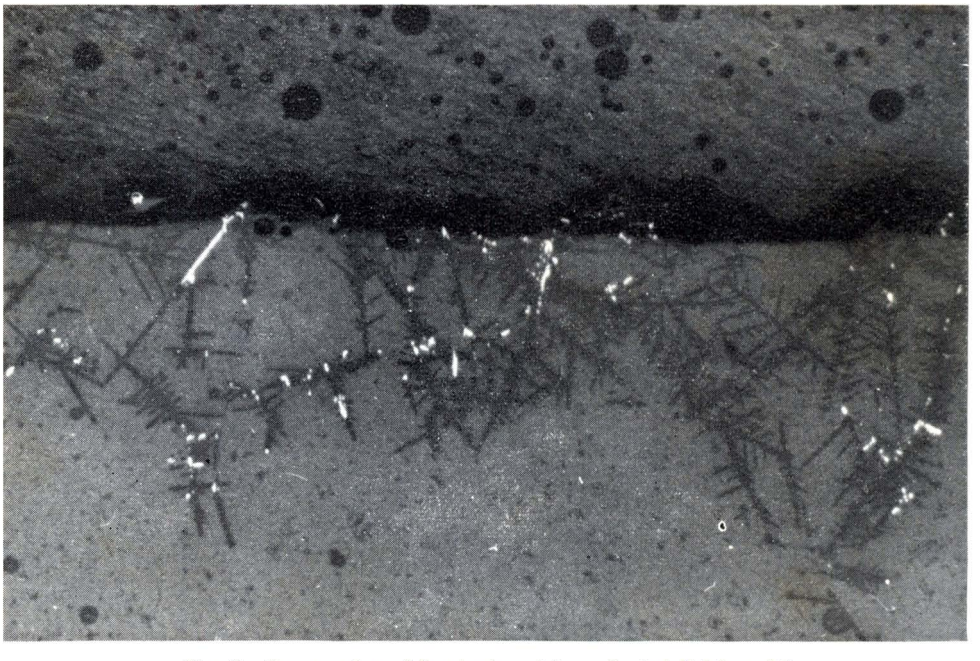

Fig. 8. Lower edge of bead viewed by reflected light × 75

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Glacial acetic acid.
Dioxan.
All of the above reagents were of analytical-reagent grade.
Formaldehyde solution (formalin 40 per cent.).
Hydrochloric acid, 0·01 N.
Talc, acid purified—British Drug Houses Ltd.
Silica gel—Merck Chemical Corporation.
Riboflavin 5'-phosphoric acid, monosodium salt (FMN)—British Drug Houses Ltd.
Lumichrome—R. N. Emmanuel.
Riboflavin—Eastman Kodak Corporation. This was extracted with chloroform and dried *in vacuo* for 2 hours at 105 °C over phosphorus(V) oxide as described by Pearson.[10]
Lumiflavin—This was prepared from riboflavin by the method of Koziol and Knobloch,[11] care being taken to acidify the mixture at the end of the reaction with hydrochloric acid prior to extraction of the product with chloroform.
Solvent systems for thin-layer chromatography—1: Toluene - methanol - acetic acid (50 + 45 + 5, v/v); 2: benzene - methanol - butanol - acetic acid (70 + 20 + 5 + 5, v/v).[12]

PREPARATION OF STANDARD SOLUTIONS—

Make up the standard solutions of the flavins (25 mg l^{-1}) in acetate buffer (pH 4·65) and 25 per cent. v/v aqueous dioxan solution. These solutions are stable for periods of 1 month when kept in the dark in a refrigerator.

PREPARATION OF TALC COLUMNS—

Remove the fines from the acid-purified talc by successive washing with water and make up the columns (0·8 × 6 cm high) by following Wahba's procedure.[9]

PREPARATION OF THIN-LAYER PLATES—

Spread thin layers of silica gel (250 μm thick) on 20 × 20-cm glass plates, store overnight at room temperature and dry for 1 hour at 110 °C immediately before use.

PROCEDURE—

In order to minimise photochemical decomposition of the flavins the determination should, if possible, be performed in diffused light.

Measure the total volume (V_v) of the urine and add 100 mg of oxalic acid to every 25 ml of the sample as soon as possible after collection, in order to ensure a pH value of less than 6 as riboflavin has maximum stability at acidic pH values. To a volume of the acidified urine (V_t), normally 10 ml, add consecutively 0·2 g of zinc acetate and 0·2 ml of formalin solution to remove urinary constituents that interfere in the thin-layer chromatography and subsequent densitometry.[9] Allow the mixture to stand for 30 minutes and then centrifuge it.

Apply the supernatant liquid to a talc column that is connected via a side-arm test-tube receiver to a water-pump working at sufficient pressure to maintain a flow-rate of 4 to 6 ml $minute^{-1}$. The riboflavin is adsorbed at the top of the column and is clearly visible. Remove the urinary pigments from the column by washing it successively with a little distilled water, 10 ml of 0·01 N hydrochloric acid and 10 ml of 5 per cent. v/v aqueous dioxan solution. Now elute the riboflavin from the column with 25 per cent. v/v aqueous dioxan solution. Collect the eluate as the yellow flavin band approaches the bottom of the column and continue collecting it until it becomes colourless again. (In all of the samples examined to date in this laboratory the riboflavin has been clearly visible as a yellow band.)

Place 10 or 20-μl volumes of the yellow eluate solution 1·5 cm from the bottom of a thin-layer plate, keeping the spot size as small as possible,[13] and develop the plate in a saturated chamber containing solvent system 1. Remove the plate when the solvent front moves a distance of 13 cm, usually after 1 hour, and dry it in a forced-draught oven maintained at 60 °C for 15 minutes. Determine the volume of the riboflavin eluate solution (V_e) carefully by placing it in a sufficiently large graduated flask (normally 10 ml will suffice; see column 4, Table II), and then make up to the mark by adding a measured volume of 25 per cent. v/v aqueous dioxan solution from a burette.

Identify the riboflavin spot on the thin-layer chromatogram by observing the fluorescence on the plate under ultraviolet light at the position corresponding to a R_F value of 0·44 (Table I). Make the riboflavin spot on the thin-layer plate quantitative by using the technique of fluorescence densitometry.[14,15] In this laboratory a "Chromoscan" recording densitometer was used for this purpose as follows: the instrument was used in the reflectance mode and a piece of non-fluorescent filter-paper (Whatman No. 1) was placed behind the plate during the measurements. Irradiation of the plate with light of the correct wavelength (365 nm), from a quartz - iodide lamp source in combination with a Wood's filter, caused the riboflavin spot to fluoresce at 530 nm. Any non-specific reflected light originating from the exciting radiation was removed with a Kodak Wratten gelatin filter 2B. A standard optical density wedge (0·5 cm thick) was used to achieve a satisfactory balance with the reference beam. The aperture setting used was 1005 (slit 10 × 0·5 mm) and the gear ratio was 1:2. All measurements were carried out in a direction perpendicular to the direction of chromatography, but the automatic integrator was not used because it was shown in earlier work to be inaccurate.[13] Instead, the area under the densitometric curve was obtained from the product of the peak height and the width of the band at half-maximum height.[16]

A standard graph of the densitometric area of the riboflavin spot *versus* the amount of riboflavin in micrograms was obtained by applying aliquots of the standard solution to the plate and then following the procedure for development and densitometry given above. All of these calibration graphs passed through the origin but different gradients were obtained for each batch of plates. An indication of the range of densitometric areas as a function of the amount of riboflavin in a spot can be obtained from an examination of columns 2 and 3 in Table II.

Calculation of the riboflavin content of urine—

The total amount of riboflavin F (in micrograms) in a urine sample can be calculated from the volume measurements, the densitometer area measurements and the standard calibration graph by using the following general equation—

$$F = 10^3 \frac{V_v \cdot V_e \cdot x}{V_t \cdot Y}$$

where F is the total amount of riboflavin, in micrograms, in urine sample; V_v the volume, in millilitres, of urine sample or voiding; V_e the volume of aqueous dioxan eluate, in millilitres, from the talc column; x the amount of riboflavin, in micrograms, in the fluorescent spot (R_F = 0·44 on the thin-layer plate, as determined from the densitometer area measurements via the standard calibration graph; V_t the volume of treated urine, in millilitres, applied to the talc column; and Y the volume of aqueous dioxan eluate, in microlitres, applied to the thin-layer plate.

This general equation can be further simplified to

$$F = 5 \cdot V_v \cdot V_e \cdot x$$

if constant sample volumes of the urines are applied to the talc column (V_t = 10 ml) and if constant volumes of the aqueous dioxan eluates are applied to the thin-layer plates (Y = 20 μl).

Results and discussion

Specificity of the method—

This was confirmed by performing the talc adsorption and the thin-layer chromatography on solutions of riboflavin, lumichrome and the five flavins listed in Table I. It was found that all seven compounds were adsorbed on the talc column and eluted together by the aqueous dioxan solvent mixture. The flavins have maximal absorption in the range 445 to 449 nm in 25 per cent. aqueous dioxan solution, with $\epsilon_{max.}$ ranging from 6 000 to 12 000 mole^{-1} cm^{-1}. This combination of identical elution volumes and similarity in absorption spectra leads to the conclusion that Wahba's spectrophotometric method[9] conducted at 446 nm is not suitable for determining the riboflavin content of urine that contains riboflavin metabolites.[1]

The separation and identification of the various compounds in the eluate from the talc column was achieved by thin-layer chromatography, by using solvent systems 1 and 2.

The R_F values in solvent system 2 (Table I) are in agreement with those estimated from the diagram given by Treadwell, Cairns and Metzler.[12] Additional confirmation of the identity of the fluorescent spots on the thin-layer plates has been obtained in this laboratory by electron-impact mass spectrometry, and these observations form the subject of a paper to be published shortly.[17]

TABLE I

R_F VALUES OF THE RIBOFLAVIN, LUMICHROME AND RIBOFLAVIN METABOLITES ON THIN LAYERS OF SILICA GEL

Compound	R_F in solvent system 1	R_F in solvent system 2
Flavin mononucleotide (FMN)	0·0	0·0
Flavin adenine dinucleotide (FAD)	0·0	0·01
Riboflavin	0·44	0·14
Hydroxyethylflavin (HEF) (7,8-dimethyl-10-(2′-hydroxyethyl)isoalloxazine)	0·55	0·32
Lumiflavin	0·60	0·39
Formylmethylflavin (FMF) (7,8-dimethyl-10-formylmethylisoalloxazine)	0·63	0·41
Lumichrome	0·68	0·58

TABLE II

DETERMINATION OF RIBOFLAVIN CONTENT OF CHILDREN'S URINE FOLLOWING ORAL ADMINISTRATION OF A VITAMIN TABLET CONTAINING 4 mg OF RIBOFLAVIN

Time after administration/hours	Area/mm²	Amount, x, of riboflavin in eluate sample (20 μl)/μg	Total volume of eluate, V_e/ml	Total riboflavin in eluate, 50 × V_e/μg	Concentration of riboflavin in urine, 5 × V_e/μg ml^{-1}	Total volume of urine in voiding, V_v/ml	Total riboflavin in voiding, F = 5 $V_v V_e x$/μg
Subject A—							
00·05	662	0·056	4·4	12·3	1·23	26	32·0
03·05	1975	0·167	6·7	55·9	5·59	158	883
05·25	4503	0·404	11·0	222·0	22·2	25	555
08·50	860	0·072	5·0	18·0	1·8	80	142
12·40	580	0·048	4·4	10·6	1·06	96	101
Subject B—							
02·30	2020	0·172	8·4	72·2	7·22	93	671
07·00	2968	0·252	6·1	76·9	7·69	56	431
09·00	915	0·076	3·3	12·5	1·25	12	15·0
11·30	860	0·072	3·7	13·3	1·33	15	20·0
14·00	1248	0·104	4·0	20·8	2·08	10	20·8
18·00	1721	0·146	3·2	23·4	2·34	10	23·4

TABLE III

COMPARISON OF RESULTS OBTAINED BY ABSORPTION SPECTROPHOTOMETRY WITH THOSE OBTAINED BY FLUORIMETRIC DENSITOMETRY

Amount of riboflavin added to urine/μg	Amount found/μg		Percentage recovery	
	Spectrophotometry	Fluorimetry	Spectrophotometry	Fluorimetry
14·75	Not detectable	15·2	—	103
29·50	27·5	28·12	93	95
35·40	34·3	35·2	97	99
41·3	38·4	40·32	93	97
147·5	151·15	143·1	102	97
247·8	248·0	236·0	101	95
443·0	408·0	432·0	92	98

APPLICATION OF THE METHOD—

As an example of the application of this method, Table II summarises some results obtained in this laboratory on the rate of urinary excretion of riboflavin by two children (aged 2 to 3 years) following oral administration of vitamin B_2 tablets.

