# THE ANALYST

THE JOURNAL OF

# The Society for Analytical Chemistry

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ANALYTICAL CHEMISTRY

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## VOL. 88

1963

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# THE ANALYST

# INDEX TO VOLUME 88

## INDEX TO AUTHORS

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## ERRATA:

VOL. **88**, 1963:

p. 28, 25th line. *For* "0·1 N" *read* "0·01 N."

p. 61, 8th line. *For* "130·13 *VC*/178" *read* "130·13 *VC*/356."

p. 67, 23rd line from bottom. *For* "0·7 mg" *read* "0·07 mg."

pp. 162, 163 (twice), 164 and 165, references. *For* "British Standard 3406 : Part 2 : 1962" *read* "British Standard 3406 : Part 2 : in the press."

pp. 174, last line, and 175, 13th line. *For* "British Standard 3406 : Part 3 : 1962" *read* "British Standard 3406 : Part 3 : 1963."

p. 179, 7th line. *For* "British Standard 3406 : Part 4 : 1962, p. 196" *read* "British Standard 3406: Part 4 : in the press."

p. 215, 13th line. *For* "$\nless$ 6 litres" *read* "$\nless$ 6 changes."

p. 273, Fig. 3 (*a*). The connection to the cathode of the EN 91 valve should be shown joined to the right-hand heater connection by the insertion of a dot at the point where the two connections cross.

p. 397, 21st line from foot of page. *For* "sodium hydroxide" *read* "iodine."

p. 540, line 3. *For* "0·1-mm cells" *read* "1·0-mm cells."

p. 546, line 2. *For* "differences" *read* "difference."

p. 547, Fig. 3. The equation "$Y = 0{\cdot}272 - 0{\cdot}935x$" *should read* "$Y = 0{\cdot}272 + 0{\cdot}935x$."

p. 738, foot of page, present address of T. Doran. *For* "Sunbury-on-Thames, Surrey," *read* "Sunbury-on-Thames, Middlesex."

p. 768, penultimate line. *For* "specific gravity" *read* "specific activity."

p. 822, lines 22–24. The "Report of the Proceedings of the Thirteenth Session of the International Commission for Uniform Methods of Sugar Analysis" is obtainable only from M. R. Saunier.

p. 829, author's address. *For* "64–68" *read* "64–78."

p. 833, reference 10. *For* "26" (the page number of the reference) *read* "36."

p. 834, reference 23. *Delete* "Berry, M. M., and Kent, A."

p. 899, reference 2. *For* "71" (the page number of the reference) *read* "76."

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# The ANALYST

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# THE ANALYST

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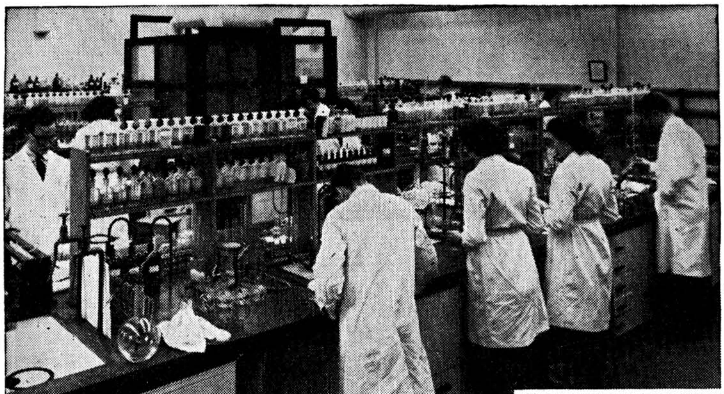
Volume 88, No. 1042 January, 1963

# Incidental information

*No. 17*



***Items of interest from our laboratory notebooks***

**9-(4-Dimethylaminophenyl)-2,3,7-trihydroxy-6-fluorone**

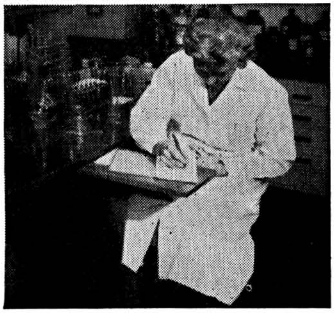
A reagent for the spectrophotometric determination of **Ta** in the presence of **Zr, Nb** or **Ti.** (see V. A. Nazarenko and M. B. Shustova, *Zavodskaya Lab.* **2B,** 1283 (1957); C.A. **53,** 13874c (1959)). CODE 3862·3. Price 1g 9/9, 5g 40/-.

**1,3-Diphenylpropan-1,3-dione** (Dibenzoylmethane)

A reagent for the spectrophotometric determination of **U.** A procedure that includes ion-exchange separation from **Th** is described by H. J. Seim *et al. Anal. Chem.* **31**, 957 (1959). CODE 4004·45. Price 5g 7/6, 10g 13/6.

**4-(2-Pyridylazo)-resorcinol, sodium salt** (PAR)

An extremely sensitive reagent for the colorimetric determination of **Co, Pb** and **U.** (see F. H. Pollard *et al. Anal. Chim. Acta* **20,** 26 (1959)). CODE 7275·5. Price 1g 6/-, 5g 22/9, 10g 44/-.



***o*-(Hydroxymercuri)-benzoic acid**

A titrimetric reagent for $S^{2-}$, **thiourea, xanthates, mercaptans** and many other organic sulphur compounds. (see M. Wronski *Analyst* **83,** 314 (1958)). CODE 4691. Price 1g, 12/6, 5g 52/6.

**Phenyl 2-pyridyl-ketoxime**

A colorimetric reagent for the determination of iron in strong alkalis and of **"oxidised" iron** in the presence of metallic iron. (see F. Trusell and H. Diehl *Anal. Chem.* **31,** 1978 (1959)). CODE 6711. Price 1g 30/-, 5g 135/-.

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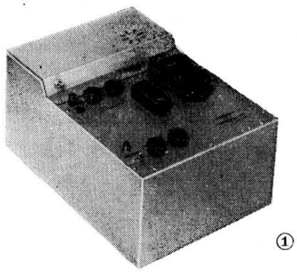
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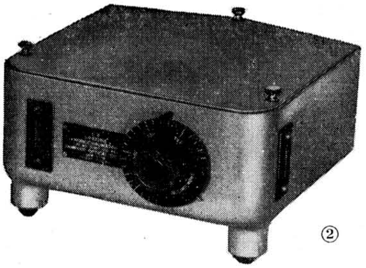
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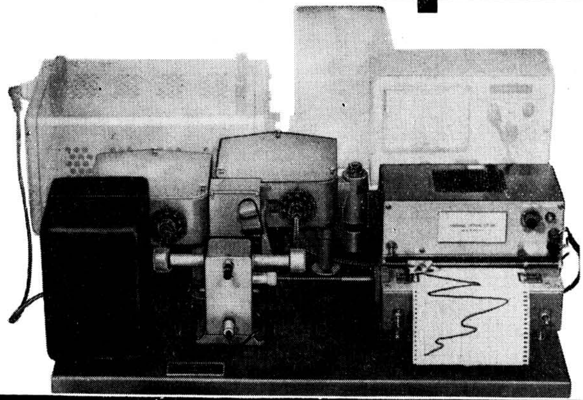
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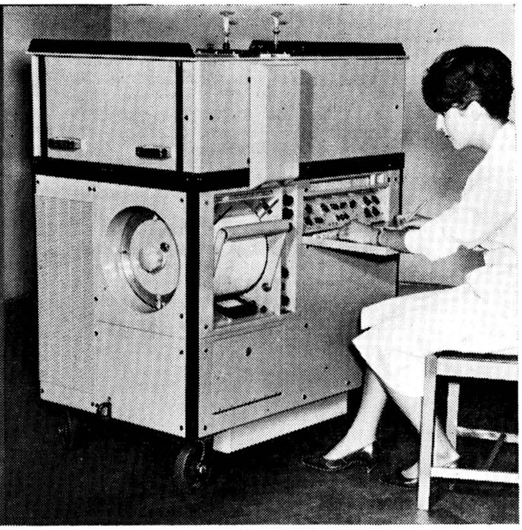

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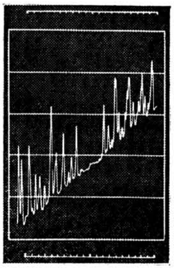

Fig. 1 *(left)*
Single-Beam Spectrum of atmospheric water vapour in SP.100 cell well. 1600 cm-1.
Instrument Pressure — $10^{-3}$ mm Hg
Cell Well Pressure — 760 mm Hg

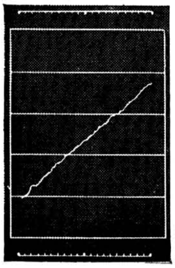

Fig. 2 *(right)*
Showing the effect of evacuating the cell well.
Instrument Pressure — $10^{-3}$ mm Hg
Cell Well Pressure — $10^{-1}$ mm Hg

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Dünnschicht-Chromatographie
Ein Laboratoriumshandbuch

Bearbeitet von

H. R. Bolliger
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H. Gänshirt
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H. Seiler
E. Stahl
D. Waldi

Herausgegeben von

Professor Dr. Egon Stahl

Institut für Pharmakognosie
der Universität des Saarlandes,
Saarbrücken

Mit 197 Abbildungen und 2 Farbtafeln.
XVI, 534 Seiten Gr.-8°. 1962.

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■ **Bitte Prospekt anfordern!**

Die neue Methode zur schnellen Trennung winziger Mengen anorganischer oder organischer Substanzen hat auf so vielen Gebieten der Naturwissenschaften und der Medizin Anwendung gefunden und so viel Erfahrungsmaterial ist gesammelt, daß eine buchmäßige Zusammenfassung einem großen Bedürfnis entspricht. Schwerlich kann ein Einzelner die erforderliche Übersicht und Laboratoriumserfahrung haben, um ein der Praxis angemessenes Handbuch verfassen zu können, das sowohl dem Anfänger als auch dem Spezialisten wirkliche Hilfe bringt. Aus diesem Grunde fand sich eine internationale Gruppe von Kollegen zusammen, die sich bemühte, das Bestmögliche zu schaffen. Es erscheint bei dem derzeitigen Entwicklungsstand der Dünnschicht-Chromatographie besonders wertvoll, daß die Autorengemeinschaft zahlreiche, noch unveröffentlichte, eigene Arbeiten mit aufgenommen hat.

**Inhaltsübersicht**

**Allgemeiner Teil:** Entwicklungsgeschichte der Dünnschicht-Chromatographie · Geräte zur Dünnschicht-Chromatographie und ihre Handhabung · Sorptionsmittel für die Dünnschicht-Chromatographie · Spezielle Arbeitstechniken · Dokumentation der Dünnschicht-Chromatogramme · Quantitative Auswertung von Dünnschicht-Chromatogrammen · Isotopentechnik · Theoretische Grundlagen der Dünnschicht-Chromatographie.

**Spezieller Teil:** Einleitung · Aliphatische Lipide · Terpenderivate, ätherische Öle, Balsame und Harze · Vitamine · Steroide (Sterine, Pregnan-, Androstan-, Oestran-Verbindungen, Gallensäuren und Herzglykoside) · Organische Basen: Alkaloide; „Einfache“ Indolderivate; Amine; Teer-Basen · Arzneimittel · Dünnschicht-Chromatographie in der klinischen Diagnostik und Pharmakologie · Organische Synthetica (Hilfsstoffe der Industrie) · Hydrophile Pflanzeninhaltsstoffe, insbesondere von Arzneipflanzen · Aminosäuren und Derivate · Dünnschicht-Ionophorese (Dünnschicht-Elektrophorese) · Nucleinsäuren und Nucleotide · Zucker und Derivate · Dünnschicht-Chromatographie anorganischer Ionen · Reagentien für die Dünnschicht-Chromatographie · Fachausdrücke (106 häufig verwendete Ausdrücke in der DC; englisch-deutsch-französisch).