It should be noted (Table II, column 7) that with this method the riboflavin content of small volumes of urine, as opposed to the 100-ml urine samples required for Wahba's method, can be determined.[9] This is of particular importance for the examination of samples obtained from small children and probably accounts for the fact that while results for riboflavin excretion rates have been reported for adults[18] and adolescents,[19] Table II contains the first results reported for infants.[20]

EFFICIENCY AND SENSITIVITY OF THE METHOD—

To compare the efficiency of this method with the earlier method of Wahba, known amounts of riboflavin were added to normal male adult urine samples and the percentage recovery was determined by both methods. The results of these experiments are tabulated in Table III. A study of the results given in this table shows that while both methods gave comparable percentage recoveries of the added riboflavin, the densitometric procedure can be used over a larger range of concentrations because of its greater sensitivity.

As a result of a considerable number of experiments the concentration limit of this specific method, when 10-ml samples of urine are applied to the talc column and 20-μl aliquots of the aqueous dioxan eluate are applied to the thin-layer plates, has been found to be 0·25 μg of riboflavin per ml of urine. The normal range[21] of adult urinary riboflavin concentration is 0·4 to 0·9 $\mu g\ ml^{-1}$ so that the method is suitable for the determination of the riboflavin content of normal human urine. As the method is not continuous it cannot be fully automated, but large numbers of samples can be handled in batches relatively easily and it is now being used routinely in this laboratory in a study of the riboflavin excretion patterns in infants.

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Analysis of Steroids.

Part XVII. A Differential Spectrophotometric Variant of the Diethyl Oxalate Method for the Determination of Ketosteroid Contaminants

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A method that consists of spectrophotometry following a Claisen condensation with diethyl oxalate was used for the determination of ketosteroid contaminants. Absorption of the main steroid component was eliminated by a differential spectrophotometric procedure. The proposed method is useful for the determination of ketosteroid contaminants present at the level of 0·1 per cent.

Recently, the present author reported a method for the spectrophotometric determination of ketosteroids, based on a Claisen condensation of these compounds with diethyl oxalate.[1] The resulting glyoxalyl derivatives showed an intensive and characteristic absorption band between 285 and 325 nm, which permitted the determination of the parent ketosteroids, as well as the analysis of steroid mixtures and ketosteroid formulations.

A differential spectrophotometric variant of the method for the determination of ketosteroid contaminants in various steroids of other types is reported in the present paper.

Experimental

Apparatus—

Spectrophotometer—Spektromom 202 ultraviolet spectrophotometer with 1-cm quartz cells.

Reagents—

All reagents were of analytical-reagent grade.

Solvent mixture—Mix 900 ml of t-butyl alcohol, distilled over sodium, with 100 ml of cyclohexane. The water content of the mixture should not exceed 0·05 per cent.

Sodium t-butoxide reagent, 0·25 N—Dissolve, by boiling, 2·88 g of sodium in 400 ml of the above solvent mixture and dilute the solution to 500 ml. When a crystalline precipitate is observed (this has occasionally occurred), the reagent is gently warmed before use. The reagent can be used for several months if it is stored in a well closed bottle. The reagent may be used provided its sodium hydroxide content is less than 30 per cent. of the total basicity. The procedure for the determination of sodium hydroxide has already been given.[1]

Diethyl oxalate reagent, M—Dilute 75 g (67·3 ml) of re-distilled diethyl oxalate to 500 ml with the above solvent mixture.

Ethanol, 96 *per cent. v/v.*

Hydrochloric acid, 0·5 N.

Procedure for the determination of oestrone 3-methyl ether contamination in oestrone 3-methyl ether 17-ethylene ketal—

Accurately weigh about 0·03 g of the material to be tested into a 50-ml calibrated flask. Add 2 ml of the solvent mixture, 2 ml of cyclohexane, 0·5 ml of diethyl oxalate reagent and finally, after dissolving the sample, 2·5 ml of sodium t-butoxide reagent. Allow the mixture to stand for 15 minutes at room temperature, then add 3 ml of 0·5 N hydrochloric acid and make up to the mark with ethanol.

Prepare the reference solution in a similar manner, the only difference being that the substance to be tested is weighed into the flask only after the addition of hydrochloric acid. The difference between the two weighings should not exceed 1 per cent.

Measure the difference between the extinctions at 295 nm and calculate the oestrone 3-methyl ether content by using the value $E_{1\,cm}^{1\,per\,cent.} = 376$.

The oestrone 3-methyl ether contamination in oestradiol 3-methyl ether and oestrone contamination in oestradiol can be determined in the same manner. In the latter case use $E_{1\,cm}^{1\,per\,cent.} = 395$ for the calculation.

PROCEDURE FOR THE DETERMINATION OF ANDROSTA-1,4-DIENE-3,17-DIONE CONTAMINATION IN ANDROSTA-1,4-DIENE-3,17-DIONE 17-ETHYLENE KETAL—

Proceed as in the above case, with the difference that before addition of the sodium t-butoxide reagent place the flask containing the mixture in an ice-bath and prolong the reaction period for 30 minutes under ice cooling.

Calculate the amount of the contaminant from the difference between the extinctions measured at 294 nm, by using the value $E_{1\,cm}^{1\,per\,cent.} = 398$.

PROCEDURE FOR THE DETERMINATION OF METHYLTESTOSTERONE CONTAMINATION IN METHANDIENONE—

Proceed as above, with the difference that in this case the final volume of solution should be 25 ml; the reaction period lasts only for 10 minutes (with ice cooling).

Measure the difference between the extinctions at 324 nm. Use $E_{1\,cm}^{1\,per\,cent.} = 223$ for the calculation.

RESULTS

The results of some model experiments are summarised in Table I. The agreement between calculated and found values is good. The reproducibility of the method is also satisfactory. The method is simple and rapid, and it has been used in routine analysis of the above substances.

TABLE I

RESULTS OF MODEL EXPERIMENTS TO DETERMINE CONTAMINANTS IN SOME STEROID DERIVATIVES

Main component	Contamination	Amount of contaminant		Standard deviation*
		Taken, per cent.	Found, per cent.	
Oestrone 3-methyl ether 17-ethylene ketal	Oestrone 3-methyl ether	0·12	0·14	
		0·39	0·36	±0·05
		0·94	0·97	
		1·52	1·55	
		2·08	2·10	±0·06
		2·66	2·64	
Oestradiol 3-methyl ether	Oestrone 3-methyl ether	0·18	0·20	
		0·55	0·58	
		1·04	1·03	
Oestradiol	Oestrone	0·15	0·16	±0·03
		0·30	0·30	
		0·67	0·63	
		1·21	1·19	±0·05
		1·90	1·85	
Androsta-1,4-diene-3,17-dione 17-ethylene ketal	Androsta-1,4-diene-3,17-dione	0·18	0·21	
		0·36	0·37	±0·04
		0·89	0·93	
		1·76	1·80	±0·07
		3·50	3·49	
Methandienone	Methyltestosterone	0·20	0·23	
		0·39	0·37	
		0·79	0·77	±0·05
		1·34	1·38	
		2·11	2·14	

*Six parallel runs.

Discussion

Our earlier paper[1] contains the details of the determination of optimum conditions for the condensation reaction and spectrophotometric measurement.

In some cases this method could be used directly for determination of ketosteroid contaminants in the presence of large amounts of steroids that do not react with diethyl oxalate, *e.g.*, for the determination of norethisterone contamination in ethynodiol, dehydroepiandrosterone contamination in 17α-methylandrost-5-ene-3β,17β-diol, etc. In these cases the steroid, being present in large excess, had no appreciable light absorption in the range 285 to 325 nm and therefore the determination could be carried out selectively on the basis of the light absorption of the glyoxalyl derivative. In several cases, however, the main component shows considerable absorption in this area whereby direct use of the diethyl oxalate method is not possible.

For example, in the case of determination of free oestrone 3-methyl ether contamination in oestrone 3-methyl ether 17-ethylene ketal the latter is present in a 100 to 1 000-fold excess and its spectrum (Fig. 1, curve A) partly overlaps that of 16-glyoxalyloestrone 3-methyl ether (Fig. 1, curve B), which forms selectively.

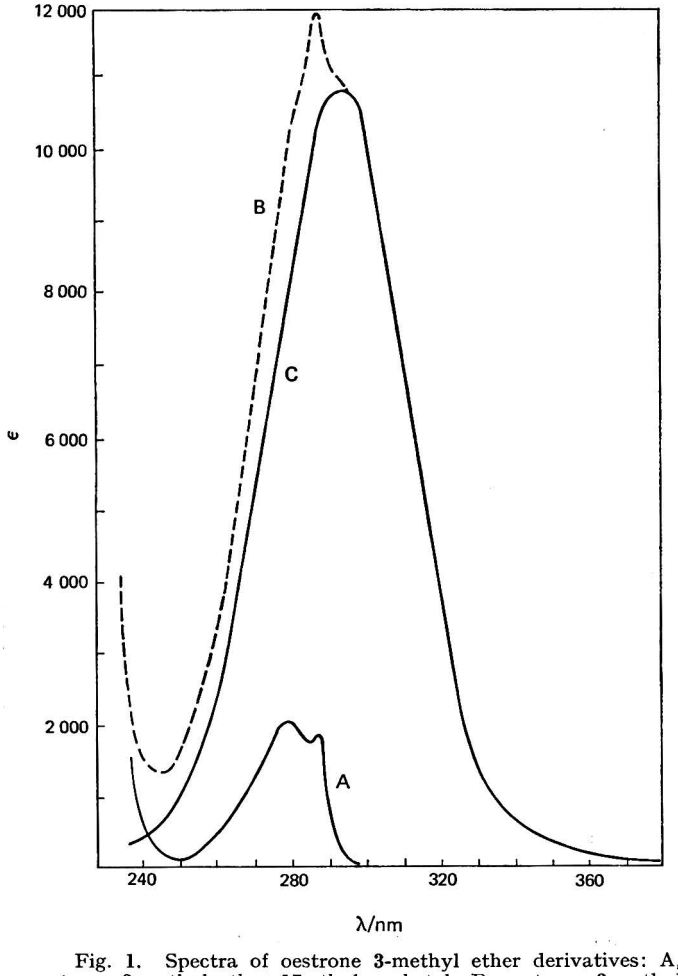
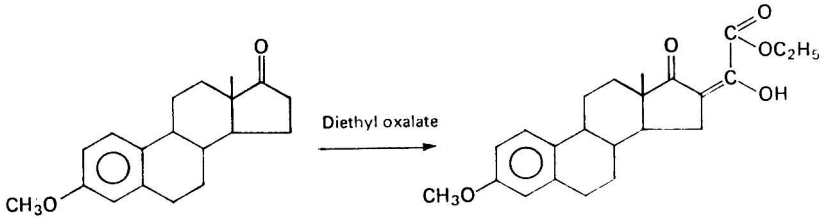
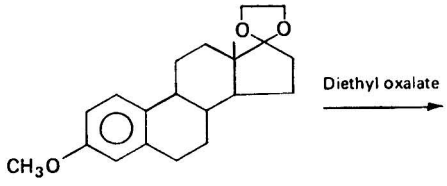


Fig. 1. Spectra of oestrone 3-methyl ether derivatives: A, oestrone 3-methyl ether 17-ethylene ketal; B, oestrone 3-methyl ether after treatment with diethyl oxalate; and C, oestrone 3-methyl ether after treatment with diethyl oxalate (difference spectrum)



Oestrone 3-methyl ether

16-Glyoxalyloestrone 3-methyl ether



No change

Oestrone 3-methyl ether
17-ethylene ketal

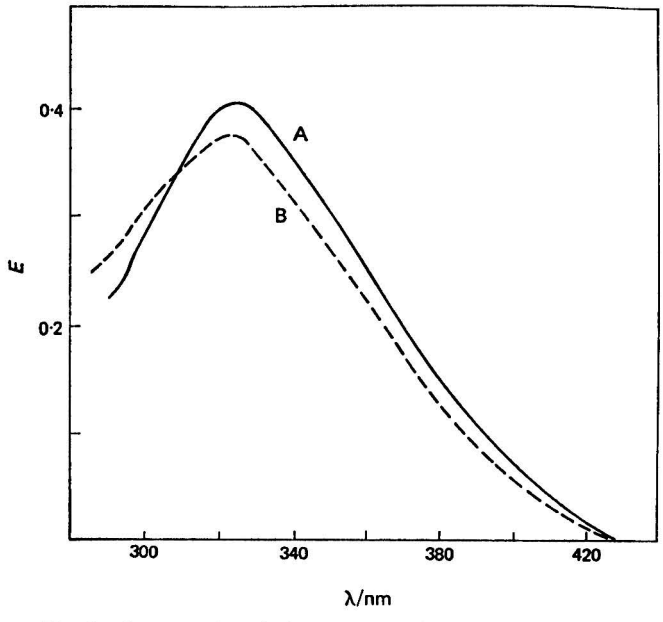


Fig. 2. Spectra of methyltestosterone derivatives: A, spectrum of methyltestosterone after treatment with diethyl oxalate (difference spectrum); and B, spectrum of methandienone containing 1·34 per cent. of methyltestosterone, after treatment with diethyl oxalate (difference spectrum)

In this case, the selective determination can be performed by differential spectrophotometry. The difference spectrum of 16-glyoxalyloestrone 3-methyl ether (Fig. 1, curve C) was taken in such a manner that the reference solution contained all the reagents and also oestrone 3-methyl ether at a concentration identical with that in the test solution; however, oestrone 3-methyl ether was added only after acidification of the reaction mixture in order to prevent its reaction with diethyl oxalate. The resulting difference curve shows the characteristic spectrum of 16-glyoxalyl-17-keto steroids ($\lambda_{max} = 295$ nm; $\Delta\epsilon = 10\,700$); the contribution of the phenolic ring A to curve B has been eliminated.

Determination of oestrone contamination in oestradiol and of oestrone 3-methyl ether contamination in oestradiol 3-methyl ether can be carried out on the same basis.

A similar problem arose during the determination of androsta-1,4-diene-3,17-dione contamination in androsta-1,4-diene-3,17-dione 17-ethylene ketal. As the 1,4-diene-3-keto system possesses no active methylene group, androstadienedione reacts selectively with diethyl oxalate at $C_{(16)}$. In this case the measurement at 294 nm was also disturbed by the main component ($\epsilon_{294} = 104$). In order to eliminate this interference, the determination was performed by differential spectrophotometry. As the main component was also found to react, although very slowly, with diethyl oxalate (presumably at $C_{(6)}$), the condensation was carried out under ice cooling, whereby the interfering reaction could be avoided while the 17-keto contamination reacted quantitatively.

Methyltestosterone contamination in methandienone (Δ^1-methyltestosterone) could also be determined by the above method (the British Pharmacopoeia gives a semi-quantitative thin-layer chromatographic method[2]). Because of the considerable light absorption of methandienone ($\epsilon_{324} = 48$), 2-glyoxalylmethyltestosterone could be measured only by differential spectrophotometry. Curve A of Fig. 2 shows the difference spectrum of pure methyltestosterone while curve B is the difference spectrum of a methandienone sample containing 1·34 per cent. of methyltestosterone. The satisfactory agreement between these curves indicates that the interfering light absorption has been cancelled out. The results obtained by this method are in good agreement with the values obtained by the method of the British Pharmacopoeia 1963.[3]

It is to be noted that the described method is not suitable for the determination of hydrocortisone contamination in prednisolone because of the interference of the dihydroxyacetone side-chain.

Finally, it is worth mentioning that most methods described in the literature for the determination of ketosteroids are not suitable for solving the above problems as they are applicable only in acidic media (thiosemicarbazide, 2,4-dinitrophenylhydrazine, etc.) and the ketals are hydrolysed by acids. From the assay methods carried out in alkaline media, the classical *m*-dinitrobenzene procedure of Zimmermann[4] cannot be used because of its relatively low sensitivity; the trinitrobenzene method of Pesez and Bartos[5] is much better in every respect (chromophore stability and sensitivity), but this method cannot be used in this case because of solubility problems.

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NOTE—Reference 1 is to Part XIV of this series.

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The Application of Anion-selective Membrane Electrodes in Pharmaceutical Analysis.

Part II. Determination of Cyanocobalamin in Pharmaceutical Preparations

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Quantitative reduction or illumination of cyanocobalamin are used to liberate hydrogen cyanide, which is then determined by using the cyanide-selective membrane electrode. The results from this procedure are very satisfactory. The method is applicable to very low concentrations not only of pure cyanocobalamin but also of pharmaceutical preparations.

CHAIET, Miller and Boley[1] have reported that microbiologically active non-cobalamins are usually found along with cyanocobalamin. Therefore, a highly specific analytical method is required that would allow cyanocobalamin to be differentiated from its analogues and determined in their presence.

The application of the anion-selective membrane-electrode method[2] in pharmaceutical analysis has introduced a sensitive and reliable method for the microdetermination of cyanide or drugs containing the cyano group.

Theoretically, every halide-membrane electrode can be converted into a cyanide electrode, but in practice the cyanide electrode based upon silver iodide is most suitable for the determination of cyanide because of its high selectivity. The electrochemical behaviour of the electrode may be expressed by the following equation[3]—

$$E = E_0 + \frac{RT}{F} \ln [a_{CN} + k \cdot a_{CN}^2]$$

where a_{CN} is the cyanide activity in the solution; k is the dissolution constant of the cyanide electrode; E_0 is the normal potential of the electrode; E is the measured potential; R, F are constants; and T is the absolute temperature.

As the electrode indicates only cyanide and not hydrogen cyanide, the pH of the standardising and measured solution should be kept above 10·5 if the total cyanide content is to be measured ($pK + 1 = 10{\cdot}5$, where K is the dissociation constant of hydrogen cyanide).

Beside direct measurements, the cyanide electrode can also be used as an indicator electrode in the titrimetric determination of cyanide. The titration curve has usually two inflection points, either of which can be used for determining the cyanide content of the compound.

LITERATURE REVIEW

The biological method is the principal one for cyanocobalamin assay. At the same time, although the microbiological method is useful for pharmaceutical products containing cyanocobalamin or cyanocobalamin-like substances, it does not differentiate between cyanocobalamin and its analogues. Konova, Neronova, Ierusalimskii and Borisova[4] used a tedious microbiological method for the determination of cyanocobalamin. Brink[5] assayed cyanocobalamin spectrophotometrically. Also, a polarographic method has been used for its quantitative determination[6]. Monnier, Saba and Galiounghi[7] irradiated an aqueous sample of cyanocobalamin with ultraviolet light and then determined it spectrophotometrically at 630 nm. Dowd, Killard, Pazdera and Ferrari[8] used a similar method in which diluted samples of cyanocobalamin were mixed with sulphuric acid. Brustier, Castaigne,

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de Montety and Amselem[9] determined cyanocobalamin from its cobalt content by ashing and colorimetric determination of the liberated cobalt. In another approach,[10] the cyanocobalamin sample was decomposed with sulphuric acid and hydrogen peroxide and then the cobalt was precipitated as cobalt(III) hydroxide and titrated iodimetrically. The cobalt content has also been determined quantitatively by EDTA titrations[11] and by radioactivity measurements.[12] Carbon-14 has been used for the determination of cyanocobalamin.[13]

Brink, Kuehl and Folkers[14] state that 1 mole of cyanide can be liberated from each mole of cyanocobalamin. This was confirmed later by Boxer and Rickards.[15]

Accordingly, hydrogen cyanide was quantitatively liberated and determined electrochemically by using the cyanide-membrane electrode.

Experimental

Reagents—

The pure cyanocobalamin used was of pharmaceutical grade* and all other chemicals and reagents were of analytical-reagent grade.

Apparatus—

The apparatus included a cyanide-membrane electrode (System Pungor *et al.* type OP-711†), a saturated calomel electrode as the reference electrode, an agar-agar bridge and an ordinary precision pH meter (type OP-203†), having an input resistance higher than 10^{12} ohms and pH values between 6 and 8 with an extended scale.

The reaction vessel in which the experiments were carried out was a Quickfit all-glass apparatus consisting of a round-bottomed flask of 100-ml capacity with three short necks. The middle neck was connected to a condenser, which was attached to a right-angled delivery tube with a fine tip. One of the other necks was connected to a nitrogen source, and the third neck was used for the addition of the materials and reagents and was stoppered during the experiments.

Procedure—

A calibration curve was first plotted by using the cyanide-membrane electrode, previously soaked for about 5 hours in a buffer solution 0·1 M in potassium nitrate adjusted to pH 11 with 0·01M sodium hydroxide. The calibration curve was obtained by using 10^{-1} to 10^{-6} M potassium cyanide solutions, pH 11, whose ionic strengths had been adjusted to 0·1 by the addition of potassium nitrate. When the cyanide activity of sample solutions of unknown cyanide content is being determined, the same ionic strength is necessary and also the pH of the solution should be adjusted to about 11 in all cases.

For the indirect determination of the cyanide content, potentiometric titration is carried out with a measuring circuit made up in the usual manner and with the above equipment and apparatus.

Hydrogen cyanide was quantitatively liberated from cyanocobalamin by two different methods—

Reduction method—

Reductive liberation of the cyano group of cyanocobalamin was carried out in the reaction apparatus described above. Known volumes of 10^{-4}, 10^{-5} and 10^{-6} M freshly prepared aqueous solutions of cyanocobalamin were placed in the round-bottomed flask. This usually represented amounts varying from about 7 to 700 μg of cyanocobalamin. Various amounts of reducing agents were added and the total volume was made up to 25 ml with distilled water. A known calculated volume of the buffer solution (0·1 M with respect to potassium nitrate and 0·01 M with respect to sodium hydroxide) was placed in a collection tube with the end of the delivery tube under the surface of the buffer solution. The reacton solution was then heated to boiling-point with a micro burner and refluxed for about 30 minutes. The reflux condenser is necessary in order to avoid transfer of moisture from the reaction flask to the collection tube. A gentle, steady stream of nitrogen was allowed to pass through the reaction solution during the experiment in order to drive off the liberated hydrogen cyanide into the buffer solution in the collection tube. To ensure that there were no impurities present in

* Supplied by Chemical Works of Gedeon Richter Ltd., Budapest, Hungary.
† Supplied by Radelkis, Budapest, Hungary.

the nitrogen that could interfere with the results, the nitrogen, before it entered the reaction flask, was passed through a washing tower half filled with 0·1 M sodium hydroxide solution to trap and eliminate any traces of hydrogen cyanide that might be present. When the experiment was completed, the sodium cyanide formed in the collection tube was measured either directly or indirectly by potentiometric titration with standard silver nitrate solution. The amounts of reducing agents used in 25 ml of total volume of solution that gave quantitative results were: 2 g of ascorbic acid, 1 g of tin(II) chloride in the presence of 1 ml of 2 N hydrochloric acid, 2 g of calcium hypophosphite with 2 ml of 2 N hydrochloric acid or 2 g of calcium hypophosphite with 2 ml of 2 N sulphuric acid.