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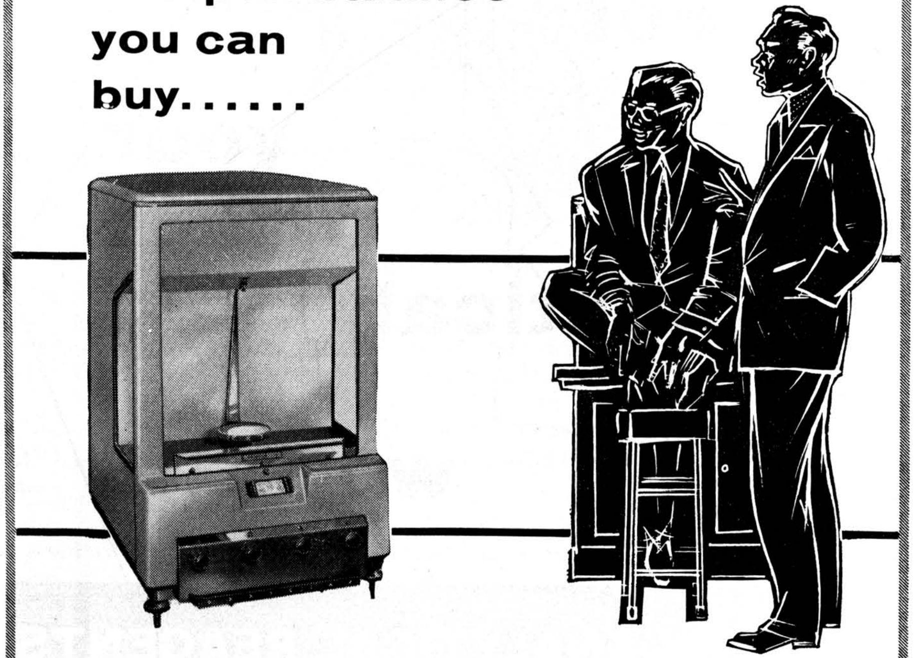

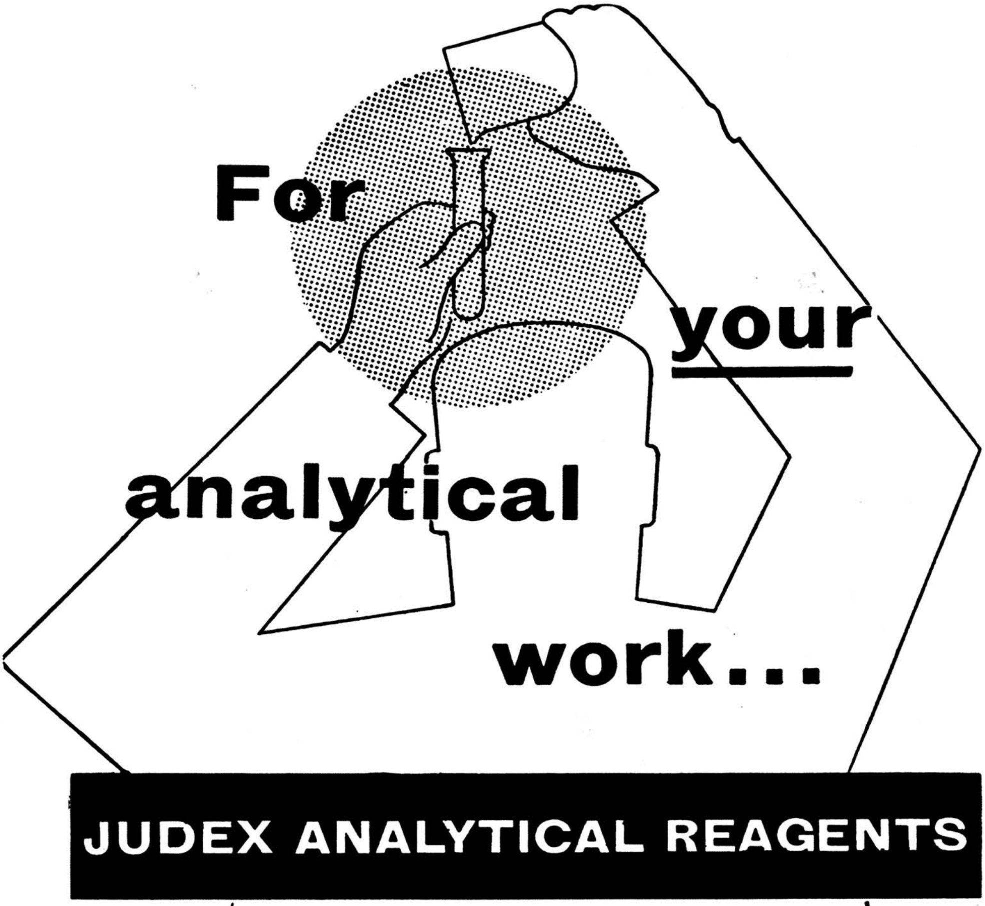
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JANUARY, 1963 Vol 88, No. 1042

THE ANALYST

# PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

## NEW MEMBERS

### ORDINARY MEMBERS

John Robert Belbin, M.A. (Cantab.), A.R.I.C.; Edmund John Huskisson Birch, M.A. (Oxon.), F.R.I.C.; Ernest Palfrey Bridge, B.Sc. (Wales); Margaret White Kenyon, A.I.S.T.; Frederick Ifeanyichukwu Ozoh, A.I.S.T., M.R.S.H.; Geoffrey Frederick Phillips, M.Sc. (Lond.), A.R.I.C.; Jaroslav Soucek, Ing.Chem. (Brno); Harold Edgar Stagg, B.Sc. (Lond.), F.R.I.C.

### JUNIOR MEMBERS

Arnold John Bloom, M.Sc.Tech. (Manc.); Helen Shirran Gilmour, B.Sc. (Aber.); John William Saich.

## DEATHS

WE record with regret the deaths of

Robert Emrys Jones
William Joseph Sommerville Pringle.

## NORTH OF ENGLAND SECTION AND PHYSICAL METHODS GROUP

A JOINT Meeting of the North of England Section and the Physical Methods Group was held at 2.15 p.m. on Saturday, December 8th, 1962, at the Old Nag's Head Hotel, Lloyd Street, Manchester. The Chair was taken by the Chairman of the North of England Section, Mr. J. Markland, B.Sc., F.R.I.C.

A lecture on "Nuclear Magnetic Resonance" was given by Professor E. R. Andrew, M.A., Ph.D.

## SCOTTISH SECTION

A JOINT Meeting of the Scottish Section with the Chemical Society, the Society of Chemical Industry and the Royal Institute of Chemistry was held at 6 p.m. on Friday, December 7th, 1962, in Room 24, Royal College of Science and Technology, Glasgow. The Chair was taken by the Chairman of the Glasgow Section of S.C.I., Professor J. Monteath Robertson, C.B.E., M.A., Ph.D., D.Sc., F.R.S.

The following paper was presented and discussed: "Thoughts on Meat," by E. C. Bate-Smith, M.Sc., Ph.D., M.Inst.R.

## WESTERN SECTION

A JOINT Meeting of the Western Section with the Cardiff and District Section of the Royal Institute of Chemistry was held at 7 p.m. on Friday, December 7th, 1962, at University College, Cardiff. The Chair was taken by the Vice-Chairman of the Western Section, Mr. E. A. Hontoir, B.Sc., A.I.M.

The following paper was presented and discussed: "Gem Stones and Jewels, Natural and Synthetic," by R. C. Chirnside, F.R.I.C.

## PHYSICAL METHODS GROUP

THE Eighteenth Annual General Meeting of the Group was held at 6.30 p.m. on Tuesday, November 27th, 1962, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the Vice-Chairman of the Group, Mr. L. Brealey, B.Sc., F.R.I.C. The following appointments were made for the ensuing year: *Chairman*—Dr. W. Cule Davies. *Vice-Chairman*—Mr. L. Brealey. *Hon. Secretary and Treasurer*—Dr. T. L. Parkinson, Product Research Department, Beecham Food and Drink Division Ltd., Harpenden Rise, Harpenden, Herts. *Members of Committee*—Mr. B. B. Bach, Dr. J. A. W. Dalziel, Mr. J. H. Glover, Mr. W. C. Hanson, Mr. D. Moore and Mr. D. C. M. Squirrell. Dr. D. C. Garratt and Mr. C. A. Bassett were re-appointed as Honorary Auditors.

The Annual General Meeting was followed by the Eighty-fifth Ordinary Meeting of the Group, at which the subject of the meeting was "Recent Improvements in Separation Techniques" and the following papers were presented and discussed: "High-voltage Paper Electrophoresis," by D. Gross, Ph.D. (see summary below); "Solvent Extraction of Inorganic Compounds, with Special Reference to Synergism," by Professor H. M. N. H. Irving, M.A., D.Phil., D.Sc., F.R.I.C., L.R.A.M.

### HIGH-VOLTAGE PAPER ELECTROPHORESIS

DR. D. GROSS said that paper electrophoresis had proved very useful in the field of high-molecular compounds, particularly proteins. In the field of low-molecular compounds progress had been much slower. In order to be competitive with paper chromatography, paper electrophoresis had to offer definite advantages. This it did in an impressive way for proteins, but not markedly for low-molecular compounds, apart from the different sequence of positions.

The introduction of the high-voltage technique employing 5 to 10 kV or potential gradients of 100 to 200 volts per cm had changed the situation significantly. Reduction in running time and hence minimisation of adverse diffusion effects had turned paper electrophoresis into a rapid and convenient tool of a high resolving power, which by itself or as a complement of paper chromatography was proving extremely valuable in many analytical problems.

The lecturer then gave briefly the theoretical background to the development of the high-voltage technique and stressed the difficulties encountered in designing suitable equipment. The main points to be observed were efficient heat dissipation and overall pressure control, where required. He first dealt with various designs currently used and then described in more detail the equipment he had developed over the last 10 years, which resulted in an apparatus of the double-cooled sandwich type with highly reproducible pressure control. In several years of service it had proved to be reliable, convenient, robust and safe, and required surprisingly little maintenance.

The lecturer next discussed some results of the application of high-voltage paper electrophoresis to problems in the fields of inorganic and organic chemistry. Limitation of time did not allow him to refer to more than one or two examples for each group of compounds such as inorganic cations—particularly the alkali and alkaline-earth metals, complex inorganic anions—particularly oxy-acids of phosphorus and sulphur, sugars, amino-acids, amines, peptides, non-volatile organic acids and lower fatty acids. Both uni- and two-dimensional techniques were employed. In some instances, he demonstrated results obtained by high-voltage electrophoresis side by side with those obtained by low-voltage electrophoresis under equivalent conditions, showing a convincingly better resolution by the high-voltage technique.

Finally, Dr. Gross summarised the advantages and difficulties of the new technique and said that he was quite confident that there was a definite place for it in modern analytical chemistry, particularly for low-molecular compounds.

# The Determination of Small Amounts of Iron

## Some Aspects of New Colorimetric Methods*

By H. J. CLULEY

(*The General Electric Company Limited, Central Research Laboratories, Hirst Research Centre, Wembley, England*)

AND E. J. NEWMAN

(*Hopkin & Williams Limited, Analytical Department, Chadwell Heath, Essex*)

Three colour reactions that seemed to merit particular attention have been examined. Certain interferences in the determination of ferrous iron with 4,7-diphenyl-1,10-phenanthroline have been overcome. Some interferences in the determination of ferrous iron with *syn*-phenyl 2-pyridyl ketoxime are reported, and the application of this reagent to the determination of iron in glass sand has been confirmed. The colour reaction between ferric iron, ethylenediaminetetra-acetic acid and hydrogen peroxide is recommended for determining larger amounts of iron, particularly in copper and its alloys.

It is not easy to select suitable methods for determining small amounts of iron from the many available in the literature. This paper deals with three methods, each of which appears to offer some distinct advantage.

Of the established reagents, 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline) is probably the most outstanding on the basis of sensitivity and selectivity.

A newer reagent, *syn*-phenyl 2-pyridyl ketoxime, has the distinctive property of giving a sensitive colour reaction with ferrous iron in highly alkaline solutions. It can be used, for example, to determine iron in alkali-metal hydroxides and in the melts resulting from alkaline fusions of silicates.

Ferric iron reacts with ethylenediaminetetra-acetic acid and hydrogen peroxide in alkaline solution to produce a colour. This provides a method for determining iron that, although not particularly sensitive, is capable of yielding accurate results. It is our opinion that this reaction has not received the attention it deserves.

## DETERMINATION OF IRON WITH BATHOPHENANTHROLINE

Bathophenanthroline reacts with ferrous iron within the pH range 2 to 9 to form the bright red *tris*-(4,7-diphenyl-1,10-phenanthroline) - ferrous ion, which has a maximum light absorption at 533 m$\mu$. It can be extracted as an ion-association system with a suitable anion into any one of several organic solvents. The molar extinction coefficient of the complex is about 22,000, the exact value depending on the solvent used. This is approximately twice the value of the molar extinction coefficient of the familiar ferrous - 1,10-phenanthroline complex in aqueous solution. Moreover, the ease of extraction of the ferrous - bathophenanthroline complex confers two extra advantages over the use of 1,10-phenanthroline. Concentration of the iron complex into an organic solvent of smaller volume than that of the test solution results in an effective increase in the sensitivity of the test. Also, the blank values can be reduced by treating certain of the reagents used in the test procedure with bathophenanthroline, followed by extraction with an organic solvent to remove any iron.

Bathophenanthroline was first introduced as a reagent for iron by Smith, McCurdy and Diehl,[1] who used it for determining iron in water. It has since been used for determining serum iron[2,3] and for determining traces of iron in tungsten,[4,5] uranium-bearing materials,[5,6] copper,[7] bismuth,[8] germanium and its dioxide,[9] petroleum oils,[10] vanadium, chromium, titanium, niobium and tantalum metals, their alloys and compounds[5] and sexavalent molybdenum compounds.[11] It has also been used for determining ferrous iron in the presence of ferric iron[12,13] and for the determinations of corticoids,[14] hydrogen peroxide[15] and tocopherols,[16] all of which depend on the use of the reagent for determining ferrous in the presence of ferric iron.

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* Presented in part at the meeting of the Society on Thursday, February 8th, 1962.

Various workers have reported interferences in the determination of iron with bathophenanthroline. Smith, McCurdy and Diehl reported the formation of a yellow complex with cobalt,[1] but this is not extracted into organic solvents. Cuprous copper also gave a yellow colour in neutral or alkaline solutions, but below pH 7 a colourless complex formed. Relatively large amounts of these metals would therefore cause considerable interference by competing with the iron for the reagent. Zinc and nickel have also been reported to interfere similarly and to cause low results when there is insufficient bathophenanthroline to react with the iron *plus* the interfering metal.[9] Ammonium, alkali and alkaline-earth metal ions cause no interference, nor do $Be^{2+}$, $Hg_2^{2+}$, $Al^{3+}$, $Ce^{3+}$, $Pb^{2+}$, $Cr^{3+}$, $UO_2^{2+}$ and $Mn^{2+}$ ions when present in at least 10-fold excess over the amount of iron. It is apparent from the applications of bathophenanthroline that many other metal ions can be tolerated.