A blank experiment was carried out and was found to give negligible values in all cases. The results are shown in Table I.

TABLE I

RECOVERY OF CYANOCOBALAMIN AT DIFFERENT CONCENTRATIONS FOR VARIOUS REDUCING AGENTS

Reducing agent	Cyanocobalamin concentration/M	Amount taken/μg	Amount recovered*/μg	Percentage recovery
Ascorbic acid	10^{-4}	675	644·0	95·5
	10^{-5}	67·5	64·4	95·5
	10^{-6}	6·75	6·37	94·4
Tin(II) chloride in hydrochloric acid	10^{-4}	675	675	100·0
	10^{-5}	67·5	70·5	104·4
	10^{-6}	6·75	7·05	104·4
Calcium hypophosphite in hydrochloric acid	10^{-4}	675	637	94·4
	10^{-5}	67·5	64·4	95·5
	10^{-6}	6·75	6·59	97·7
Calcium hypophosphite in sulphuric acid	10^{-4}	675	644	95·5
	10^{-5}	67·5	70·5	104·4
	10^{-6}	6·75	7·05	104·4

* Each value given is the average of a minimum of six experiments and the relative standard deviation is 0·541 per cent.

Illumination method—

The method was carried out with the same apparatus used in the previous method but without the condenser. Five millilitre volumes of aqueous solutions of cyanocobalamin containing from about 7 μg to about 27 μg of the compound were placed in the reaction flask and were made up to 25 ml total volume with phosphate - citric acid buffer solution at pH 3, 4 or 5, these being the optimum pH conditions for quantitative liberation of hydrogen cyanide from cyanocobalamin solution. The reaction solution was then exposed to two 500-W lamps for 60 minutes. So that the solution should not become hot due to the powerful source of illumination, the reaction flask was cooled during the experiment by being immersed in a large funnel full of running tap water. The liberated hydrogen cyanide was driven off by a steady stream of nitrogen as in the previous method. The sodium cyanide formed in the collection tube was determined either directly or indirectly. A blank experiment was carried out and was found to give negligible values in all cases. The results are shown in Table II.

TABLE II

RECOVERY OF CYANOCOBALAMIN AT DIFFERENT CONCENTRATIONS AND pH VALUES BY THE ILLUMINATION METHOD

pH	Cyanocobalamin concentration/M	Amount taken/μg	Amount recovered*/μg	Percentage recovery
3	10^{-5}	27·0	26·5	97·7
4	10^{-5}	27·0	26·5	97·7
5	10^{-5}	27·0	25·8	95·5
3	10^{-6}	6·75	6·45	95·5
4	10^{-6}	6·75	6·45	95·5
5	10^{-6}	6·75	6·37	94·4

* Each value given is the average of a minimum of six experiments and the relative standard deviation is 0·715 per cent.

A few experiments with different concentrations and different amounts of cyanocobalamin were carried out to compare the direct and the indirect methods. The differences between the methods were found to be within the acceptable experimental errors and did not exceed 0·04 pCN.

Application to Pharmaceutical Preparations

The following preparations were used:

*Gerovit capsules**—A multivitamin preparation each capsule of which contains 0·5 μg of cyanocobalamin.

Sirepar vials†—A liver hydrolysate preparation with 10 μg ml^{-1} of cyanocobalamin.

Vitamin B_{12} + B_1 ampoules†—Each 2-ml ampoule contains 1 000 μg of cyanocobalamin.

The cyanocobalamin contents of these preparations were determined by the two methods with the same equipment and apparatus. In the illumination method, as there was no significant difference between the results given in Table II by using different pH values, only pH 4 was used. The results are shown in Table III.

Table III

Recovery at pH 4 of vitamin B_{12} from various sources by reduction and by illumination

Compound	Method	Amount taken/μg	Amount recovered,* μg	Amount recovered,* per cent.
Gerovit capsules	Reduction with ascorbic acid	3	2·79	90·2
	Reduction with $SnCl_2$ *plus* HCl	3	2·79	93·3
	Reduction with $Ca(H_2PO_2)_2$ *plus* HCl	3	2·73	91·2
	Reduction with $Ca(H_2PO_2)_2$ *plus* H_2SO_4	3	2·86	95·5
	Illumination	3	2·89	96·6
Sirepar vials	Reduction with ascorbic acid	5	4·56	91·2
	Reduction with $SnCl_2$ *plus* HCl	5	5·22	104·5
	Reduction with $CaH_2PO_2)_2$ *plus* HCl	5	4·56	91·3
	Reduction with $Ca(H_2PO_2)_2$ *plus* H_2SO_4	5	5·00	100·0
	Illumination	5	4·88	97·7
Vitamin B_1 + B_{12} ampoules	Reduction with ascorbic acid	5	5·30	106·0
	Reduction with $SnCl_2$ *plus* HCl	5	5·22	104·5
	Reduction with $Ca(H_2PO_2)_2$ *plus* HCl	5	5·35	107·0
	Reduction with $Ca(H_2PO_2)_2$ *plus* H_2SO_4	5	5·35	107·0
	Illumination	5	5·20	104·0

* Each value given is the average of a minimum of six experiments and the relative standard deviation is 0·633 per cent.

From the results it is concluded that both the reduction and the illumination methods in combination with the cyanide-selective membrane electrode can be adopted for the determination of cyanocobalamin not only in the pure form, but also in pharmaceutical preparations. The method has been proved to be accurate and reproducible as well as simple and rapid for routine use. Moreover, it is highly specific even towards traces of cyanide, so that it responds to very dilute concentrations, which represents a great advantage as cyanocobalamin is usually prescribed in microgram doses. The application of the two methods to vitamin B_{12} determination depends naturally on the presence of only one cyano group in the cyanocobalamin molecule.

The error of the results lies within the limits ±5 per cent.

Conclusions

The quantitative liberation of hydrogen cyanide from cyanocobalamin in the pure form as well as in pharmaceutical preparations was achieved by applying two methods. The first was the release of the cyano group by reduction with different reducing agents under reflux. The second was by exposure to a strong source of visible light at room temperature.

* Supplied by United Works of Pharmaceutical and Dietetic Products, Budapest, Hungary.
† Supplied by Chemical Works of Gedeon Richter, Ltd., Budapest, Hungary.

The use of the cyanide-selective membrane electrode proved to be of great value. The method simplifies such determinations at very low concentrations. Impurities that usually interfere in other methods do not affect this method. Therefore, it is recommended for adoption as a quick routine analytical control method not only for the determination of cyanocobalamin but also for the differentiation of this compound from its analogues.

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A Method for the Determination of Carbon Monoxide, Carbon Dioxide, Sulphur Dioxide, Carbonyl Sulphide, Oxygen and Nitrogen in Furnace Gas Atmospheres by Gas Chromatography

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A method is described for the routine analysis of mixtures containing any of the named gases by using a dual-column system and a katharometer detector. The method is suitable for 1-ml gas samples that may contain from 100 per cent. of one of the gases down to at least 7×10^{-2} per cent. of any of them, provided that the ratio of carbon monoxide to carbon dioxide or nitrogen to carbon monoxide does not exceed 50:1.

In copper matte smelting the loss of copper to the slag is significantly influenced by the oxidation states of the slag and matte. Hence, when studying the matte - slag system it is necessary to determine the oxygen and sulphur dioxide contents of the surrounding atmosphere. A silica-saturated iron silicate slag may sustain oxygen pressures of from 10^{-12} to 10^{-6} atmospheres at 1 300 °C; a satisfactory method of obtaining such oxygen partial-pressures in the laboratory is to use the equilibrium between carbon monoxide and carbon dioxide according to the following equation—

$$CO_2 \leftrightharpoons CO + \tfrac{1}{2}O_2 \quad (1)$$

The oxygen pressure is derived from the carbon monoxide and carbon dioxide partial-pressures, determined in turn by analysis, and the equilibrium constant for reaction (1) by using the equation—

$$P_{O_2} = \left[\frac{KP_{CO_2}}{P_{CO}}\right]^2 \quad (2)$$

Similarly, the sulphur partial-pressure can be calculated from the oxygen and sulphur dioxide pressures in the atmosphere from the equation—

$$SO_2 \leftrightharpoons \tfrac{1}{2}S_2 + O_2 \quad (3)$$

or from the carbonyl sulphide and carbon monoxide partial-pressures by using the reaction—

$$COS \leftrightharpoons \tfrac{1}{2}S_2 + CO \quad (4)$$

Thus, for these studies, it became necessary to develop a rapid routine method for the determination of carbon monoxide, carbon dioxide, sulphur dioxide and carbonyl sulphide in air that could be used for small amounts of any of them.

The analysis of gas mixtures containing nitrogen, oxygen, carbon monoxide and carbon dioxide has been studied by many workers, but little information on the analysis of mixtures containing carbon monoxide, carbon dioxide and sulphur dioxide is available. No single adsorbent or column support material appears to be suitable for the complete resolution of these gases by gas chromatography.

Molecular sieves have been used to separate nitrogen, oxygen and carbon monoxide[1,2,3,4,5,6] but under normal operating conditions carbon dioxide is irreversibly adsorbed. The use of silica gel enables carbon monoxide and carbon dioxide to be separated but it will not allow the complete resolution of nitrogen and oxygen[7,8] and, in addition, it leads to the production of asymmetrical sulphur dioxide peaks with very long retention times. Relatively long columns containing silicone oil or di(2-ethylhexyl) sebacate on diatomaceous earth supports

have been used to separate carbon dioxide and sulphur dioxide.[5,9,10] Porous beads of polystyrene cross-linked with divinylbenzene, initially developed for gel-permeation chromatograpy, were used by Hollis[11] in a study of the gas-chromatographic behaviour of some light gases. It was found that the separation of the components appeared to involve direct partition throughout the polymer beads. A material of this type, Porapak Q,* has been shown to be suitable for the resolution of carbon dioxide and sulphur dioxide[12,13] (and also by B. H. M. Billinge, private communication).

A review of the literature showed that previous workers have used complex systems involving multiple analysis, multiple column and detector combinations and flow switching.[3,14,15] In developing the present method the simplest combination of columns and detectors was sought and was incorporated into a commercial gas chromatograph that was already available for development work.

Experimental

Apparatus—

A Pye Series 104, Model 34, dual-column chromatograph, equipped with a heated head dual-katharometer detector and an isothermal column oven, was used for this work. The out-of-balance signal from the katharometer bridge, which could be attenuated as required by a factor of up to one thousand times, was fed to a Kent Chromalog 1 Integrator and to a Telsec Series 700 flat-bed potentiometric recorder†. A polarity change-over switch on the katharometer power supply allowed separations to be made on either column. The carrier gas was freed of moisture and volatile hydrocarbons by passing it through a gas-purifying bottle containing Union Carbide Molecular Sieve 13 × (−80 + 100 mesh). The carrier gas flow-rates from the two sides of the detector were continuously monitored by capillary-type flow meters containing dibutyl phthalate as manometric fluid. These flow meters also provided a convenient check for septa leakages. Samples were introduced through silicone rubber septa by using a series of glass gas syringes (0 to 30 μl, 0 to 100 μl, 0 to 250 μl and 0 to 500 μl)‡.

Initially, three 1·5 m × 4 mm bore glass columns were packed with Porapak Q (−100 +120 mesh), Porapak R (−100 +120 mesh) and Union Carbide Molecular Sieve, Type 5A (−85 + 100 mesh), respectively. After packing, the columns were purged with carrier gas at 200 °C for 24 hours.

The use of helium as carrier gas would be expected to yield the best sensitivity but it was considered too expensive for routine use, therefore three other carrier gases, argon, nitrogen - hydrogen (3 + 1) and high purity (99·99 per cent.) hydrogen, were used in the development studies.

The molecular sieve column was connected to one side of the dual-katharometer detector and the Porapak Q column was connected to the other side for the first stage of the initial tests; in the second stage the Porapak Q column was replaced by the one containing Porapak R. The following values of the instrument variables were set for the initial tests: carrier gas flow-rate, 40 ml minute^{-1}; katharometer bridge current, 130 mA; column-oven temperature, 120 °C; and detector-oven temperature, 150 °C. For testing, known volumes of nitrogen (oxygen free),§ oxygen,§ carbon monoxide,‖ carbon dioxide,¶ carbonyl sulphide** and sulphur dioxide** were injected into each column in turn and the retention time, peak shape and peak height were observed for each gas. The tests were repeated for all three carrier gases. Argon led to poor sensitivity at low bridge currents and bridge currents greater than 130 mA gave double peaks for sulphur dioxide. The best sensitivity was obtained by using hydrogen as carrier gas and this was used for the remaining part of the study.

The chromatograms thus obtained showed that Porapak Q could resolve carbon dioxide, carbonyl sulphide and sulphur dioxide but gave asymmetrical peaks for sulphur dioxide. This tailing was considerably reduced by use of the more polar Porapak R, but the carbon

* Obtainable from Waters Associates Ltd., Portwood, Stockport, Cheshire.

† Obtainable from Telsec Industries Ltd., Oxford.

‡ Obtainable from Scientific Glass Engineering Pty. Ltd., London, N.20.

§ Supplied by British Oxygen Co. Ltd.

‖ Supplied by Air Products Ltd.

¶ Supplied by Distillers Co. Ltd.

** Supplied by B.D.H. (Chemicals) Ltd.

dioxide peak was not fully separated from the composite nitrogen, oxygen and carbon monoxide peak. This situation was improved by using a 2·75 m × 4 mm bore column of Porapak R. Symmetrical peaks and adequate resolution of nitrogen, oxygen and carbon monoxide were obtained with the 1·5-m molecular sieve, Type 5A, column and carbon dioxide and sulphur dioxide were irreversibly adsorbed.

Further tests were carried out with the 1·5-m molecular sieve column and the 2·75-m Porapak R column connected one to each of the two sides of the dual-channel detector. Samples of the gases were injected into the appropriate column over a range of column and detector-oven temperatures and katharometer bridge currents. The optimum conditions finally chosen for routine analysis were carrier gas flow (hydrogen) 40 ml minute^{-1}, bridge current 250 mA, column-oven temperature 160 °C and detector-oven temperature 170 °C. Sharpening of the peaks and improved sensitivity resulted from the higher column temperature and bridge current. Typical chromatograms obtained under these conditions are shown in Fig. 1.

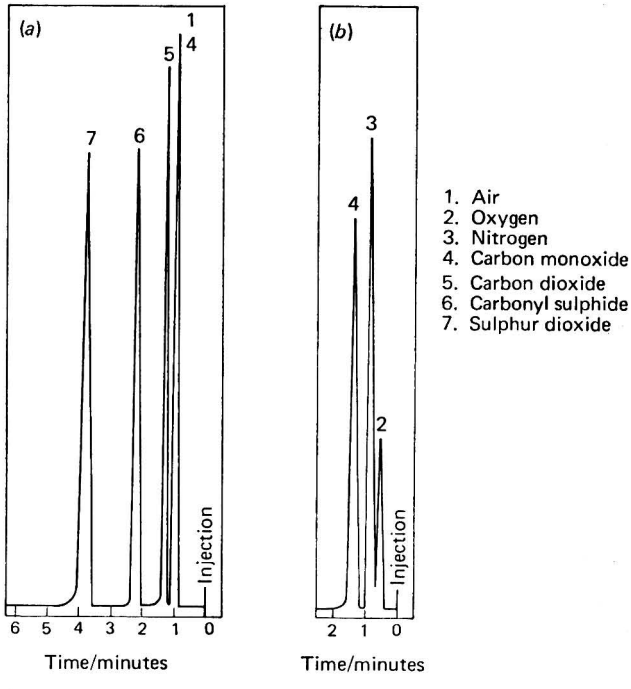

Fig. 1. Typical gas chromatograms with: (*a*) Porapak R; and (*b*) Union Carbide Molecular sieve 5A

CALIBRATION—

The katharometer detector was calibrated for the six gases by injecting several volumes of each gas and determining the resultant peak areas from the digital integration print-out of the Chromalog. Calibration graphs of volume injected against peak areas were plotted for each attenuation setting of the instrument. In the course of routine measurements calibration graphs were checked periodically.

PRECISION AND SENSITIVITY—

During the development of this technique of analysis it was found that the error in repeatability of syringe operation could be as little as ±1 per cent. with a practised operator. This can be considerably reduced by using a repeating adaptor, which is a commercial device that allows the operator to pre-set accurately the volume delivered by the syringe. In all quantitative measurements the attenuation of the detector output is adjusted so that the

peak height is greater than 10 per cent. of the full-scale recorder deflection. The working measurement limit of analysis for any component has been based upon a peak height of 10 per cent. of full-scale deflection at the minimum attenuation setting (×1). The working limits for the six gases examined are shown in Table I. The ultimate detection limit has been defined as the amount of the component producing a peak height equivalent to twice the instrument short-term noise at the lowest attenuation, *i.e.*, 2 × 1 per cent. at attenuation ×1, which is equivalent to about one fifth of the working limit for this instrument.

TABLE I

RETENTION TIMES AND WORKING ANALYSIS LIMITS OF GASES

Number in Fig. 1	Gas	Retention time/s		Working analysis limits at attenuation × 1	
		Porapak R	Molecular sieve 5A	μl	p.p. atm.
1	Air	59·2	—	—	—
2	O_2	—	37·0	0·2	2×10^{-4}
3	N_2	—	45·0	0·2	2×10^{-4}
4	CO	60·6	76·6	0·2	2×10^{-4}
5	CO_2	79·2	—	0·2	2×10^{-4}
6	COS	135·0	—	0·2	2×10^{-4}
7	SO_2	223·2	—	0·7	7×10^{-4}

CONCLUSION

From the results of this work a method of routine analysis was established in which the Porapak R column was used for the determination of carbon dioxide, carbonyl sulphide and sulphur dioxide, and the molecular sieve column for nitrogen, oxygen and carbon monoxide. In the recommended procedure a 0·5 or 1·0-ml sample of furnace gas mixture is injected into the Porapak R column with the detector attenuation set to a suitable value, and the appropriate peak areas for carbon dioxide, carbonyl sulphide and sulphur dioxide are measured by the integrator. The detector polarity is changed and a similar sample is injected into the molecular sieve column, the peak areas for nitrogen and carbon monoxide being measured in the same way. Conversion of the peak areas into component percentages is carried out with the aid of previously determined calibration values. When filling the gas syringe it is most important to ensure that the syringe is fully flushed with the gas mixture; and when the sample for analysis is taken the syringe should be filled completely, quickly removed from the sampling point and adjusted to the pre-set volume immediately prior to injection to ensure minimal atmospheric contamination.

After extended use of the system it was found that the molecular sieve column became saturated with respect to carbon dioxide, carbonyl sulphide and sulphur dioxide. This resulted in some base-line disturbance in the form of a broad peak that began about 15 minutes after injection and was eluted over a period of about 5 to 10 minutes. The column can readily be re-activated by heating at 200 °C for 8 hours with a carrier gas purge. However, it is possible to continue using the column in the saturated condition because neither its resolution nor its sensitivity appears to be affected.[12]

Gas samples from the present slag equilibrium studies had carbon monoxide to carbon dioxide ratios that ranged from 10:1 to 1:69. The results of analysis of known gas mixtures within this range showed that in all instances carbon monoxide was resolved from carbon dioxide on the Porapak R column. Further experiments have shown that the limit of resolution with the 2·75-m column occurs at a ratio of about 50:1, while similar experiments with known mixtures of nitrogen or air and carbon monoxide have shown that the limit of resolution with the present molecular sieve column occurs at a ratio of nitrogen to carbon monoxide greater than 50:1. Samples of this type have not been encountered in the present study.

Within the ratio limits quoted above the analysis procedure offers a rapid quantitative means of analysing gas mixtures containing as little as 0·7 μl ml^{-1} of sulphur dioxide and 0·2 μl ml^{-1} of any of the other five gases.

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The Determination of Phosphate in Detergents by Cool-flame Emission Spectroscopy

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The total phosphate content of detergent materials is determined by measurement of the emission of the HPO molecular species at wavelength 528 nm in a cool hydrogen - nitrogen diffusion flame. Preliminary treatment with cation-exchange resin is necessary to remove interference by metals. Analytical results on detergent samples containing up to 20 per cent. of phosphates (expressed as P_2O_5) indicate a precision of the order of 2 to 4 per cent. for the method.

PHOSPHATES of various types are included, as builders, in many detergent formulations. The determination of total phosphate, as described in British Standard 3762 : 1964,[1] involves the destruction of organic matter by ashing, conversion of the phosphate present entirely into the ortho form by hydrolysis with hydrochloric acid, followed either by titration with standard alkali or gravimetric determination as magnesium pyrophosphate. The presence of borates, silicates and heavy metals requires additional separation stages in the procedure.

The work of Dagnall, Thompson and West[2] on the determination of phosphorus by molecular-emission spectroscopy in a cool nitrogen - hydrogen diffusion flame indicated the possibility of developing a very rapid and convenient method for the determination of phosphate in detergents, which would be ideally suited to samples that could readily be taken into aqueous solution.

In earlier publications, several authors have described methods for the determination of phosphorus by flame-emission spectroscopy. Brite[3] and Davis, Dinan, Lobbett, Chazin and Tufts[4] used an oxy-hydrogen flame to produce the phosphorus oxide continuum in the 540 nm region, but wide slits were necessary to provide sufficient energy, and interference from other metallic elements occurred; the use of an ion-exchange resin to remove cations such as Na^+, K^+, Ca^{2+}, Pb^{2+}, Fe^{2+} and Fe^{3+} was recommended. Skogerboe, Gravatt and Morrison[5] investigated the analytical applications of the phosphorus monoxide band emission at 246·4 nm and also the atomic phosphorus emission at 253·6 and 255·3 nm but found that sensitivity was generally poor, with the exception of the band emission at 246·4 nm in an air - hydrogen flame, with which, with a wide slit, a detection limit of 5 p.p.m. of phosphorus was achieved.

Brody and Chaney[6] utilised the emission at 526 nm attributed to the HPO species as the basis of a specific gas-chromatographic detector for phosphorus, and the same emission species has been further studied by several authors.[2,7,8,9] The emission at 526 nm has been shown to be sensitive, selective and quantitatively related to total phosphorus concentration, with a limit of detection of the order of 0·1 to 0·2 p.p.m. of phosphorus in an argon - hydrogen or nitrogen - hydrogen flame; the emission intensity has been shown to vary linearly with concentration up to at least 500 p.p.m. of phosphorus. Recently, Aldous, Dagnall and West[10] have described a simple photometer incorporating a selective filter (peak transmission at 520 nm) for the determination of phosphorus; this instrument is capable of achieving, with a heated nebulisation chamber, a limit of detection of 0·007 p.p.m. for phosphorus.

In the present work we have followed essentially the technique described by Dagnall, Thompson and West.[2] Our original interest was in the analysis of detergent powders to the requirements of Defence Specification DEF-114-A;[11] in this case the builders may include either pentasodium triphosphate or tetrasodium pyrophosphate, and a total phosphate content, expressed as phosphorus pentoxide, is required to be determined. Analysis of other detergents has subsequently been investigated and results are included. In all examples, a preliminary cation-exchange procedure has been followed.