The anions $Cl^-$, $I^-$, $NO_3^-$, $SO_4^{2-}$, $ClO_4^-$, $S_2O_3^{2-}$, $SCN^-$ and acetate do not interfere. Cyanide interferes by reacting with the bathophenanthroline - ferrous complex to produce the violet dicyano-*bis*-(4,7-diphenyl-1,10-phenanthroline) - ferrous complex. It is possible that this reaction could be used for determining traces of cyanide.

There have been reports that the formation of the iron - bathophenanthroline complex is hindered by phosphate,[4,9] and that low results are obtained in the presence of oxalate,[4] citrate[4,9] and tartrate.[11] We also observed serious interference from lactate, fluoride and pyrophosphate, traces of which caused extremely low recoveries of iron.

The main objective of the work reported here was to overcome the interference caused by these anions.

## EXPERIMENTAL

The usual procedure for determining iron with bathophenanthroline is to add the reagent to the acetate-buffered test solution of pH 4 to 6, containing iron that has been reduced to the ferrous state; hydroxyammonium chloride is the most common reductant. After the colour has developed the complex can be extracted into one of several solvents. We prefer to use chloroform because it is denser than the aqueous phase. The chloroform extracts are diluted to a standard volume with absolute industrial ethanol, and the optical density of the solution is measured at 533 m$\mu$. Modifications of this general procedure, designed to overcome interference by tartrate[11] and citrate,[4,5] have been described, but no method has been described in the literature for eliminating interference from lactate, oxalate, fluoride, phosphate or pyrophosphate.

Galliford and Newman[11] complexed sexavalent molybdenum with tartrate to prevent precipitation of the molybdenum during determinations of iron with bathophenanthroline, but this raised the problem of the slightly low results that tartrate caused. These low results arise because tartrate hinders the extraction of the complex in some way. It is widely known that the ferrous - 1,10-phenanthroline complex can be extracted into the polar solvent nitrobenzene by adding perchlorate ion to the test solution. The ion-association system formed between the ferrous - phenanthroline complex and perchlorate ion is reasonably soluble in nitrobenzene. By analogy, Galliford and Newman added perchlorate to the test solutions of molybdates containing tartrate. In this way they increased the solubility of the bathophenanthroline - ferrous complex in chloroform, and so overcame the interference from tartrate.

We have applied a similar procedure to the determination of iron in molybdates, sodium tungstate, antimony trichloride and tartaric acid. Tartrate was used to prevent precipitation in tests on the molybdates, tungstate and antimony trichloride. This procedure is referred to below as the "tartrate method." We could not overcome the rather more serious interferences caused by citrate, lactate, oxalate, fluoride, phosphate and pyrophosphate simply by adding perchlorate to the test solution. The use of ascorbic acid, which is a more powerful reducing agent than hydroxyammonium chloride, caused a slight improvement in the results, but they were still extremely low. We presumed that these anions were forming complexes with the ferric ions and so preventing their reduction to the ferrous state. The formation of such complexes might be expected to be considerably reduced in fairly concentrated acid solution, and we decided, therefore, to reduce the ferric iron to ferrous under rather acid conditions. The test solutions were adjusted to 1 or 2 M in perchloric acid before adding ascorbic acid to reduce the iron. The bathophenanthroline was then added to the solution, the pH was raised to between 4 and 6, and the determination was completed in the usual way. The results obtained by this method were encouraging. The method is referred to below as the "acid reduction method."

The results obtained by these procedures are shown in Tables I, II, III and IV, and comparison is made with results obtained by other methods, all of which involved a final colorimetric determination of ferric iron by a thiocyanate method. The only serious discrepancy is in the results obtained on sodium pyrophosphate. Details are also given of recovery experiments carried out with known amounts of added iron.

TABLE I

RESULTS OBTAINED BY THE TARTRATE METHOD

A 1-g sample was used for each test

| | Iron found by— | |
|---|---|---|
| Sample | tartrate method, % | thiocyanate method, % |
| Antimony trichloride .. .. .. | 0·0012 | 0·0013 |
| Molybdic acid .. .. .. | 0·00021 | 0·0002 |
| Sodium molybdate .. .. .. | 0·00017 | 0·0002 |
| Tartaric acid .. .. .. | 0·00018 | 0·0002 |

TABLE II

RESULTS OF RECOVERY EXPERIMENTS BY THE TARTRATE METHOD

A 1-g sample was used for each test

| Sample | Iron added, µg | Iron found, µg | Recovery, µg |
|---|---|---|---|
| Antimony trichloride .. .. | 0 | 12 | |
| | 10 | 21 | 9 |
| Molybdic acid .. .. .. | 0 | 2·0, 2·1 | |
| | 10 | 12·1, 12·3 | 10·1 |
| Sodium molybdate .. .. | 0 | 1·7, 1·6 | |
| | 10 | 11·9, 11·6 | 10·1 |
| Sodium tungstate .. .. | 0 | 0 0 | |
| | 10 | 10·0, 9·8 | 9·9 |
| Tartaric acid .. .. .. | 0 | 1·8 | |
| | 5 | 6·8 | 5·0 |
| | 10 | 11·7 | 9·9 |

TABLE III

RESULTS OBTAINED BY THE ACID REDUCTION METHOD

| | | Iron found by— | |
|---|---|---|---|
| Sample | Weight taken for acid reduction method, g | acid reduction method, % | thiocyanate method, % |
| Citric acid .. .. | 1 | 0·00064 | 0·0008 |
| Lactic acid .. .. | 2·5 | 0·00027 | 0·0003 |
| Oxalic acid .. .. | 1 | 0·00035 | 0·0003 |
| Orthophosphoric acid .. | 1 | 0·00072 | 0·0008 |
| Sodium fluoride .. | 0·5 | 0·0022 | 0·002 |
| Sodium oxalate .. | 0·5 | 0·00080 | 0·0006 |
| Sodium pyrophosphate | 0·1 | 0·0010 | Not detected |

In each instance the bathophenanthroline method was a considerable improvement on the thiocyanate method. Most of the procedures ending with a thiocyanate determination were tedious; for example, the tests on the organic materials required a preliminary destruction of the organic matter.

## METHODS

### REAGENTS—

All reagents should be of analytical grade when appropriate.

*Ammonia solution, approximately* 5 N.

*Ascorbic acid, B.P.*

*Ethanol*—Industrial methylated spirit, 74° O.P.

TABLE IV

RESULTS OF RECOVERY EXPERIMENTS BY THE ACID REDUCTION METHOD

The weights of sample used are as shown in Table III

| Sample | Iron added, µg | Iron found, µg | Recovery, µg |
|---|---|---|---|
| Citric acid .. .. .. .. | 0 | 6·4 | |
| | 5 | 11·4 | 5·0 |
| | 10 | 16·5 | 10·1 |
| Lactic acid* .. .. .. .. | 0 | 9·9 | |
| | 5 | 14·9 | 5·0 |
| | 10 | 20·0 | 10·1 |
| Oxalic acid .. .. .. .. | 0 | 3·5 | |
| | 5 | 8·4 | 4·9 |
| | 10 | 13·5 | 10·0 |
| Orthophosphoric acid .. .. | 0 | 7·2 | |
| | 5 | 12·3 | 5·1 |
| | 10 | 17·3 | 10·1 |
| Sodium fluoride .. .. .. | 0 | 11·1 | |
| | 5 | 15·7 | 4·6 |
| | 10 | 21·2 | 10·1 |
| Sodium oxalate* .. .. .. | 0 | 5·8 | |
| | 5 | 10·7 | 4·9 |
| | 10 | 15·4 | 9·6 |
| Sodium pyrophosphate .. .. | 0 | 1·0 | |
| | 5 | 6·3 | 5·3 |
| | 10 | 10·7 | 9·7 |

* Different batch from that used in Table III.

*Perchloric acid, 60 per cent. w/w, sp.gr.* 1·54—This is approximately 10 M.

*Potassium sodium tartrate.*

*Sodium acetate, hydrated.*

*Sodium hydrogen carbonate.*

*Bathophenanthroline solution,* 0·001 M—Dissolve 0·0332 g of 4,7-diphenyl-1,10-phenanthroline in 100 ml of ethanol.

*Chloroform.*

*Hydrochloric acid, approximately* 5 N.

*Hydroxyammonium chloride - ammonium perchlorate solution*—Dissolve 50 g of hydroxyammonium chloride and 160 g of ammonium perchlorate in sufficient water to produce 1 litre; adjust the pH to between 3 and 4, if necessary, by adding ammonia solution. Transfer the solution to a large separating funnel, add 10 ml of bathophenanthroline solution and set aside for 15 minutes. Extract any pink colour by shaking vigorously with successive 10-ml portions of chloroform until the extracts are colourless. Discard the extracts, and store the solution in an iron-free glass or polythene bottle.

*Sodium hydroxide solution, approximately* 5 N.

*Tartrate solution*—Dissolve 63 g of tartaric acid in sufficient water to produce 1 litre. Add 20 ml of hydroxyammonium chloride - ammonium perchlorate solution, adjust the pH to between 3 and 4 with sodium hydroxide solution, and transfer to a large separating funnel. Purify and store the solution as described for the hydroxyammonium chloride - ammonium perchlorate solution.

*Standard iron solution*—Dissolve 8·65 g of ammonium ferric sulphate in 50 ml of nitric acid, sp.gr. 1·42, and sufficient water to produce 1 litre.

$$1 \text{ ml} \equiv 1 \text{ mg of iron.}$$

*Dilute standard iron solution*—Dilute 10 ml of the standard iron solution to 1 litre with water; this solution must be freshly prepared before use.

$$1 \text{ ml} \equiv 10\ \mu\text{g of iron.}$$

PREPARATION OF CALIBRATION GRAPH—

By pipette or burette place amounts of dilute standard iron solution to cover the range 0 to 40 $\mu$g of iron in separate 150-ml separating funnels, and treat each aliquot as described below. Dilute with 20 ml of water, add 20 ml of hydroxyammonium chloride - ammonium perchlorate solution and 10 ml of bathophenanthroline solution, and set aside for 15 minutes. Add 5 ml of chloroform, insert the stopper, shake the funnel vigorously for 30 seconds, and allow the layers to separate. Run the chloroform layer through a glass-wool plug supported in a small funnel into a 25-ml calibrated flask. Add another 5 ml of chloroform to the contents of the separating funnel, extract by shaking for a few seconds, allow the layers to separate, and add this extract to the first.

Dilute the combined extracts to the mark with ethanol, mix, and measure the optical density at 533 m$\mu$ in a 1-cm cell against the solution containing no added iron. Plot a graph relating optical density to amount of iron present (in micrograms); the graph should be linear and pass through the origin.

Note that an identical calibration graph can be prepared with ascorbic acid as reducing agent in place of the hydroxyammonium chloride. Calibration graphs prepared in accordance with the various procedures described below were identical.

PROCEDURES—

*For sodium molybdate and sodium tungstate*—Dissolve 1 g of sample in 40 ml of tartrate solution in a 150-ml separating funnel. Add 20 ml of hydroxyammonium chloride - ammonium perchlorate solution, and adjust the pH of the solution to about 4 with hydrochloric acid or ammonia solution. Add 10 ml of bathophenanthroline solution, mix, and set aside for 15 minutes. Add 5 ml of chloroform, and complete the determination as described under "Preparation of Calibration Graph"; use as blank a solution prepared from the same amounts of reagents.

*For molybdic acid*—Dissolve 1 g of sample in approximately 10 ml of water and sufficient sodium hydroxide solution to give a pH of about 10·5, boil for a few minutes, and then cool. Transfer the solution to a 150-ml separating funnel, add 40 ml of tartrate solution, and complete the determination as described above for sodium molybdate and sodium tungstate.

*For tartaric acid*—Dissolve 1 g in 25 ml of water, and add 20 ml of hydroxyammonium chloride - ammonium perchlorate solution and sufficient ammonia or sodium hydroxide solution to give a pH between 4 and 6. Add 10 ml of bathophenanthroline solution, and set aside for 15 minutes. Add 5 ml of chloroform, and complete the determination as described above.

The iron content of tartaric acid can also be determined by the acid reduction method with ascorbic acid, as described below.

*For antimony trichloride*—Dissolve 1 g of sample in a mixture of 4 ml of hydrochloric acid solution and 0·5 ml of perchloric acid. Add this solution slowly, with stirring, to a solution of 5 g of potassium sodium tartrate in 40 ml of water. Add 0·1 g of ascorbic acid and 10 ml of bathophenanthroline solution. Add sodium hydrogen carbonate in small portions until the pH is about 7. Warm the solution until it is clear, cool, and adjust the pH again if necessary to about 7 by adding sodium hydrogen carbonate. Heat the solution on a steam-bath for 15 minutes, cool, and transfer to a 150-ml separating funnel. Add 5 ml of chloroform, and complete the determination as described above.

If sodium hydroxide solution is used to neutralise the acid solution, a temporary region of high pH may occur in the solution, causing precipitation of the antimony; once precipitated, the antimony does not readily redissolve. Neutralisation with sodium hydrogen carbonate avoids this difficulty.

ACID REDUCTION METHOD—

(This method was applied to the determination of iron in citric, lactic, oxalic and phosphoric acids, sodium fluoride, sodium oxalate and sodium pyrophosphate. The weights used of each sample are shown in Table III.)

Dissolve the sample in sufficient water and perchloric acid to give a clear solution between 1 and 2 M in perchloric acid. Add 5 ml of freshly prepared 10 per cent. ascorbic acid solution and 10 ml of bathophenanthroline solution. Add 0·5 g of sodium acetate, raise the pH of the solution to between 4 and 6 by adding ammonia solution, and set aside for 20 minutes (for oxalates it is also advisable to heat the solution on a steam-bath for 5 minutes). Transfer the solution to a 150-ml separating funnel, add 5 ml of chloroform, and complete the determination as described above.