Experimental

Apparatus and operating conditions—

All emission-intensity measurements were made on a Unicam SP900 flame photometer fitted with a standard air - propane burner head; the top of the burner head was sited 1 cm below the bottom of the monochromator entrance slit. Nitrogen, at 15 p.s.i., was introduced through the normal air-nebulising system of the instrument and hydrogen, at 2-cm pressure on the dibutyl phthalate manometer, was introduced through the normal fuel system. The nebulisation rate was of the order of 2·5 ml minute^{-1}. Other instrumental conditions were: slit width 0·3 mm, electrical band width 3 and gain setting approximately 5 (adjusted as necessary to give a convenient galvanometer response).

Reagents—

Analytical-reagent grade orthophosphoric acid was used to provide the phosphorus reference standard. After standardisation, an accurate 0·002 M orthophosphoric acid solution was prepared by dilution (1 ml contains 62·0 μg of phosphorus).

Dowex 50W-X8 cation-exchange resin (hydrogen form), in a column 20 cm long and 1·5 cm i.d., was used for the preliminary separation of metal ions, and was adequate for at least fifteen sample treatments. The resin was regenerated by stirring for 8 hours with 0·3 N hydrochloric acid, decanting the spent acid, and washing the resin with water until free of acid.

Procedure—

Dissolve 2·50 $\pm$ 0·01 g of the detergent sample in 200 ml of distilled water in a 400-ml beaker, transfer the solution and washings to a 1-litre calibrated flask and dilute to volume. Transfer a 25-ml aliquot of this solution by pipette to the ion-exchange column and collect the effluent in a 250-ml calibrated flask. Wash with successive 25-ml portions of distilled water to a final adjusted volume of 250 ml (Note 1). Measure two aliquots (A ml) of this solution by pipette into two 100-ml calibrated flasks (Note 2) and to one aliquot add 10 ml of the standard phosphorus solution. Dilute both solutions to volume.

With the SP900 flame photometer set at the wavelength corresponding to peak response for the phosphorus emission, about 528 nm (Note 3), nebulise the two solutions alternately and, from at least three replicate measurements, record the mean galvanometer deflection for each. Adjust the gain control, on a test run, so that the doped solution gives a reading of abour 90 per cent. of full-scale deflection. Spray distilled water after each pair of solutions to restore the galvanometer zero (Note 4).

From the mean readings for the sample (S_0) and for the sample *plus* added phosphorus (S_1), calculate the phosphorus content of the sample. The percentage of phosphorus, expressed as P_2O_5, is given by the equation (in full)—

$$\text{Phosphorus, as } P_2O_5\text{, per cent.} = \frac{S_0}{S_1 - S_0} \times \frac{620}{100} \times \frac{1\,000}{\text{sample weight(g)}} \times \frac{250}{25} \times \frac{100}{A} \times \frac{1}{10^4} \times f$$

where f (= 2·29) is the factor for converting phosphorus into P_2O_5. For a fixed sample weight of 2·50 g the equation reduces to—

$$\text{Phosphorus, as } P_2O_5\text{, per cent.} = \frac{S_0}{S_1 - S_0} \times \frac{567{\cdot}9}{A}$$

Notes—

1. An effluent flow-rate of 10 ml minute^{-1} is recommended.

2. The volume of the aliquot should be chosen at this stage to give a phosphorus content of not more than 5 to 6 μg ml^{-1} due to the sample in the final test solution. For samples containing 12 to 20 per cent. of P_2O_5, take 25-ml aliquots; for those containing 6 to 12 per cent., 50-ml aliquots; and for those containing less than 6 per cent., 90-ml aliquots, *i.e.*, 10 ml of water or 10 ml of standard phosphorus solution.

P_2O_5 in sample, per cent.	Aliquot (A ml)	Phosphorus, as P_2O_5, per cent., given by
12 to 20	25	$\frac{S_0}{S_1 - S_0} \times 22{\cdot}72$
6 to 12	50	$\times$ 11·36
<6	90	$\times$ 6·31

Alternatively, for low phosphate contents (less than 6 per cent. of P_2O_5) the sample weight can be increased.

3. The peak-response wavelength should be found by scanning between 530 and 526 nm while nebulising a test solution containing 5 to 10 $\mu g\ ml^{-1}$ of phosphorus.

4. A control solution, containing all the detergent ingredients with the exception of pentasodium triphosphate gave no measurable response at the phosphorus wavelength when taken through the full preparative procedure. Therefore, no interference correction is necessary, and distilled water can be used for zero control.

Results

Synthetic detergent mixtures—

Portions (2·50 g) of various laboratory-prepared detergent mixtures were analysed by the suggested procedure. In each instance the mixture contained sodium tetraborate, sodium carbonate, sodium sulphate, sodium carboxymethylcellulose, Polyox (polyoxyethylene ethers) and pentasodium triphosphate, the proportion of the last ingredient being varied to give samples with several different phosphate levels within the range normally encountered.

The specimen of pentasodium triphosphate used to prepare these mixtures was also analysed by the same procedure, the final aliquot (*A*) in this case being 10 ml. As the recovery was slightly higher than theoretical, the experimentally determined result was used to correct the results obtained on the synthetic mixtures.

The results, summarised in Table I, indicated satisfactory recovery of phosphate for the requirements of most detergent analysis. There was no evidence of interference by the other ingredients of the mixtures. (See also Note 4 above.)

Table I

Determination of phosphate in synthetic detergent mixtures

Synthetic mixture	Phosphate, as P_2O_5, per cent.* Added	Found
A	8·4	8·6
B	14·0	14·2
C	8·3	8·2
D	14·1	14·3
E	4·5	4·4

* Analysis of the pentasodium triphosphate additive by the same method gave 45·7 per cent. calculated as P_2O_5. (Theoretical, for $Na_5P_3O_{10}.6H_2O$ = 44·6 per cent.) Results are calculated on the basis of this analysis. Each result is the mean of three determinations.

Proprietary detergents—

In Table II, analytical results are shown for various commercial or Service Department detergents; phosphate determinations were made by both the present flame-photometric procedure and by the standard chemical method.[1]

Table II

Comparison of flame-photometric and chemical determinations of phosphate in proprietary detergents

	Phosphate, as P_2O_5, per cent., by flame-photometric method	chemical method[1]
Laundry detergent powder to DEF-141-A[11] ..	16·9	16·8
Commercial washing powder	10·8	10·9
Laboratory detergent powder	12·9	12·1
Laboratory detergent liquid	2·7	3·2

Chemical results were the means of either two or three determinations and flame-photometric results were the means of at least six determinations. The comparison, taken in conjunction with the recoveries on synthetic detergents (Table I), suggests that the accuracy of the method is reasonable; a wider range of samples and more chemical results would be needed to make a firmer judgment than this.

SAMPLE HOMOGENEITY—

The high sensitivity of detection of phosphorus given by the flame-photometric method indicates that very small samples could be taken for analysis, *i.e.*, less then 0·1 g, compared with the 10-g sample recommended for the chemical method,[1] but it became clear early in the present investigation that powder-detergent samples were non-homogeneous at this level. This was demonstrated by the experiment shown in Table III; the precision obtained on 2·50-g samples (diluted to 1 litre) was better than that obtained on 0·250-g samples (diluted to 100 ml), and the former procedure was adopted.

TABLE III

TEST OF SAMPLE HOMOGENEITY

Procedure	Number of replicates	Phosphate, as P_2O_5, found, per cent.	Standard deviation	Coefficient of variation, per cent.
2·50 g to 1 litre (standard method)	8	13·0	±0·28	±2·1
0·250 g to 100 ml (then as standard method)	8	13·4	±0·62	±4·6

PRECISION—

By following the proposed method exactly, with a sample weight of 2·50 g, a series of precision tests gave coefficients of variation ranging from 1·7 per cent. for a liquid sample to 3·7 per cent. for a sample containing 16·9 per cent. of phosphate, as P_2O_5.

DISCUSSION

The materials examined included both ionic and non-ionic surfactant molecules, and there was evidence in the early stages of the investigation that the former tended to depress the phosphorus emission while the latter exerted an enhancing effect. These trends were not markedly affected by the preliminary cation exchange, and the standard addition technique was adopted as a safeguard against interference effects arising either in the sample solution or in spectral emission.

It is important in the standard addition method that the ratio $S_0/(S_1 - S_0)$ is of the order of unity.[12,13] The added phosphorus concentration should be neither very large nor very small relative to the sample concentration, otherwise precision is lost and, also, interference effects may not be compensated for.

The SP900 galvanometer read-out, with fixed wavelength setting, has been found rapid and convenient for this analysis. An alternative system involving the use of a 10-mV potentiometric recorder, with wavelength scanning, was tested but with no useful advantage. However, it is interesting to note that the peak response for the phosphorus emission, which has been attributed to HPO emission at 526·2 nm,[6] occurred on our equipment at 528 nm, as reported elsewhere.[2] Wavelength calibrations obtained by nebulising solutions containing barium (553·5 nm), thallium (535·0 nm) and magnesium (518·4, 517·3 and 516·7 nm) indicated wavelengths of 560, 528 and 512 nm for the three peaks attributed to the HPO system. The thallium atomic emission at 535·0 nm is strong in the cool flame and could perhaps form an internal standard for high-precision phosphorus determinations in other applications.

CONCLUSION

The advantages of the cool-flame emission method, as applied to the determination of phosphate in detergent materials, are speed and simplicity. The only pre-treatment involved is the cation-exchange stage, whereas the preliminaries to the standard chemical procedures can be very time consuming. A determination by the suggested method can be completed within 2 hours.

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The Identification of Polyol Base Compounds in Polyurethane Polyethers by Gas Chromatography

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The identification of polyol base compounds of polyurethane polyethers has been carried out by gas chromatography after conversion into their corresponding acetates. The polyethers are allowed to react with the mixed anhydride of acetic and toluene-*p*-sulphonic acids (a cleavage reagent), and converted into the acetates. With gas chromatography the acetate peaks of the polyol base compounds appear at different positions, thus enabling these compounds to be easily distinguished.

POLYETHERS that are widely used in the reaction with polyisocyanates to form polyurethane foams are ethylene oxide or propylene oxide adducts of polyols (*e.g.*, propylene glycol, glycerol, trimethylolpropane, sorbitol, etc.). In the analysis of the polyurethanes identification of these base compounds is very important, but has proved to be difficult because they form a relatively small part of the molecules of polyethers.

The proportions of oxyethylene and oxypropylene in the polyethers have been determined by cleaving the latter with hydriodic acid,[1] hydrobromic acid,[2,3] phosphoric acid,[4,5] pyrolysis gas chromatography,[6,7] infrared spectrometry[8] and nuclear magnetic resonance spectrometry.[9,10] However, these methods, except for the Mathias - Mellor hydrobromic acid fission - gas-chromatographic method, are not satisfactory for the detection of base compounds.

Mixed sulphonic - carboxylic anhydrides have been used as active acylating agents.[11,12] Recently, Karger and Mazur[13] reported that the mixed anhydride of acetic and toluene-*p*-sulphonic acids, prepared from acetic anhydride and anhydrous toluene-*p*-sulphonic acid, is an effective reagent for cleavage of the ether linkages.

In the present study Karger and Mazur's method has been extended to enable polyol base compounds in polyurethane polyethers to be identified by gas chromatography after cleavage of the polyethers with acetic toluene-*p*-sulphonic anhydride.

EXPERIMENTAL

CLEAVAGE REAGENT—

A mixture of 126 g of toluene-*p*-sulphonic acid, which had been dried under reduced pressure at room temperature for 20 hours, and 150 g of acetic anhydride was refluxed in a four-necked flask at 130 °C for 30 minutes. The product consisting of acetic toluene-*p*-sulphonic anhydride, acetic acid and excess of acetic anhydride was used without further purification.