### OTHER APPLICATIONS OF BATHOPHENANTHROLINE

We also use the acid reduction method for the routine determination of iron in lead, cerium, thorium, chromium and uranium compounds.

When ethanolic bathophenanthroline solution is added to aqueous test solutions a turbidity is often produced that makes visual estimations of traces of iron impossible. However, while determining iron in ascorbic acid we noticed that, when not less than 0·5 g of ascorbic acid was present, the test solution remained clear on addition of the bathophenanthroline solution. A simple test for the visual estimation of iron was developed, as described below.

> Transfer the solution containing iron to a 50-ml Nessler cylinder, and add 0·5 g of ascorbic acid. Adjust the pH of the solution to between 4 and 6, and add 4 ml of bathophenanthroline solution. Dilute to 50 ml with water, and set aside for 15 minutes. Compare any red colour produced with similarly prepared standards containing known amounts of iron.

The minimum amount of iron that can be detected by this procedure is about 0·1 $\mu$g.

## DETERMINATION OF IRON WITH *syn*-PHENYL 2-PYRIDYL KETOXIME

Phenyl 2-pyridyl ketoxime (PPK) forms coloured water-soluble compounds with several transition-metal ions in neutral and alkaline solution, most of which can be extracted into organic solvents. Red derivatives are produced with both ferrous and ferric iron, but the lowest concentration of ferric iron that can be detected in solution with this reagent is about 5 p.p.m., whereas only 0·1 p.p.m. of ferrous iron produces a detectable colour. Phenyl 2-pyridyl ketoxime is applied, therefore, to the determination and detection of iron in the ferrous state.[17]

The ferrous - PPK complex contains three molecules of the oxime and one atom of iron. It is water soluble and has a wavelength of maximum absorption at 545 m$\mu$. It is formed in neutral and highly basic solutions, and from solutions more than molar in sodium hydroxide it can be extracted into pentanol or chloroform. In the presence of a little ethanol, one extraction with pentanol serves to remove the iron complex from the aqueous phase, and the pentanol solution of the complex exhibits maximum absorption at 550 m$\mu$.

Aqueous solutions of the iron complex are said to be unstable, whereas solutions in pentanol are stable and show a negligible diminution in absorption after standing for 24 hours.[17] Solutions of the iron compound in chloroform are light sensitive, but we found that they could be stabilised by adding ethanol. The solution of the iron complex in the chloroform - ethanol mixture had an absorption maximum at 550 m$\mu$. Two extractions with chloroform are needed to remove the iron complex completely from the aqueous phase; on the other hand, chloroform has an advantage over pentanol in being denser than the aqueous phase. The practical work described below was carried out by the chloroform extraction method, and most of the results were compared with those obtained via extraction with pentanol; the results by both procedures were identical. The molar extinction coefficient of the complex is about 15,000 in both solvents, and Beer's law is obeyed at least over the range 0 to 2·8 $\mu$g of iron per ml.

The advantage of phenyl 2-pyridyl ketoxime is its colour reaction with ferrous iron in strongly alkaline conditions. It has been used by Trusell and Diehl[17] for determining iron in lithium, sodium and potassium hydroxides and in sodium carbonate. The time required for full development of the iron colour depends on the concentration of hydroxide. In fairly concentrated sodium hydroxide solutions the colour development is slow, but it can be hastened by heating the solution.

The measurement of the colour could be made on the aqueous solution, but there are two complications; as mentioned above the aqueous solutions of the complex are not particularly stable and the colour intensity depends on the alkalinity. The molar extinction coefficient of the iron - PPK complex is a maximum in 3 M sodium hydroxide, but decreases fairly rapidly in concentrations of sodium hydroxide on both sides of this value. Therefore, unless strict control of alkalinity is feasible it is recommended that the extraction procedure should be adopted.

To reduce iron to the ferrous state in these strongly alkaline conditions it is necessary to use sodium dithionite. This chemical usually contains appreciable amounts of iron, so that solutions prepared from it must be made free from iron before use. The solution is not stable under normal conditions and must be prepared freshly before use. We attempted to use several other reducing agents, but without success.

## METHOD

The method is based on that of Trusell and Diehl.[17]

### REAGENTS—

*Chloroform.*

*Ethanol*—Industrial methylated spirit, 74° O.P.

*Phenyl 2-pyridyl ketoxime solution, 0·2 per cent.*—Dissolve 2 g of *syn*-phenyl 2-pyridyl ketoxime in 1 litre of 0·1 M hydrochloric acid.

*Sodium dithionite solution, 10 per cent. (iron-free)*—Dissolve 2 g of sodium dithionite in 10 ml of water, add 5 ml of phenyl 2-pyridyl ketoxime solution and 5 ml of 10 M sodium hydroxide, and set aside for 10 minutes. Extract the iron - PPK complex into 10 ml of chloroform and 5 ml of ethanol, and discard the extract.

*Sodium hydroxide solution, 10 M (iron-free)*—Dissolve 400 g of sodium hydroxide in 800 ml of water, add 10 ml of sodium dithionite solution and 50 ml of PPK solution, and set aside for 10 minutes. Extract the iron - PPK complex into 100 ml of pentanol and 25 ml of ethanol, and discard the extract. Dilute to 1 litre with water, and store in a polythene bottle.

*Standard iron and dilute standard iron solutions*—Prepare as described under the procedures for the determination of iron with bathophenanthroline, p. 6.

### PREPARATION OF CALIBRATION GRAPH—

By pipette or burette, place amounts of dilute standard iron solution to cover the range 0 to 70 $\mu$g of iron in separate 50-ml beakers, and treat each aliquot as described below. Dilute with water to produce 10 ml, add 25 ml of 10 M sodium hydroxide, 2 ml of 10 per cent. sodium dithionite solution and 5 ml of 0·2 per cent. phenyl 2-pyridyl ketoxime solution. Heat the solution on a steam-bath for 5 to 10 minutes, cool to room temperature, and transfer to a 150-ml separating funnel with 10 ml of water. Add 5 ml of ethanol and 5 ml of chloroform, extract by shaking vigorously for 30 seconds, and then allow the layers to separate. Run the chloroform layer through a glass-wool plug supported in a small funnel into a 25-ml calibrated flask. Add another 5 ml of chloroform to the contents of the separating funnel, and extract and separate as before.

Dilute the combined extracts to the mark with ethanol, mix, and measure the optical density at 550 m$\mu$ in a 1-cm cell against the solution containing no added iron. Plot a graph relating optical density to amount of iron present (in micrograms); the graph should be linear and pass through the origin.

Note that an identical calibration graph can be prepared with pentanol as extractant in place of the chloroform. In this instance extract once with 10 ml of pentanol and dilute the extract to 25 ml with pentanol.

### PROCEDURE—

Dilute the solution of the sample with water or sodium hydroxide solution to a volume of 35 ml, the concentration of sodium hydroxide being about 7 M. Add 2 ml of 10 per cent. sodium dithionite solution and 5 ml of 0·2 per cent. phenyl 2-pyridyl ketoxime solution. Heat the solution on a steam-bath for 5 to 10 minutes, cool to room temperature, and transfer to a 150-ml separating funnel with 10 ml of water. Complete the determination as described under "Preparation of Calibration Graph"; use as blank a solution prepared from the same amounts of the reagents used.

### INTERFERENCES—

Recovery experiments were carried out by the procedure described above on 40-$\mu$g amounts of iron in the presence of various salts. The weights used and the results obtained are shown in Table V.

TABLE V

RECOVERY OF 40-$\mu$g AMOUNTS OF IRON IN THE PRESENCE OF VARIOUS SALTS

| Salt added | Weight of salt taken, g | Recovery of iron, $\mu$g |
|---|---|---|
| Sodium acetate | 1 | 40·4 |
| Sodium arsenate | 1 | Heavy precipitate |
| | 0·1 | 36·5* |
| Sodium chloride | 1 | 40·1 |
| Trisodium citrate | 1 | 39·7 |
| Sodium fluoride | 0·002 | 40·1 |
| Sodium molybdate | 1 | 32·7 |
| | 0·5 | 35·9 |
| Sodium nitrate | 1 | 40·0 |
| Sodium oxalate | 0·1 | 31·0* |
| Ammonium perchlorate | 1 | 39·8 |
| Disodium hydrogen orthophosphate | 1 | 40·1* |
| Sodium sulphate | 1 | 39·0 |
| Potassium sodium tartrate | 1 | 27·9 |
| | 0·5 | 35·6 |
| Sodium tetraborate | 1 | 40·1 |

* A precipitate or turbidity formed during the test, and the iron was extracted from the filtered test solution.

Recovery experiments with 40-$\mu$g amounts of iron were also carried out in the presence of some other transition-metal cations known to give colour reactions with PPK. The results are shown in Table VI.

TABLE VI

RECOVERY OF 40-$\mu$g AMOUNTS OF IRON IN THE PRESENCE OF OTHER TRANSITION METALS

| Ion added | Weight of ion added, $\mu$g | Recovery of iron, $\mu$g |
|---|---|---|
| $Co^{2+}$ | 40 | 38·1 |
| $Cu^{2+}$ | 40 | 36·5 |
| $Mn^{2+}$ | 40 | 38·1 |
| $Ni^{2+}$ | 40 | 36·1 |
| $Pt^{2+}$ | 100 | 38·4 |

Quantitative recoveries of 40-$\mu$g amounts of iron were obtained in the presence of 10-mg amounts of cerium$^{III}$ and mercury$^{I}$ and 5-mg amounts of aluminium, calcium and zinc. However, mercury gave a black precipitate and calcium a white precipitate, and it was necessary to extract with pentanol from solutions containing these metals (because pentanol has a lower density than the aqueous phase) in order to obtain good recoveries.

Precipitates were also given by cadmium (yellow), lead (black) and magnesium (white), and low results were obtained in the presence of 5 mg of cadmium and 10 mg each of the other two metals. The interference caused by magnesium was particularly serious.

## DETERMINATION OF IRON IN GLASS SAND

Trusell and Diehl[17] described the application of PPK to the determination of iron in glass sand. The sample was fused in a platinum vessel with a mixture of equimolecular amounts of sodium carbonate and sodium tetraborate. When the melt was cool it was dissolved in water, treated with sodium dithionite and PPK solution, and the determination was completed in the usual way. They obtained excellent results on NBS glass sand.

In our hands this method gave low and variable results. It was thought that dissolution of the carbonate - borate melt in water gave a solution insufficiently alkaline to permit quantitative extraction of the ferrous - PPK complex. A further trial of the method was made in which sodium hydroxide solution was added to the solution of the melt in water; development and extraction of the colour then gave quantitative recovery of the iron. The method, with this minor modification, is quick, simple and accurate. The advantage over conventional methods is that removal of the silica (with hydrofluoric acid) is unnecessary.

REAGENTS—

*Fusion mixture*—An equimolecular mixture of anhydrous sodium carbonate and sodium tetraborate.

The other reagents required are those listed on p. 9.

PROCEDURE—

Weigh 0·1 g of the finely ground sand into a platinum dish previously rendered free from iron. Add 1 g of the fusion mixture, and blend it intimately with the sample. Place the dish in a muffle furnace at 1000° C for 10 minutes. Allow the melt to cool, and dissolve it by warming with 10 ml of water. Transfer the solution to a small beaker with 25 ml of 10 M sodium hydroxide, and add 2 ml of 10 per cent. sodium dithionite solution and 5 ml of 0·2 per cent. phenyl 2-pyridyl ketoxime solution. Heat the solution on a steam-bath for 5 to 10 minutes, cool to room temperature, and transfer to a 150-ml separating funnel with 10 ml of water. Complete the determination as described under "Preparation of Calibration Graph," p. 9; use for the blank test 1 g of the fusion mixture and the same amounts of reagents as used in the test.

RESULTS—

The results obtained by this procedure were compared with those obtained by the method described in British Standard 2975:1958, "Sand for making colourless glasses." In the B.S. method the silica is removed by the conventional hydrofluoric acid and sulphuric acid fuming procedure, and the iron is determined in the residue with thioglycollic acid. The results in Table VII show close agreement between the two methods.

The PPK method is appreciably quicker, as no evaporation stage is involved. A further advantage of the PPK method is that it is unnecessary to grind the sand to the extremely fine state essential in conventional methods for achieving ready solubility of the sand in hydrofluoric acid.

TABLE VII

DETERMINATION OF FERRIC OXIDE CONTENT OF GLASS SAND BY THE BRITISH STANDARD AND PHENYL 2-PYRIDYL KETOXIME METHODS

| | Ferric oxide found by— | |
|---|---|---|
| | B.S. method, % | PPK method, % |
| Sample A | 0·034 | 0·034 |
| | 0·034 | 0·035 |
| Sample B | 0·076 | 0·077 |
| | 0·076 | 0·076 |

## DETERMINATION OF IRON BY COLOUR REACTION WITH EDTA AND HYDROGEN PEROXIDE

Metals that form coloured cations usually give more intense colours in the form of their EDTA complexes. For this reason EDTA and related complex-forming compounds have occasionally been suggested as reagents for the colorimetric determination of certain metals, but such procedures obviously have little selectivity. However, Cheng and Lott[18] observed that addition of hydrogen peroxide to an ammoniacal solution containing ferric iron and EDTA resulted in the formation of a distinctive purple colour. Only cobalt (blue colour) was found to react similarly, though metals forming peroxide complexes (not EDTA - peroxide complexes), such as chromium, molybdenum, uranium and vanadium, could interfere. These workers later advocated the use of the ferric - EDTA - peroxide colour for the colorimetric determination of iron in limestone and clay.[19]

The Fe - EDTA - $H_2O_2$ colour was also subsequently investigated by Ringbom, Siitonen and Saxén.[20] These workers concluded that the purple colour was due to a peroxy derivative of the ferric - EDTA complex, but the blue colour formed under similar conditions by cobalt was attributed to the cobalt$^{III}$ - EDTA complex, the peroxide serving merely to oxidise the cobalt to the higher valency state.