SAMPLES—

All of the following polypropylene glycol samples used are commercially available: PP-2000 (M.W. about 2 000); GP-3000, based on glycerol (M.W. about 3 000); TP-4000, based on trimethylolpropane (M.W. about 4 000); and SP-750, based on sorbitol (M.W. about 750).

APPARATUS—

A Simadzu gas chromatograph, Model GC-4APT, was used for this study. The gas - liquid chromatographic column consisted of a 2-m length of 3 mm i.d. stainless-steel tubing packed with 25 per cent. w/w Apiezon L coating on 60 to 80-mesh Chromosorb W.AW. The conditions used were: column temperature, from 180 to 260 °C, with programming rate 6 °C minute^{-1}; and flow-rate of the carrier gas (helium) 30 ml minute^{-1}.

PROCEDURE—

A mixture of 5 g of reagent and 1 g of sample was refluxed at 130 °C for 2½ hours. The reaction product was extracted with diethyl ether and the ether solution washed several times with water to remove the excess of reagent. One microlitre of the ether extract was injected into the chromatograph.

RESULTS

The gas chromatograms of the reaction products of polyethers based on propylene glycol, glycerol, trimethylolpropane and sorbitol are shown in Fig. 1.

In Fig. 1*a* the peak of the product from the base compound, propylene glycol diacetate, and that of propylene glycol diacetate produced by cleavage of the polyoxypropylene carbon chain overlap and cannot be distinguished from each other, but no peaks corresponding to other base compounds appear.

In Fig. 1 (*b* and *c*) the peaks of glycerol triacetate (triacetin) and trimethylolpropane triacetate appear with the large peaks of propylene glycol diacetate produced by the cleavage of the ether linkages of polyoxypropylenes.

In Fig. 1*d* characteristic peaks, which consist of the acetates of sorbitol, appear with the peak of propylene glycol diacetate.

As shown in Fig. 1, acetate peaks corresponding to each of the polyol compounds appear at different positions, so that the base compounds of polyethers can now easily be distinguished. The peak of propylene glycol diacetate produced by the cleavage of polyoxypropylene does not interfere except in the case of the polyether based on propylene glycol.

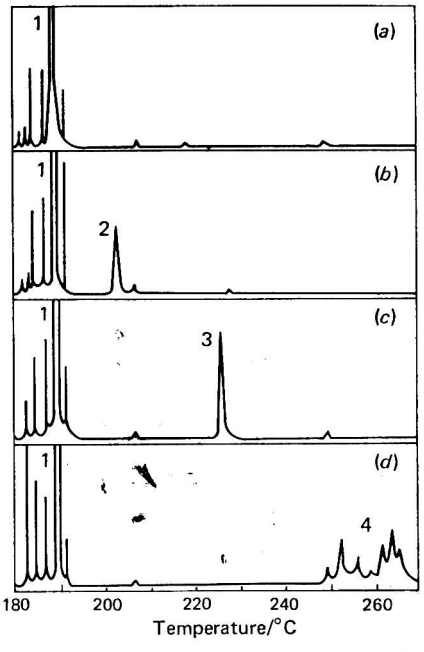

Fig. 1. Gas chromatograms of reaction products of polyethers. 1, Propylene glycol diacetate; 2, glycerol triacetate (triacetin); 3, trimethylolpropane triacetate; and 4, acetate of sorbitol

DISCUSSION

Identification of the polyol base compounds of polyurethane polyethers by gas chromatography was carried out after conversion into their corresponding acetates by using mixed sulphonic-carboxylic anhydrides.

When polyethers react with the reagent, toluene-*p*-sulphonic esters will be produced as well as acetates. With the ethyl ether the peak of ethyl toluene-*p*-sulphonate appeared with the acetate peak in the gas chromatogram. With ethers of higher molecular weight, however, it is difficult to detect the toluene-*p*-sulphonic ester produced under the general gas-chromatographic conditions because of the high boiling-points of these esters. Moreover, the proportions of the sulphonic esters increase with the increase in functional groups of polyols and those of the acetates decrease. Nevertheless, the gas chromatograms (Fig. 1) are satisfactory for identification of base compounds of polyethers.

When the reagent and ethyl toluene-*p*-sulphonate were refluxed together, ethyl acetate was produced, indicating that acetylation and sulphonic ester formation are in a state of equilibrium under certain conditions.

This reagent was used by Karger and Mazur[13] for the cleavage of ethers of low molecular weight, but it is clear that it can also be used for cleavage of polyethers.

Acetyl chloride can be used instead of acetic anhydride, but with the latter the preparation procedure is much easier and the performance of the reagent more efficient.

This method can be applied not only to the identification of polyol base compounds but also to the determination of the hydrophobic groups in oxyethylene-type non-ionic surface-active agents.

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An Electrometric Method for the Determination of Soil Moisture

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An electrometric method for determining soil moisture, based on the reduction of the specific resistance of propan-2-ol that occurs on addition of moisture to it, is described. The soil sample is shaken with pure propan-2-ol and from the fall in the specific resistance of the filtered extract the percentage of moisture in the soil is read off.

Pure alcohols, such as propan-2-ol, have a specific resistance of about 2 MΩ at 25 °C, which is considerably reduced in the presence of water or salts, or both. The specific resistance of propan-2-ol containing increasing amounts of water, and after shaking the aqueous alcoholic samples with excess of pure sodium chloride, was therefore determined at 25 °C. Double-distilled water that had a specific resistance of 5 MΩ at 25 °C was used. A platinised platinum conductivity cell connected to an electronic a.c. resistance bridge was used to measure the specific resistance. The results obtained are given in Table I and a graph of the specific resistance of the saline aqueous alcoholic mixtures is shown in Fig. 1.

Table I

Specific resistance of propan-2-ol at 25 °C when mixed with varying amounts of water and shaken with excess of sodium chloride

Water added to 10 ml of alcohol/g	Specific resistance at 25 °C	
	Water alone/MΩ	Water *plus* NaCl/kΩ
0·0	2·00	90·00
0·2	1·30	28·00
0·4	0·60	11·00
0·5	—	8·00
0·6	0·50	5·50
0·7	—	4·00
0·8	0·40	3·30
0·9	—	2·70
1·0	0·38	2·10

A proportionate reduction in the specific resistance of the alcohol occurs with additions of increasing amounts of water and, in presence of sodium chloride, the specific resistance, even of the pure alcohol, is considerably reduced. Probably sodium chloride is slightly soluble in the alcohol. Additions of the salt to the aqueous alcoholic solution further reduced its specific resistance, but it is to be noted that in this instance also the reduction is proportional to the amount of water added and not to the amount of salt as this is always present in excess. A typical parabolic relationship exists between the specific resistance and the amount of water added (Fig. 1).

The above behaviour of the specific resistance of the alcohol suggests a convenient technique for the rapid determination of moisture in solid materials, including soils. This paper deals only with the application of the technique to soils.

Edison, in 1961, has shown that, when soil is shaken with a sufficient excess of propan-2-ol, all of the moisture in the soil sample is extracted. From the specific resistance of the extract the amount of water extracted can be determined and, therefore, the moisture in the soil can be calculated.

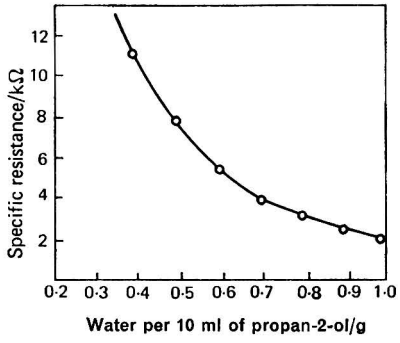

Fig. 1. Graph showing relationship between specific resistance and amount of water added

Method

The accuracy of such a method for determining soil moisture and its suitability for application to various soils were then examined. Six soil samples from different parts of India were collected. The samples were ground to pass through a 2-mm sieve as in soil analysis, and about 50 to 100 g of each soil sample were dried at 105 °C for 12 hours and were preserved in a desiccator over concentrated sulphuric acid.

Calculated amounts of each of the oven-dried soil samples were weighed into test-tubes and mixed with the requisite amounts of water to give exactly 2 g of the wet soil, the tubes being then stoppered with rubber corks and left overnight to attain equilibrium. The following morning, each wet soil sample was treated with 10 ml of pure propan-2-ol and the mixture was thoroughly shaken and filtered through a dry filter-paper. The filtrates were collected over 0·5 g of finely powdered dry sodium chloride, and the supernatant clear liquid was taken for measurement of its specific resistance. The results obtained are presented in Table II.

Table II

Specific resistance at 25 °C of propan-2-ol extracts of wet soil samples

Water in 2 g of wet soil/g	Water, per cent.	Barhe soil	Gwalior soil	Durg soil	Ujjain soil	Sagar soil	Rewa soil
		Specific resistance/kΩ					
0·00	0·0	91·0	89·3	88·0	90·0	92·0	88·9
0·40	20·0	11·0	—	—	11·0	11·0	11·0
0·44	22·0	—	—	9·6	—	—	—
0·50	25·0	—	7·2	—	—	—	—
0·60	30·0	5·7	—	—	6·0	6·0	5·5
0·64	32·0	—	—	4·7	—	—	—
0·70	35·0	—	4·2	—	—	—	—
0·80	40·0	3·1	—	—	3·3	3·3	3·2
0·84	42·0	—	—	3·0	—	—	—
0·90	45·0	—	2·7	—	—	—	—
1·00	50·0	2·0	2·1	2·1	2·2	2·2	2·1

From these values the amount of water extracted by the alcohol was determined by reference to the graph (Fig. 1) and the percentage of moisture in the wet soil samples was calculated. In Table III are given the added and the determined moisture percentages in the soils together with their pH values and salinities.

It can be seen from Table II that with increasing percentages of moisture in any of the soil samples there is considerable reduction (several kiloohms) in the specific resistance of the alcoholic extract. However, the reduction in specific resistance at any level of soil moisture is the same irrespective of the nature of the soil.

TABLE III

MOISTURE PERCENTAGE IN SOIL SAMPLES (CALCULATED FROM FIG. 1)

Soil	pH	Salinity/ $m\Omega^{-1}$	Moisture, per cent.				
Barha	7·6	0·1	Traces (0·0)	20·0 (20·0)	29·0 (30·0)	40·5 (40·0)	50·5 (50·0)
Gwalior	7·0	0·1	Traces (0·00)	26·0 (25·0)	34·5 (35·0)	44·5 (45·0)	50·0 (50·0)
Durg	6·9	0·8	Traces (0·00)	21·9 (22·0)	31·8 (32·0)	42·0 (42·0)	50·0 (50·0)
Ujjain	7·9	0·1	Traces (0·00)	20·0 (20·0)	28·8 (30·0)	40·0 (40·0)	49·0 (50·0)
Sagar	7·1	0·1	Traces (0·00)	20·0 (20·0)	28·8 (30·0)	40·0 (40·0)	49·0 (50·0)
Rewa	6·3	0·1	Traces (0·00)	20·0 (20·0)	30·0 (30·0)	40·5 (40·0)	50·0 (50·0)

Figures in brackets indicate the percentages of water added to the dry soil samples (Table II, column 2).

It is clear from Table III that the moisture percentages determined agree closely with the added moisture in all of the soil samples. The pH values of the soils varied from 6·3 to 7·6 and the salinity from 0·1 to 0·8 $m\Omega^{-1}$, but they did not affect the accuracy of the moisture determinations. Salinity and pH are known to interfere in other electrical methods for determining soil moisture.

The method described above is rapid, easy to manipulate, does not require the use of complicated or costly instruments and yields results accurate to within 1 per cent. over a wide range of soil moistures and with a variety of soils. Extension of the method to other solid materials is under investigation.