Ringbom *et al.* pointed out that the Fe - EDTA - $H_2O_2$ complex could be used for the colorimetric determination of iron even in the presence of metals forming coloured EDTA (but not EDTA - peroxide) complexes. This was achieved by suitable treatment of a sample aliquot with EDTA, ammonia and peroxide to develop the iron colour, and by using as reference solution a further aliquot of the sample solution to which only EDTA and ammonia were added; the difference in optical densities was that due to the Fe - EDTA - $H_2O_2$ complex. Ringbom *et al.* further observed that the copper - EDTA complex had a favourably low absorption at wavelengths at which the iron colour could be measured, so that determination of iron in the presence of substantial amounts of copper appeared practicable. These workers quoted some results for the iron content of brass, directly determined in this manner, but gave little experimental detail.

The use of the Fe - EDTA - $H_2O_2$ complex for the colorimetric determination of iron has attracted little attention. However, the possible application in the presence of large amounts of copper has obvious merit, particularly as many reagents for iron, such as 1,10-phenanthroline and 2,2′-bipyridyl and their derivatives, suffer interference from copper. Some observations on the Fe - EDTA - $H_2O_2$ method and on its application to copper alloys are described below.

## EXPERIMENTAL

### ABSORPTION SPECTRA—

The procedure of Ringbom *et al.*[20] was used for developing the Fe - EDTA - $H_2O_2$ colour. To a solution containing 0·5 mg of ferric iron was added in turn 10 ml of 0·01 M EDTA (disodium salt), one drop of ammonia solution, sp.gr. 0·88, to make the solution alkaline, 2·0 ml of 100-volume hydrogen peroxide and 3·0 ml of ammonia solution; water was then added to give a final volume of 25 ml. These conditions were subsequently shown to give full development of the iron colour. A corresponding solution of the Fe - EDTA complex was prepared in the same manner, but omitting the hydrogen peroxide. To study the absorption spectrum of the copper - EDTA complex, a 25-ml solution containing 100 mg of copper (as sulphate) and an excess of EDTA, together with ammonia but no peroxide, was also prepared. The optical densities of these three solutions were measured over the range 400 to 600 mμ in 4-cm cells with water as the reference.

The absorption spectra plotted from these results are shown in Fig. 1. The Fe - EDTA - $H_2O_2$ complex shows a broad absorption peak at about 500 mμ with maximum absorption

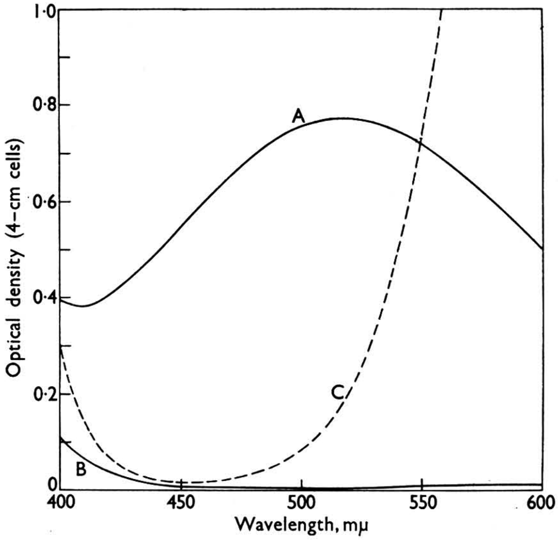

Fig. 1. Absorption spectra: curve A, 0·5 mg of iron, as the Fe - EDTA - $H_2O_2$ complex, in 25 ml; curve B, 0·5 mg of iron, as the Fe - EDTA complex, in 25 ml; curve C, 100 mg of copper, as the Cu - EDTA complex, in 25 ml

at 520 m$\mu$, as reported by Cheng and Lott[18] and by Ringbom *et al.*[20] At 500 to 520 m$\mu$, the Fe - EDTA complex has zero absorption. The curve obtained for the Cu - EDTA complex showed that, despite its intense colour at the concentration employed, the absorption is low in the region where the iron colour can be measured; as found by Ringbom *et al.*[20] the absorption is minimal at 450 m$\mu$.

To determine iron in the presence of much copper by the use of a suitable reference solution to eliminate the absorption due to copper, the choice of wavelength lies between 450 m$\mu$, at which absorption due to copper is least, and 520 m$\mu$, at which absorption of the iron colour is greatest. With the solution containing 100 mg of copper, the optical densities measured against water were 0·01 at 450 m$\mu$ and 0·20 at 520 m$\mu$; from this point of view measurement at 450 m$\mu$, as suggested by Ringbom *et al.*, is preferable. On the other hand, the absorption of the iron complex at 450 m$\mu$ is only about two-thirds that at 520 m$\mu$.

It appeared practicable to use either wavelength and, in fact, measurement at both wavelengths was made throughout the work described here.

### EFFECT OF DIFFERENT PROPORTIONS OF REAGENTS—

Experiments were made with 0·5-mg amounts of iron in a final volume of 25 ml to ascertain the effect on the iron colour of different proportions of EDTA, peroxide and ammonia; the results are shown in Table VIII.

Variation of the volume of ammonia solution, sp.gr. 0·88, from 1 to 5 ml, corresponding to final pH values of 10·7 to 11·3, had no effect on the colour; these and other experiments confirmed the conclusion[20] that the pH should be above 10·5 for full development of the colour. Restricting the volume of 0·01 M EDTA to 1 ml, *i.e.*, little greater than the amount equivalent to 0·5 mg of iron present, resulted in incomplete development of the colour, as assessed by measurement at 520 m$\mu$. A minimum of 2 ml of the EDTA solution and 2 ml of the hydrogen peroxide appeared desirable, larger amounts having no effect on the colour. It thus appeared that the proportions of EDTA, hydrogen peroxide and ammonia could be varied appreciably, provided that the necessary minimum proportions were exceeded.

TABLE VIII

EFFECT OF DIFFERENT PROPORTIONS OF REAGENTS ON THE Fe - EDTA - $H_2O_2$ COLOUR

Each solution of final volume 25 ml contained 0·5 mg of iron; optical densities measured in 4-cm cells

| Volume of 0·01 M EDTA, ml | Volume of 100-volume hydrogen peroxide, ml | Volume of ammonia solution, sp.gr. 0·88, ml | pH of final solution | Optical density at— | |
|---|---|---|---|---|---|
| | | | | 520 m$\mu$ | 450 m$\mu$ |
| 10 | 2 | 1 | 10·7 | 0·763 | 0·520 |
| 10 | 2 | 3 | 11·1 | 0·772 | 0·523 |
| 10 | 2 | 5 | 11·3 | 0·769 | 0·520 |
| 1 | 1 | 1 | Not measured | 0·710 | 0·536 |
| 10 | 1 | 1 | Not measured | 0·756 | 0·510 |
| 10 | 3 | 5 | Not measured | 0·771 | 0·523 |
| 1 | 2 | 3 | Not measured | 0·756 | 0·536 |
| 2 | 2 | 3 | Not measured | 0·768 | 0·521 |
| 4 | 2 | 3 | Not measured | 0·767 | 0·518 |

It is noteworthy that the two experiments in which only 1 ml of 0·01 M EDTA was used gave incomplete development of the colour as indicated by the optical density at 520 m$\mu$, but at 450 m$\mu$ enhanced absorptions were observed. It would appear that under these conditions a different complex is being formed.

For calibration purposes, and in later experiments, the amounts of reagents used for development of the iron colour were 10 ml of 0·01 M EDTA, 2 ml of 100-volume hydrogen peroxide and 3 ml of ammonia solution, sp.gr. 0·88.

### EFFECT OF TIME—

The formation of the Fe - EDTA - $H_2O_2$ colour appears to be virtually instantaneous, and the optical densities of solutions of the complex can be measured immediately after preparation. Re-measurement of the optical densities of two solutions after 1 hour's standing in artificial light gave the following results, the original optical densities being given in brackets: at 520 m$\mu$, 0·640 (0·646) and 0·634 (0·632); at 450 m$\mu$, 0·436 (0·434) and 0·432 (0·432).

The colours are therefore stable for at least an hour, but were observed to undergo appreciable fading overnight. The use of smaller amounts of hydrogen peroxide in development of the colour results in more rapid fading, the fading presumably being attributable to decomposition of the excess hydrogen peroxide in the alkaline solution; after it has faded the colour can be instantly regenerated by addition of peroxide.

### CALIBRATION—

For calibration purposes colours were developed for 0·1- to 0·5-mg amounts of iron and measured against appropriate reference solutions, *i.e.*, containing the same amounts of iron and other reagents, but no peroxide. The results are shown in Table IX. Beer's law is obeyed for measurement both at 520 m$\mu$ and at 450 m$\mu$, but, as observed earlier, measurement at the shorter wavelength results in a loss of sensitivity of about one third.

TABLE IX

CALIBRATION RESULTS

Final volume of solution 25 ml; optical densities measured in 4-cm cells

| Iron present, mg | Optical density at 520 m$\mu$ | Optical density per mg of iron | Optical density at 450 m$\mu$ | Optical density per mg of iron |
|---|---|---|---|---|
| 0·1 | 0·154 | 1·54 | 0·103 | 1·03 |
| 0·2 | 0·306 | 1·53 | 0·206 | 1·03 |
| 0·3 | 0·461 | 1·54 | 0·311 | 1·04 |
| 0·4 | 0·614 | 1·54 | 0·413 | 1·03 |
| 0·5 | 0·772 | 1·54 | 0·523 | 1·05 |
| | | Mean 1·54 | | Mean 1·04 |

### EFFECT OF COBALT—

From the earlier published work it was apparent that cobalt was the element most likely to interfere with the iron colour. Lott and Cheng[19] stated that the cobalt colour could be inhibited by cyanide, but gave no practical details. In this work a few experiments were made to assess the extent of interference by cobalt and to examine its inhibition by cyanide.

With a solution of 0·5 mg of cobalt, use of the colour development procedure adopted for iron gave a pure blue colour, which was not discharged on addition of cyanide. However, if the cyanide was added at an earlier stage, *i.e.*, after the addition of EDTA and a slight excess of ammonia to a cobalt solution, there resulted a pale yellow colour, presumably that of the cobalt - cyanide complex; subsequent addition of hydrogen peroxide and more ammonia caused no change in the colour. The formation of the blue cobalt colour can be thus inhibited if cyanide is added at the correct stage.

Some quantitative results on the interference of cobalt and its inhibition are shown in Table X; the optical densities quoted were measured against reference solutions containing neither iron nor cobalt, so that the total absorptions of the colours obtained could be observed. These results show that the colour due to cobalt, if not inhibited, gives an absorption equivalent to about $\frac{1}{4}$ and $\frac{1}{9}$ of the same weight of iron for measurements at 520 and 450 m$\mu$, respectively. The yellow colour resulting from the complexing of cobalt with cyanide was found to give zero absorption at 520 m$\mu$, but a small absorption at 450 m$\mu$; the latter could be effectively eliminated by using as a reference solution an aliquot of the cobalt-containing sample solution treated with EDTA, ammonia and cyanide, but not peroxide. It was apparent that development of the Fe - EDTA - $H_2O_2$ colour was unaffected by the presence of cyanide.

TABLE X

INTERFERENCE OF COBALT AND ITS INHIBITION BY CYANIDE

| Iron present, mg | Cobalt present, mg | Volume of 10 per cent. potassium cyanide added, ml | Optical density at— | |
|---|---|---|---|---|
| | | | 520 m$\mu$ | 450 m$\mu$ |
| 0 | 0·5 | 0 | 0·180 | 0·060 |
| 0 | 0·5 | 1 | 0 | 0·041 |
| 0·5 | 0 | 0 | 0·772 | 0·523 |
| 0·5 | 0 | 1 | 0·768 | 0·515 |
| 0·5 | 0·5 | 1 | 0·774 | 0·562 |

### MEASUREMENT OF THE IRON COLOUR IN THE PRESENCE OF MUCH COPPER—

Attention was then directed to the specific application of determining iron in the presence of a large amount of copper. To assess the accuracy of measurement of the iron colour in this context, solutions containing 100 mg of copper (as sulphate) and various amounts of iron were prepared in duplicate. In one solution of each pair, the iron colour was developed with EDTA, hydrogen peroxide and ammonia as previously described, except that the amount of EDTA used was increased to 12 ml of a 0·15 M solution to cater for the large amount of copper present. The other solution of each pair was treated similarly, except that no peroxide was added, to serve as the reference solution for the spectrophotometric measurements.

The results obtained, corrected for the small amount of iron found in the copper sulphate and other reagents, are shown in Table XI. The amounts of iron deduced from measurements at either wavelength are of acceptable accuracy, and show that as little as 0·05 mg of iron can readily be measured in the presence of 100 mg of copper, *i.e.*, equivalent to 0·05 per cent. of iron in copper.