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Book Reviews

SELECTED READINGS IN CHROMATOGRAPHY. Edited by R. J. MAGEE. Pp. viii + 129. Oxford, New York, Toronto, Sydney and Braunschweig: Pergamon Press. 1970. Price (hard-cover) £1·75; $5.50; (flexi-cover) £1·25; $4.00.

This small, but very interesting, book contains reprints of six of the original papers published in various branches of chromatography. A mere catalogue of the names of the authors and the titles of their papers can convey only part of what is in store for readers but, nevertheless, it is worth giving such a catalogue in a short review because these names are legendary in all laboratories where chromatography is studied rather than just used.

The editor's foreword leads on to the paper by Tswett "Zur Chemie des Chlorophylls," which is followed by the classical paper of Martin and Synge. The paper of Kirchner, Miller and Keller "Separation and Identification of Some Terpenes by a New Chromatographic Technique" follows with deceptive ease and leads to one of the first papers dealing with electrophoresis, that of Hangaard and Kroner, in which they describe the partition chromatography of amino-acids under the influence of an applied voltage.

Although out of chronological sequence, the paper of Thompson, Harvey, Choppin and Seaborg (1954) dealing with ion-exchange chromatography and the chemical properties of elements 99 and 100, is well suited to precede what to me has always been a fascinating paper, that published in 1952 by James and Martin and dealing with gas - liquid partition chromatography.

It is fascinating to read these papers, in the light of one's own background of general practical chromatography, and to see how whole technologies have been built up as a result of these fundamental discoveries. It is also salutary to consider these few papers in the light of the effect they, and some others, have had on the progress of chemistry as a whole and analytical chemistry in particular.

The editor is to be congratulated on the brief introduction made to each paper and technique; he is also to be thanked for including the paper of Tswett in the original German, with an excellent vocabulary aid (for many of us, a help—if not a necessity).

I cannot remember when I found a book so worthy of recommendation. I would not hesitate to recommend it to any student of chemistry from the sixth form level to postgraduate level. Indeed, I have already done so. L. S. BARK

MARINE CHEMISTRY. VOLUME II. THEORY AND APPLICATIONS. By DEAN F. MARTIN. Pp. xii + 451. New York: Marcel Dekker Inc. 1970. Price $9·50; £4·50.

According to the author's preface, the present volume was developed from an introductory course in marine chemistry presented to advanced undergraduates and postgraduates from various backgrounds. It is intended to cover both the theoretical and applied aspects of the subject, the practical aspects having been treated in the first volume. Because of its origin perhaps, the book reads like a rather disjointed collection of lecture notes. Some of the arguments are not at all well presented, and the reader is left with the impression that the author has little grasp of many aspects of the subject. The selection of subject material is poorly balanced; thus, dissolved oxygen receives only passing mention, and dissolved and particulate organic carbon, which are of great importance in the marine ecosystem, are not discussed at all. In contrast, a disproportionate amount of space is devoted to topics that are of little or no direct relevance to the subject, *e.g.*, tides, waves, the Plimsoll line, optical activity, the ownership of the sea-floor (8 pages on this!) and the now discredited thermal anomalies in the properties of water. Although most of the book is very disappointing, the chapters on the nitrogen and phosphorus cycles are reasonably good. That concerned with the model ocean is interesting, but the author fails to warn the reader that many of the mechanisms presented are speculative and not by any means generally accepted. The chapter on organic production is too short and scrappy to be of any value.

The book is marred throughout by errors of fact and misprints, which are too numerous to mention, and also by much loose writing (thus, the reader may be perplexed to read (p. 137) that "oxidation of phytoplankton is estimated to require about 276 atoms of oxygen"). The price of the book seems high considering that it is reproduced by an offset process. This system of reproduction is hard on the eyes and results in very few words to the page. In view of these various deficiencies, the reviewer is unable to recommend this book for anyone requiring a balanced account of the field of modern marine chemistry. J. P. RILEY

INTRODUCTION TO MOLECULAR SPECTROSCOPY. By E. F. H. BRITTAIN, W. O. GEORGE and C. H. J. WELLS. Pp. xii + 387. London and New York: Academic Press. 1970. Price £6.

The object of this volume is to present a balanced treatment of the principal methods of molecular spectroscopy together with selected practical work. The authors draw on their experience in teaching the principles and applications of molecular spectroscopy to undergraduates. Some subjects, however, are covered in more detail than would normally be presented to undergraduates; these have formed part of an M.Sc. course in molecular spectroscopy taught by the authors.

The introductory chapter of the book is concerned with fundamental theory common to all branches of spectroscopy, and gives a brief account of the theory of the interaction of electromagnetic radiation with matter, wave mechanics and symmetry concepts. The subsequent chapters deal in turn with electronic and vibrational spectroscopy, nuclear magnetic resonance and mass spectrometry. Each chapter is divided into sections that treat principles, instrumentation and experiments. The experiments chosen illustrate topics discussed in the principles and instrumentation sections. These experiments are selected to provide a well balanced view of the use of each technique in different branches of chemistry. Thus, in the chapter on vibrational spectroscopy, for example, there are examples of interest to teachers of physical chemistry (vibration-rotation spectra of HCl, CO_2, etc., and their interpretation), inorganic chemistry (vibrational spectra of triangular, pyramidal and tetrahedral molecules and ions), organic chemistry (characteristic group vibrations and effect of substituents, hydrogen bonding and deuterium exchange studies) and analytical chemistry (analysis of two- and four-component mixtures, attenuated total reflectance). At the conclusion of each experiment pertinent questions concerning the interpretation of the results are raised in a separate discussion section.

This volume is well written and deserves widespread readership among those concerned with the teaching of practical chemistry to undergraduates. G. F. KIRKBRIGHT

ANALYTICAL CHEMISTRY IN SPACE. Edited by RICHARD E. WAINERDI, P.E., Ph.D. International Series of Monographs in Analytical Chemistry, Volume 35. Pp. viii + 275. Oxford, London, Edinburgh, New York, Toronto, Sydney, Paris and Braunschweig: Pergamon Press. 1970. Price £7.

This collection of seven articles provides critical, well organised and systematic reviews of some of the instruments and techniques used in the analysis of extraterrestrial materials, the resulting data and their interpretation. The book should serve well as a research text and as a source book for advanced courses in analytical chemistry and the sciences of the solar system.

The individual chapters are as follows: Determining the Composition and History of the Solar System (Herzog, 60 pages); Space Engineering Considerations (McKannan, 28 pages); Solar System Atmospheres (Johnson, 20 pages); Mass Spectroscopy in Solar System Exploration (Herzog, 56 pages); Lunar and Planetary Surface Analysis Using Neutron Inelastic Scattering (Waggoner, 30 pages); Extraterrestrial *In Situ* 14 MeV Neutron Activation Analysis (Hislop and Wainerdi, 14 pages); and Recovered Extraterrestrial Materials (Mapper and Smales, 68 pages).

As Sir Bernard Lovell remarks in his foreword, "A start has been made . . . on the analysis of the chemical constituents of space" and that is what this book is about. The chapters should be useful and stimulating to those already in the field and to those interested in joining it. Certainly, the book is timely, for the exploration of the solar system continues to gain momentum. Space chemistry is no longer an armchair activity but is an active pursuit conducted by telescope, space vehicle and equipment at the laboratory bench. The data discussed in this book are already changing and shaping our understanding of the origin of the dynamic equilibrium of the materials of our own planet. The pace of research is now fast, and, as such, events have already overtaken the book. Thus, Mapper and Smales, when they wrote their chapter, were able to discuss only the plans for lunar sample return and subsequent analysis. Even so, the predictions and assessments made in this book, both by Mapper and Smales and by other authors, make interesting reading when compared with the results from Apollo 11 and 12. The book certainly makes a good background for chemical research and study concerned with the Moon and the rest of the solar system.

In short, this is a useful and interesting text on the analytical methods and results concerned with our attempts to unravel the chemistry of the solar system. G. EGLINTON

A Sensitive, Specific Method for Determining the Riboflavin Content of Children's Urine

A four-part method based on Wahba's technique is presented for the specific determination of the riboflavin content of children's urine. First, the interfering urinary constituents are precipitated by treating the urine sample with zinc acetate and formalin solutions; they are then removed by centrifugation. The riboflavin in the resultant supernatant liquid is then separated from the other soluble molecular constituents of urine by elution chromatography, by using a talc column. The riboflavin eluate is next subjected to one-dimensional chromatography on silica gel plates to confirm the identity of the fluorescent spot before the determination. Finally, the thin-layer chromatographic plate is dried and the riboflavin content of the plate is measured fluorimetrically by using the technique of reflectance densitometry.

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Analyst, 1971, **96**, 432–436.

Analysis of Steroids. Part XVII. A Differential Spectrophotometric Variant of the Diethyl Oxalate Method for the Determination of Ketosteroid Contaminants

A method that consists of spectrophotometry following a Claisen condensation with diethyl oxalate was used for the determination of ketosteroid contaminants. Absorption of the main steroid component was eliminated by a differential spectrophotometric procedure. The proposed method is useful for the determination of ketosteroid contaminants present at the level of 0·1 per cent.

S. GÖRÖG

Chemical Works of Gedeon Richter Ltd., Budapest X, Hungary.

Analyst, 1971, **96**, 437–441.

The Application of Anion-selective Membrane Electrodes in Pharmaceutical Analysis
Part II. Determination of Cyanocobalamin in Pharmaceutical Preparations

Quantitative reduction or illumination of cyanocobalamin are used to liberate hydrogen cyanide, which is then determined by using the cyanide-selective membrane electrode. The results from this procedure are very satisfactory. The method is applicable to very low concentrations not only of pure cyanocobalamin but also of pharmaceutical preparations.

YEHIA M. DESSOUKY and E. PUNGOR

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Analyst, 1971, **96**, 442–446.

A Method for the Determination of Carbon Monoxide, Carbon Dioxide, Sulphur Dioxide, Carbonyl Sulphide, Oxygen and Nitrogen in Furnace Gas Atmospheres by Gas Chromatography

A method is described for the routine analysis of mixtures containing any of the named gases by using a dual-column system and a katharometer detector. The method is suitable for 1-ml gas samples that may contain from 100 per cent. of one of the gases down to at least 7×10^{-2} per cent. of any of them, provided that the ratio of carbon monoxide to carbon dioxide or nitrogen to carbon monoxide does not exceed 50:1.

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Analyst, 1971, **96**, 447–451.

The Determination of Phosphate in Detergents by Cool-flame Emission Spectroscopy

The total phosphate content of detergent materials is determined by measurement of the emission of the HPO molecular species at wavelength 528 nm in a cool hydrogen - nitrogen diffusion flame. Preliminary treatment with cation-exchange resin is necessary to remove interference by metals. Analytical results on detergent samples containing up to 20 per cent. of phosphates (expressed as P_2O_5) indicates a precision of the order of 2 to 4 per cent. for the method.

W. N. ELLIOTT and R. A. MOSTYN

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Analyst, 1971, **96**, 452–456.

The Identification of Polyol Base Compounds in Polyurethane Polyethers by Gas Chromatography

The identification of polyol base compounds of polyurethane polyethers has been carried out by gas chromatography after conversion into their corresponding acetates. The polyethers are allowed to react with the mixed anhydride of acetic and toluene-*p*-sulphonic acids (a cleavage reagent), and converted into the acetates. With gas chromatography the acetate peaks of the polyol base compounds appear at different positions, thus enabling these compounds to be easily distinguished.

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Analyst, 1971, **96**, 457–459.

An Electrometric Method for the Determination of Soil Moisture

An electrometric method for determining soil moisture, based on the reduction of the specific resistance of propan-2-ol that occurs on addition of moisture to it, is described. The soil sample is shaken with pure propan-2-ol and from the fall in the specific resistance of the filtered extract the percentage of moisture in the soil is read off.

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Analyst, 1971, **96**, 460–462.

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