TABLE XI

MEASUREMENT OF IRON COLOURS IN THE PRESENCE OF 100 mg OF COPPER

| | Measurement at 520 mμ | | Measurement at 450 mμ | |
|---|---|---|---|---|
| Iron added, mg | Optical density corrected for blank | Iron found, mg | Optical density corrected for for blank | Iron found, mg |
| 0·055 | 0·086 | 0·056 | 0·057 | 0·055 |
| 0·145 | 0·220 | 0·143 | 0·151 | 0·145 |
| 0·300 | 0·456 | 0·296 | 0·313 | 0·302 |

Subsequently experiments were carried out to apply the procedure to the determination of iron in copper alloys. For this purpose alloys were dissolved in hydrochloric and nitric acids, tartaric acid then being added to prevent precipitation of tin when the colour was subsequently developed in ammoniacal solution. It was found desirable to restrict the amount of acid used for solution of the alloys, otherwise the amount of ammonium salts produced on neutralisation tended to buffer the solution to a pH unfavourably low for development of the iron colour. The procedure adopted was found to give a pH of about 10·7 at the final colour stage, *i.e.*, adequate for full development of the iron colour, as shown earlier.

Details of the full method evolved for the direct determination of iron in copper alloys are given below.

## METHOD

### REAGENTS—

*Standard iron solution*—Dissolve 0·1000 g of pure iron in 4 ml of diluted nitric acid (1 + 1), and dilute with water to 1 litre.

1 ml ≡ 0·1 mg of iron.

*EDTA solution, approximately* 0·01 M—Dissolve 3·7 g of disodium ethylenediaminetetraacetate dihydrate in sufficient water to produce 1 litre.

*EDTA solution, approximately* 0·15 M—Dissolve 56 g of disodium ethylenediaminetetraacetate dihydrate in sufficient water to produce 1 litre.

*Ammonia solution, sp.gr.* 0·88.

*Hydrogen peroxide,* 100 *volume.*

*Hydrochloric acid, sp.gr.* 1·18.

*Nitric acid*—Dilute nitric acid, sp.gr. 1·42, with an equal volume of water.

*Tartaric acid solution,* 10 *per cent., aqueous.*

### PREPARATION OF CALIBRATION GRAPH—

In each of two 25-ml calibrated flasks place, by pipette, 1-ml portions of standard iron solution (1 ml ≡ 0·1 mg of iron). To each add 10 ml of 0·01 M EDTA and one drop of ammonia solution. To the contents of one flask add 2 ml of hydrogen peroxide; to the contents of both flasks add 3 ml of ammonia solution, dilute to the mark with water, and mix. Measure the optical density of the iron colour in 4-cm cells at 520 mμ and at 450 mμ; use as reference the solution containing no peroxide.

Repeat the procedure with amounts of standard iron solution up to 5 ml and also in the absence of added iron. Correct the optical densities of the iron solutions for the small absorption found in the absence of added iron, and plot two calibration graphs (corresponding to the measurements at the two wavelengths) for the results. The graphs should be linear and pass through the origin.

PROCEDURE—

Weigh 1 g of sample, preferably in the form of drillings, into a 100-ml beaker (see Note 1). Add 5 ml of hydrochloric acid and 2·5 ml of diluted nitric acid (1 + 1), warm to initiate the reaction, and then remove from the source of heat. If necessary, heat again to complete the solution of the alloy, add 10 ml of water, and boil for 2 minutes to remove oxides of nitrogen. Cool, add 10 ml of tartaric acid solution, and dilute the solution to 50 ml in a calibrated flask.

By pipette, place 5-ml aliquots into each of two 25-ml calibrated flasks (see Note 2), and add to each 12 ml of 0·15 M EDTA and 1·0 ml of ammonia solution. To one solution add 2·0 ml of hydrogen peroxide, and to the other add 2 ml of water. Dilute each solution to the mark with ammonia solution, and mix.

Measure the optical density of the iron colour in 4-cm cells at 520 mμ and at 450 mμ; use as reference the solution to which no peroxide was added. Correct each reading for any blank value obtained by applying the procedure in the absence of a sample, and from the corrected optical densities deduce the iron content of the sample.

NOTES—

1. A 1-g sample is suitable for iron contents of 0·05 to 0·5 per cent.; for 0·5 to 1 per cent. of iron, use 0·5 g of sample.

2. After washing before use, shake the flasks free from superfluous water; if a millilitre or two of water remains in the flasks, the amount of ammonia required for the final dilution to 25 ml may be insufficient to give the pH necessary for full development of the iron colour.

RESULTS—

The procedure was applied to 1-g portions of pure copper, to which known amounts of iron were added after dissolution. The results (see Table XII) gave good agreement with the amounts of iron added, and showed that the method adopted for solution of samples, and in particular the use of tartaric acid, had no deleterious effect on the development of the iron colour.

TABLE XII

DETERMINATION OF KNOWN AMOUNTS OF IRON ADDED TO PURE COPPER

| | | Iron found by measurement at— | |
|---|---|---|---|
| Weight of copper taken, g | Iron added, % | 520 mμ, % | 450 mμ, % |
| 1·0 | 0·05 | 0·049 | 0·048 |
| 1·0 | 0·15 | 0·150 | 0·148 |
| 1·0 | 0·30 | 0·296 | 0·299 |

The procedure was also applied in triplicate to two standard samples of copper alloy; these were a bronze (B.C.S. No. 207, 0·06 per cent. of iron) and a manganese brass (B.C.S. No. 179, 0·91 per cent. of iron) and the amounts of sample used were, respectively, 1·0 and 0·5 g. Further, to simulate samples with intermediate iron contents, iron was determined in three mixtures of the two standard alloys.

The results obtained are shown in Table XIII. On the standard samples close agreement was obtained between replicate determinations and between the values deduced from measurements at the two wavelengths; the latter observation provided strong evidence that interference from the constituents of the alloys was not occurring. The mean values obtained on the standard alloys were lower than the certified values, but fell within the range of results from which the certified values were obtained. The results on the mixtures were completely consistent with the iron contents calculated from the mean values found for the standard alloys.

It would appear that measurement at 520 or 450 mμ can be used with equal success for determining iron in copper alloys. For samples of an unfamiliar nature, measurement at both wavelengths may be a useful precaution in that interference, for example, from an unsuspected cobalt content, would be revealed by disparity between the results obtained at 520 and 450 m$\mu$.

TABLE XIII

DETERMINATION OF IRON IN STANDARD SAMPLES OF COPPER ALLOYS

| Sample | Weight of sample, g | Iron found by measurement at— | | Certified iron content and range of results, % | Calculated iron content (for mixtures), % |
|---|---|---|---|---|---|
| | | 520 m$\mu$, % | 450 m$\mu$, % | | |
| Bronze C, B.C.S. No. 207 | 1 | 0·052<br>0·053<br>0·052<br>mean 0·052 | 0·051<br>0·053<br>0·052<br>mean 0·052 | 0·06<br>(0·05 to 0·08) | — |
| Manganese brass B, B.C.S. No. 179 | 0·5 | 0·82<br>0·82<br>0·81<br>mean 0·82 | 0·83<br>0·82<br>0·82<br>mean 0·82 | 0·91<br>(0·81 to 0·98) | — |
| B.C.S. No. 207<br>B.C.S. No. 179 | 0·5<br>0·1 | 0·18 | 0·18 | — | 0·18 |
| B.C.S. No. 207<br>B.C.S. No. 179 | 0·5<br>0·25 | 0·31 | 0·31 | — | 0·31 |
| B.C.S. No. 207<br>B.C.S. No. 179 | 0·3<br>0·6 | 0·57 | 0·57 | — | 0·56 |

## SUMMARY

The three colour reactions reviewed in this paper cover between them a wide field of application. The influences of diverse ions on iron determinations by each method are reported in more detail than previously, and practical procedures have been devised to overcome several interferences.

Bathophenanthroline is recommended for highly accurate determinations of iron in microgram amounts under most conditions. For determining iron in highly alkaline solutions, however, phenyl 2-pyridyl ketoxime gives a useful and reasonably sensitive colour reaction. For determining larger amounts of iron in the presence of many other metals, the colour reaction between ferric iron, EDTA and hydrogen peroxide is extremely useful. It can be highly recommended for determining iron in copper-bearing metals.

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# A Scheme for the Quantitative Analysis of a Phosphate Rock including the Use of a Benzoate Separation

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In a scheme for analysing a phosphate rock, group-3 metals are separated by a double precipitation as benzoates at a pH of 3·5 and calcium is separated by a double precipitation as the oxalate at a pH of 4·0. Gravimetric finishes are employed for determining aluminium, calcium and magnesium. The determination of aluminium as the oxinate in the presence of phosphate, after the separation of interfering metals by a cupferron extraction, is examined. In the colorimetric determination of iron as the ferrous - bipyridyl complex, interference by phosphate is avoided by reduction in acid solution with ascorbic acid (in place of hydroxylamine hydrochloride) followed by formation of the complex in a chloroacetate buffer solution (pH 3·0). A colorimetric determination is employed for silica, in which fluoride and phosphate can be tolerated up to certain limits. The determination of other elements is discussed.

THE scheme proposed here for analysing a phosphate rock is divided into two main sections: (*i*) a systematic gravimetric analysis, with group separations for determining aluminium, calcium and magnesium, and (*ii*) individual determinations of the other elements by specific methods on separate portions of samples.

The chief difficulty in analysing phosphate rocks lies in the systematic analysis, as the separation of minor amounts of group-3 metals from the predominant alkaline earths by the ammonia precipitation is vitiated by the presence of major amounts of phosphate.

Various procedures have been devised to overcome the problem of chemical separations in the presence of phosphate; for example, Harvey[1] removed calcium by precipitation as sulphate in alcoholic solution and then precipitated the group-3 elements as phosphates; Hoffman and Lundell[2] avoided systematic analysis and determined each element separately.

As the neutral or weakly alkaline conditions of the ammonia separation cause the phosphates of the alkaline earths to co-precipitate with the group-3 hydroxides, it would appear that precipitation at a lower pH, by an alternative reagent, might effect a clean separation.

Benzoic acid, a possible reagent for this separation, was first introduced into chemical analysis by Kolthoff, Stenger and Moskovitz[3] who used it to separate titanium and the tervalent metals from the bivalent metals in weakly acid solutions. The work of Smales[4] and Jewsbury and Osborn[5] suggests that the former elements are completely precipitated from solutions having pH values between 3 and 4. Benzoic acid has also been proposed for use in a scheme of qualitative analysis in the presence of phosphate.[6]

The investigation described here was an attempt to establish conditions for the use of this reagent in quantitative separations and to develop a scheme for the main analysis of phosphate rocks.

Of the elements determined on separate samples of phosphate rock, the alkalis, manganese, titanium, phosphate, carbonate and water present no special difficulties. The gravimetric determination of silica in the presence of fluoride is tedious and not entirely satisfactory[2] and, on balance, the direct colorimetric procedure is to be preferred. The determination of iron may give rise to some problems, and the determination of fluoride remains difficult.

## SYSTEMATIC ANALYSIS

The systematic scheme is based on the combination of separations listed below.

1. A double precipitation of the group-3 metals as benzoates at pH 3·5. Our work shows that this effectively separates these metals from calcium, magnesium and small amounts of manganese.

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2. As the benzoate precipitate contains an indeterminate amount of phosphate, simple ignition and weighing gives no useful information. Aluminium is therefore separated from phosphate and determined gravimetrically by an oxine precipitation, after removal of iron and titanium by a cupferron extraction. Chromium$^{III}$ and uranium$^{VI}$ interfere, as they are partly or completely precipitated as phosphates, benzoates and oxinates and are not removed by a cupferron extraction; however, they are only trace constituents of phosphate rocks of the apatite type.

3. The subsequent separation of calcium from magnesium and phosphate is by a double-acid oxalate precipitation as described by Hoffman and Lundell.[2]

### pH AND COMPLETENESS OF PRECIPITATION WITH BENZOIC ACID—

Benzoate precipitations (described in the method given below) were made from solutions containing 25·0 mg per 100 ml of $Al_2O_3$, $Fe_2O_3$ or $TiO_2$ (the titanium solution was in N sulphuric acid), the pH being varied. Oxidising conditions were necessary for the precipitation of iron. After filtration the concentration of each metal remaining in solution was determined colorimetrically. The figures shown in Table I indicate that precipitation of these elements is virtually complete at pH 3·0 and above.

TABLE I

COMPLETENESS OF PRECIPITATION FROM SOLUTIONS OF DIFFERENT pH VALUES

Solutions containing 25·0 mg of the metal oxides

| | Concentration of the element remaining in solution | | |
|---|---|---|---|
| pH of precipitation | Aluminium as $Al_2O_3$, mg per 100 ml | Iron as $Fe_2O_3$, mg per 100 ml | Titanium as $TiO_2$, mg per 100 ml |
| 2·0 | 0·22 | 0·37 | 0·15 |
| 2·5 | 0·09 | 0·10 | 0·03 |
| 3·0 | 0·03 | 0·02 | 0·01 |
| 3·5 | 0·01 | 0·02 | 0·01 |
| 4·0 | 0·01 | 0·02 | 0·01 |

### CONTAMINATION OF THE BENZOATE PRECIPITATE—

Benzoate precipitations were carried out at various pH values on a synthetic solution containing the group-3 elements, alkaline earths and a small amount of manganese. These precipitates were ignited and examined spectrographically for calcium, magnesium and manganese. The results in Table II show that the contamination of the precipitate increases with pH and that a double precipitation at pH 3·5 effects a good separation of the group-3 elements from the bivalent metals.

TABLE II

CO-PRECIPITATION OF CALCIUM, MAGNESIUM AND MANGANESE

Precipitations from solutions containing 100·0 mg of CaO, 10·0 mg of MgO, 25·0 mg of $Al_2O_3$, 10·0 mg of $Fe_2O_3$, 2·5 mg of $TiO_2$, 0·10 mg of MnO and 75·0 mg of $P_2O_5$ per 100 ml of solution

| | Amount found in the ignited precipitates | | |
|---|---|---|---|
| Precipitation pH | Calcium oxide, % | Magnesium oxide, % | Manganese oxide, % |
| 4·0 | 2·0 | 0·1 | 0·1 |
| 3·5 | 0·9 | 0·05 | 0·05 |
| 3·0 | 0·5 | 0·04 | 0·03 |
| 3·5 (double precipitation) | 0·1 | 0·03 | not detected |

### CHOICE OF pH FOR THE SEPARATION—

As the contamination of the benzoate precipitate increases with pH, the lowest pH at which the precipitation is complete (3·0) appears to be the most favourable. However, the final choice of pH was governed by the solubility of benzoic acid, which decreases with pH.

At the concentration used (1 g per 100 ml), benzoic acid comes out of solution at a pH of 3·0 at about 50° C and so interferes with the filtration; by working at a pH of 3·5 this difficulty is avoided.

## Determination of Aluminium

According to Lundell and Knowles,[7] aluminium can be separated from phosphate by precipitation as the oxinate from ammoniacal solution. Our work shows that this separation is also satisfactory in acetic acid - acetate solution, provided that the precipitation is made by first adding the oxine to a N hydrochloric acid solution and then raising the pH to about 5 with ammonia and ammonium acetate. Under these conditions 25·0 mg of alumina could be correctly determined in the presence of 50·0 mg of phosphorus pentoxide. If the pH is adjusted to about 5 before adding oxine, aluminium phosphate is precipitated out and results are low; prolonged standing of the solution improves the recovery as the aluminium phosphate is slowly converted to the oxinate.

In the literature, various values are given for the excess of oxine required. A considerable excess was used by Stumpf[8] (about 10 per cent.) and Miller and Chalmers[9] (about 2 mg of excess per ml); as a result of our investigations, we are in agreement with the values they suggest.

### METHOD

REAGENTS—

*Benzoic acid*—Analytical-reagent grade.

*Ammonium benzoate wash solution*—A solution containing 0·5 per cent. w/v of benzoic acid and 0·5 per cent. w/v of ammonium benzoate in water.

*Ammonium acetate solution, 50 per cent. w/v, aqueous.*

*Cupferron solution, 1 per cent. w/v in chloroform.*

*Hydroxylamine hydrochloride solution, 10 per cent. w/v, aqueous.*

*Oxine solution, 2 per cent. w/v in 4 per cent. v/v acetic acid.*

*Oxalic acid solution, 10 per cent. w/v, aqueous.*

*Ammonium oxalate wash solution*—A solution containing 0·1 per cent. of ammonium oxalate in water.

*Ammonium dihydrogen phosphate*—Analytical-reagent grade.

*Ammonium nitrate wash solution*—A solution containing 10 per cent. of ammonia solution, sp.gr. 0·880, and 2 per cent. of concentrated nitric acid in water.

DECOMPOSITION OF THE SAMPLE—

Weigh 0·5000 g of sample into a 3-inch platinum basin, add 10 ml of hydrofluoric acid, and set the solution aside overnight. Then add 10 ml of perchloric acid, and evaporate to dryness on a hot-air bath. Add a further 10 ml of perchloric acid and about 20 ml of water, heat on an air bath to dissolve the solids, and evaporate to dryness. Repeat this operation three times to ensure removal of fluoride. Dissolve the residue in 10 ml of 5 N hydrochloric acid, transfer to a 100-ml calibrated flask, and dilute to the mark with water.

PROCEDURE—

Take 50 ml of sample solution, dilute to 100 ml in a 250-ml beaker, add 5 ml of saturated bromine water (to oxidise ferrous iron), boil for 1 to 2 minutes, and then add 1 g of benzoic acid. Add diluted ammonia solution (1 + 1) dropwise, with stirring, until a faint permanent precipitate forms, complete the precipitation by adjusting the pH to 3·5 with ammonium acetate (50 per cent.), boil for about 2 minutes to coagulate the precipitate, and stand the beaker on a boiling-water bath for 30 minutes. Collect the precipitate by filtration through a Whatman No. 540 filter-paper, and wash several times with the ammonium benzoate wash solution. Wash the precipitate back into the beaker, dissolve in 20 ml of hot 5 N hydrochloric acid, dilute to 100 ml with water, and reprecipitate. Set the filtrate aside.

Dissolve the precipitate in 20 ml of 5 N hydrochloric acid, and wash the solution into a separating funnel (total volume should be about 75 ml). Extract iron and titanium with successive portions of a 1 per cent. solution of cupferron in chloroform until the extract is colourless, and wash twice with chloroform and once with light petroleum (it is essential to remove all cupferron, otherwise its oxidation products will contaminate the oxine precipitate). Transfer the aqueous solution to a 250-ml beaker. Add sufficient oxine solution to ensure an excess of 2 mg per ml and 2 ml of hydroxylamine hydrochloride solution (this inhibits the oxidation of reagents and prevents the discoloration of the precipitate); heat the solution to 70° C, and add diluted ammonia solution (1 + 1) until a faint permanent precipitate forms. Precipitate the bulk of the aluminium by slowly adding, with stirring, 10 ml of 50 per cent. ammonium acetate solution, and adjust the pH to about 5 with diluted ammonia solution (1 + 1). Stand the beaker on a water bath for 30 minutes to allow the precipitate to settle. Collect the precipitate in a sintered-glass crucible, wash several times with hot water, dry at 140° C, and weigh. Heat the combined filtrates from the benzoate separations to boiling, and add sufficient 10 per cent. w/v oxalic acid solution to give 1 g of oxalic acid per 100 ml of solution. Adjust the pH of the solution to 4·0 with diluted ammonia solution (1 + 1), boil for 2 to 3 minutes, stand on a water bath for 2 hours, and set aside overnight. Collect the precipitate on a porous porcelain crucible, and wash with a 0·1 per cent. solution of ammonium oxalate. Dissolve the precipitate in hot hydrochloric acid, and wash with water into a 250-ml beaker. Dilute the solution to 100 ml, add 1 g of oxalic acid, heat to boiling, and precipitate the calcium by raising the pH to 4·0 with diluted ammonia solution (1 + 1). Boil the solution for 2 to 3 minutes, stand on a water bath for 30 minutes, and set aside to cool for 2 hours. Collect the precipitate on the porous porcelain crucible, wash with ammonium oxalate solution, ignite at 550° C, and weigh as calcium carbonate.

To remove ammonium salts, concentrate the combined filtrates by evaporation, add 25 ml of concentrated nitric acid and, after the effervescence ceases, evaporate to dryness. Add 100 ml of water, make the solution slightly ammoniacal, and transfer to a 250-ml beaker. Add 1 g of ammonium phosphate and 20 ml of ammonia solution, sp.gr. 0·880, for each 100 ml of solution, and set aside for 2 to 3 days. Collect the precipitate in a porous porcelain crucible, wash with cold ammoniacal ammonium nitrate, dissolve in hydrochloric acid, and reprecipitate. Set the solution aside overnight, and collect the precipitate in the same crucible; ignite the precipitate at 950° C, and weigh as the pyrophosphate. Apply a correction for manganese, which is collected at this point.

### Recovery of Aluminium, Calcium and Magnesium by the Proposed Scheme

This systematic scheme was applied to a synthetic solution, and in Table III is shown the recovery of calcium, magnesium and aluminium in the presence of phosphate, titanium and iron.

TABLE III

RECOVERY OF ALUMINIUM, CALCIUM AND MAGNESIUM

The solution contained, beside the relevent elements, 10·0 mg of $Fe_2O_3$, 4·0 mg of $TiO_2$ and 75·0 mg of $P_2O_5$

| | Added, mg | Found, mg | Found, mg (corrected)* |
|---|---|---|---|
| Alumina .. .. .. | 25·00 | 24·81 | — |
| Calcium oxide .. .. | 100·00 | 99·85 | 100·18 |
| Magnesium oxide .. .. | 10·00 | 10·20 | 9·98 |

* Correction applied for calcium found in the magnesium pyrophosphate.

The calcium precipitate, after being weighed as carbonate, was ignited to the oxide and examined spectrographically; no significant amounts of impurities were detected (less than 1 part in 1000). The magnesium pyrophosphate precipitate was also examined spectrographically. Any group-3 elements escaping precipitation as benzoates would be collected with the magnesium precipitate, but none was detected, thus confirming that the precipitation of these elements by benzoic acid was complete; calcium, detected spectrographically, was determined chemically by separating it as the sulphate in 75 per cent. alcoholic solution. The amount found was 0·33 mg, calculated as calcium oxide.

## NON-SYSTEMATIC ANALYSIS

The remaining elements are determined on separate portions of solid powder or solution.

### Determination of Iron

Hoffman and Lundell[2] determined iron by a standard volumetric method, and Hillebrand and Lundell,[10] in discussing the titanous sulphate method, do not report phosphate as an interfering ion. Colorimetric methods may present difficulties owing to the formation and precipitation of iron$^{III}$ phosphate under the weakly acid conditions often employed. The common method in which the iron$^{II}$ complex of 2,2′-bipyridyl or *o*-phenanthroline is formed, with hydroxylamine hydrochloride being used as a reducing agent in an acetate buffer, is subject to this interference, and Baudemer and Schaible[11] prefer a citrate buffer with hydroquinone as the reducing agent. They emphasise the importance of adding the reducing and colorimetric reagents before the buffer. Grat-Cabanac[12] also reports that, under these conditions, hydroxylamine hydrochloride is not satisfactory, as the colour development takes 20 hours to reach a maximum, and she considers sodium dithionite a better reducing agent.

Our work shows that the order of addition of the reagents and the effectiveness of the reductant are important. To avoid error in this determination iron must be fully reduced to the ferrous state in acid solution (0·2 N hydrochloric acid), before the pH is raised by adding the buffer solution, otherwise recoveries are low; presumably iron$^{III}$ phosphate comes out of solution. We find that in 0·2 N hydrochloric acid solution hydroxylamine is a poor reducing agent at room temperature; hardly any reduction takes place after 10 minutes standing (even after 20 hours some ferric iron remains). These observations show that in the normal method effective reduction must take place simultaneously with the formation of iron$^{II}$ - bipyridyl complex in acetate buffer as colour development is complete in 15 minutes, so that when phosphate is present low results are to be expected. In boiling 0·2 N hydrochloric acid solutions hydroxylamine hydrochloride completes the reduction of iron in 20 minutes and there is no error in the determination.

The use of a more effective reducing agent, ascorbic acid, in place of hydroxylamine hydrochloride also avoids this source of error. A disadvantage is that titanium forms a yellow complex with ascorbic acid, and may cause interference if present in large amount. However, this yellow complex forms only in acetate buffer solution (pH 5·0) and not in chloroacetate buffer solution (pH 3·0). As other group-3 phosphates may precipitate in acetate buffer, the formation of the iron$^{II}$ - bipyridyl complex in chloroacetate buffer is to be preferred.

### METHOD

SAMPLE SOLUTION—

Prepare as for the systematic analysis.

REAGENTS—

*2,2′-Bipyridyl solution,* 0·15 *per cent. w/v, aqueous.*
*Chloroacetic acid solution,* 1 N.
*Ascorbic acid*—Analytical-reagent grade.

PROCEDURE—

By pipette, transfer 10 ml of the sample solution, containing not more than 1 mg of $Fe_2O_3$ to a 50-ml beaker, add 50 mg of ascorbic acid and 10 ml of bipyridyl solution, and set aside for 10 minutes. Add 5 ml of chloroacetic acid solution, and adjust the pH to 3·0 by dropwise addition of 4 N ammonia solution; wash into a 100-ml calibrated flask, and make up to the mark with water. Measure the optical density in a 1-cm cell at 525 m$\mu$. Carry out determinations on a standard solution and a blank solution at the same time.

## Determination of Titanium

As the determination of titanium by the peroxide method is often made in phosphoric acid solution to reduce interference by iron, the presence of phosphate in the sample is of little significance. The sensitivity of the method may be reduced by phosphoric acid, but according to Weissler,[13] this is avoided by measuring the optical density at 400 m$\mu$.

### METHOD

SAMPLE SOLUTION—

Prepare as for the systematic analysis, but after the final evaporation dissolve the solids in 5 ml of 60 per cent. perchloric acid before diluting to 100 ml in a calibrated flask.

REAGENTS—

*Mixed acids*—A mixture containing 20 per cent. v/v of 20 N sulphuric acid and 20 per cent. v/v of syrupy phosphoric acid.

*Hydrogen peroxide reagent*—Mixed acids containing 20 per cent. v/v of hydrogen peroxide (100 volume).

PROCEDURE—

By pipette, place 20 ml of the sample solution into a 25-ml calibrated flask, make up to the mark with hydrogen peroxide reagent, and mix. Repeat, with the mixed acids in place of the hydrogen peroxide reagent to obtain the sample blank solution. Determine the reagent blank value on 20 ml of distilled water. Measure the optical densities of solutions in 4-cm cells at 400 mμ. Deduct both the reagent blank value and the sample blank value, and compare with determinations carried out on standards at the same time.

## Determination of Manganese

Determining manganese in phosphate rock raises no special problems, and the permanganate colorimetric method is satisfactory. Various combinations and concentrations of phosphoric, sulphuric and nitric acids have been used, but we find that nitric acid inhibits the oxidation of manganese to permanganate and that this is most marked when the acid contains much nitrogen dioxide; nitric acid was therefore omitted from the acid solutions.

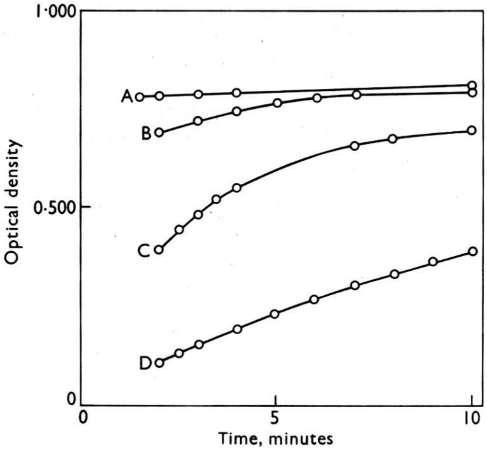

Fig. 1. Effect of fluoride on the rate of formation of silicomolybdic acid. Solutions contained 500 μg of silica, 2 ml of ammonium molybdate solution (10 per cent. w/v in 2 N sulphuric acid) and different amounts of fluoride in a total volume of 25 ml: curve A, no fluoride; curve B, 5 mg of fluoride; curve C, 7·5 mg of fluoride; curve D, 10·0 mg of fluoride.

Optical densities were measured at 400 mμ in 1-cm cells after various intervals of time

### METHOD

SAMPLE SOLUTION—

Prepared as for the titanium determination.

REAGENTS—

*Mixed acids*—A mixture containing 50 per cent. of 20 N sulphuric acid and 25 per cent. of syprupy phosphoric acid.

*Sodium periodate*—Analytical-reagent grade.

*Sodium nitrite solution, 10 per cent. w/v, aqueous.*

PROCEDURE—

By pipette, transfer 25 ml of sample solution to a 100-ml beaker, and add 10 ml of mixed acids and 0·3 g of sodium periodate. Boil the solution for 5 minutes after the permanganate colour first appears, allow to cool, transfer to a 50-ml calibrated flask, make up to the mark with distilled water, and set aside overnight. Measure the optical density of the solution in a 2-cm cell at 525 m$\mu$, add one drop of sodium nitrite solution, stir to decolorise, and measure the optical density again. The differences in these optical densities are used in the calculations. Compare with standard solutions.

### Determination of Silica

As the gravimetric procedure is difficult and tedious, a colorimetric method based on the formation of the yellow silicomolybdic acid and subsequent reduction to molybdenum blue was adopted. In this method, attention must be paid to the effect of phosphate. Fluoride suppresses the formation of silicomolybdic acid and according to Case[14] this interference can be eliminated by the addition of boric acid. However, Greenfield[15] maintains that no precautions need be taken for phosphate rocks.

Our investigation shows that fluoride interferes by retarding the formation of the silicomolybdic acid (see Fig. 1). This effect will be negligible for the amount of fluoride normally encountered in phosphate rocks, but, for safety, a period considerably in excess of the minimum time required for the formation of the hetero-poly acid was allowed in the procedure described.

Phosphate would cause positive interference, as it forms a hetero-poly acid with molybdate that is reduced to molybdenum blue. Oxalic acid decomposes phosphomolybdic acid and can be used to suppress this interference, but as it also slowly bleaches silicomolybdic acid this procedure must be carefully standardised.

There must be about twice the theoretical amount of molybdate required to form the 12 hetero-poly acids of both silicate and phosphate, otherwise there will be negative interference of the type shown in Fig. 2.

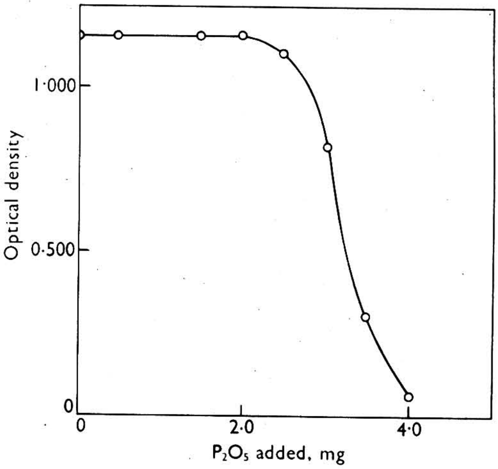

Fig. 2. Interference by phosphate in the determination of silica as molybdenum blue. Silico- and phosphomolybdic acids were formed in solutions containing 300 $\mu$g of silica, $x$ mg of $P_2O_5$ and 1 ml of ammonium molybdate solution (10 per cent. w/v in 2 N sulphuric acid) in a total volume of 25 ml. Reduction to molybdenum blue, after addition of 5 ml of 10 per cent. oxalic acid solution, was by 100 mg of ascorbic acid. The final volume was 100 ml and optical densities were measured at 810 m$\mu$ in 1-cm cells

## METHOD

REAGENTS—

*Sodium hydroxide pellets*—Analytical-reagent grade.

*Ammonium molybdate solution,* 10 *per cent. w/v in* 2 N *sulphuric acid.*

*Oxalic acid solution,* 10 *per cent., aqueous.*

*Ascorbic acid*—Analytical-reagent grade.

PROCEDURE—

Weigh 250 mg of sample (—150 mesh) into a silver crucible, add 4 g of sodium hydroxide, and gently heat with constant swirling to a dull red heat; fuse for about 5 minutes. Cool the melt, half fill the crucible with water, digest on a water bath for 1 to 2 hours, and wash into a 1-litre calibrated flask half filled with water containing 10 ml of 12 N hydrochloric acid. Clean the crucible with water containing 2 ml of 12 N hydrochloric acid, add this solution to the contents of the flask, and make up to the mark with distilled water.

Transfer a portion of this solution, containing not more than 300 $\mu$g of silica, to a 100-ml calibrated flask, add 1 ml of ammonium molybdate solution (sufficient for about 1·5 mg of phosphate), mix, and set aside for 10 minutes.

Then add 5 ml of oxalic acid solution, mix well, after 30 seconds add 100 mg of ascorbic acid, and dilute to the mark. Allow 2 to 3 hours for the reduction, and measure the optical density in a 1-cm cell at 810 and 650 m$\mu$. Compare with standards after deducting blank values (normally negligible).

## Determination of other Elements

Phosphate, carbonate, water and fluoride are determined by the usual methods. Spectrochemical arc techniques are suitable for determining most trace elements, except uranium. The method is not sensitive for this element, which can be determined by separating it from phosphate and most other elements (except iron) by anion exchange and employing a colorimetric finish. The small amounts of aklalis present are best determined by flame-photometric methods. In determining total sulphur, wet oxidation is employed for the decomposition of the sample.

## CONCLUSION

A double precipitation of the group-3 elements as benzoates at pH 3·5 effects a good separation of these elements from calcium, magnesium and manganese, in the presence of phosphate. This separation permits a modified standard scheme to be applied to the group analysis of phosphate rocks.

I thank Mr. K. L. H. Murray for the spectrographic analysis of precipitates.

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# The Removal of Phosphate in the Barium Perchlorate Titration of Sulphate

## Application of the Method to the Oxygen-flask Combustion Technique

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A procedure is described for the quantitative separation of phosphate from mixtures containing sulphuric and phosphoric acids. The free acids are first converted to the corresponding silver salts, and, after separation of the insoluble silver phosphate, the soluble silver sulphate is decomposed by treatment with a suitable ion-exchange resin. The free sulphuric acid produced is then titrated with an aqueous solution of barium perchlorate. In combination with the oxygen-flask combustion technique the method provides an accurate and precise procedure for the micro-determination of sulphur in organic compounds containing phosphorus.

It is now well established that the oxygen-flask combustion technique, in combination with a suitable volumetric procedure, provides the most rapid and convenient method for the micro-determination of halogens, sulphur, phosphorus, boron, etc., in a wide range of organic compounds.[1,2] In determining sulphur for example, the sulphuric acid obtained by decomposition of the sample can be titrated accurately with barium perchlorate,[3] if certain interfering elements, including metals, fluoride and phosphate, are absent. Metals can be removed by a suitable ion-exchange resin,[3] and interference by the fluoride ion can be suppressed by adding boric acid to the solution before titration.[2] The use of magnesium carbonate for removing phosphate has been proposed,[3] but experience suggests that complete separation cannot be achieved with this reagent. From a consideration of the relative solubilities of magnesium and silver phosphates, it was thought that a more complete separation of the phosphate could be effected by precipitation as the silver salt. This was confirmed experimentally by tests carried out with aqueous solutions containing only sulphuric and phosphoric acids. By suitable modification, including the use of a cation-exchange resin, the method was successfully applied to the separation of phosphate, the sulphuric acid then being titrated with barium perchlorate. Finally, in combination with the oxygen-flask combustion technique, the method was successfully applied to the determination of sulphur in organic compounds containing phosphorus.

## EXPERIMENTAL

### THE SEPARATION OF PHOSPHATE FROM SULPHATE BY PRECIPITATION WITH MAGNESIUM CARBONATE—

The examination of this procedure was limited to the simple experiment described below. An accurately measured volume of 0·025 N sulphuric acid was diluted to 25 ml with water, and, after the addition of 100 ml of ethanol, the solution was titrated with 0·01 N barium perchlorate solution with a mixture of thorin and methylene blue as indicator.[4] The volume of titrant required was 5·38 ml. In a further test, a similar volume of sulphuric acid was mixed with a small volume of phosphoric acid equivalent to 3 mg of phosphorus, and, after dilution to 6 ml, the solution was boiled with 120 mg of magnesium carbonate for a few minutes. After it had been cooled in ice-water for about 10 minutes, the solution was filtered into a flask containing about 20 g of moist Amberlite IR-120 cation-exchange resin, and the filter washed with 20 ml of water. The flask was shaken for 5 minutes, the solution decanted, and the resin rinsed with four 25-ml portions of ethanol. The combined solutions were then titrated as before.

The volume of titrant required was about 6·0 ml and the end-point was poorly defined. In a final experiment this procedure was repeated as far as the decantation of the solution from the resin. The resin was then washed with water instead of alcohol, and the phosphate content of the solution was determined colorimetrically.[5] The amount of phosphorus found was 0·2 mg, and, since this result showed that only about 93 per cent. of the added phosphate had been separated as the magnesium salt, the method was abandoned without further examination.

THE SEPARATION OF PHOSPHATE AS SILVER PHOSPHATE—

*Preliminary experiments*—Since many anions interfere in the titration of sulphate with barium perchlorate, especially if present in large amount, and since it was desirable that the metal content of the test solutions should be as small as possible at every stage, no attempt was made to use a soluble silver salt for precipitating phosphate from mixtures of sulphuric and phosphoric acids.

It was thought that a satisfactory method of separation might be based on the reaction of the mixture of acids with silver oxide, and this was supported by the result of the preliminary experiment described below. An excess of silver oxide was added to 5·0 ml of a solution containing sulphuric and phosphoric acids equivalent to 0·2 mg of sulphur and 0·6 mg of phosphorus per ml, respectively. The solution was heated with an excess of silver oxide, cooled, and filtered on to a few grams of cation-exchange resin. The mixture was shaken for a few minutes, the solution decanted, and the resin washed repeatedly with water. Colorimetric determination of the phosphorus content of the combined solutions showed that only 0·02 mg, or 0·7 per cent., of the added phosphorus had escaped precipitation as silver phosphate. In further experiments with similar test solutions the same procedure was employed, except that the resin was rinsed with ethanol in place of water and the free sulphuric acid in the combined solution was titrated with barium perchlorate. Unsatisfactory end-points were at first obtained, but it was found that this defect could be overcome by washing the resin with dilute sodium hydroxide solution, water and ethanol, in that order, before use. The relative merits of aqueous and alcoholic solutions of barium perchlorate were also examined at this stage, and no significant difference between them was observed in the titration of free sulphuric acid. The more convenient aqueous solution was therefore used in all subsequent work. With these minor modifications a further series of tests was carried out. For each test, 2·0 ml of a standard solution of sulphuric acid were transferred from a content pipette to a test-tube containing a small volume of phosphoric acid equivalent to 3 mg of phosphorus. The pipette was rinsed with small quantities of water to give a total volume of 10·0 ml, and the solution was heated to near boiling-point. Successive small portions of silver oxide were then added until present in excess, the solution being re-heated to near boiling-point at intervals. The solution was cooled to room temperature, filtered into a flask containing about 20 g of moist Amberlite IR-120 cation-exchange resin, and the filter washed with 15·0 ml of water. The flask was stoppered, shaken for about 5 minutes, the solution decanted, and the resin rinsed with four 25-ml portions of ethanol.

The combined solutions were then titrated with 0·01 N barium perchlorate with a mixture of thorin and methylene blue as indicator. Well defined end-points were obtained in all titrations. The results obtained were—

| | |
|---|---|
| Sulphur present, mg .. .. .. | 0·87 |
| Sulphur found, mg .. .. | 0·88, 0·87, 0·87, 0·86, 0·88, 0·87, 0·87 |
| Mean value, mg .. .. .. | 0·87 Precision (as standard deviation) ± 0·007 |

APPLICATION OF THE METHOD TO THE OXYGEN-FLASK COMBUSTION TECHNIQUE—

*Preliminary experiments*—Since hydrogen peroxide was to be used as the absorbent in the oxygen-flask combustion method, the phosphate separation procedure was applied to a series of solutions containing 1·0 ml of 30 per cent. hydrogen peroxide in addition to sulphuric and phosphoric acids equivalent to about 1·0 mg of sulphur and 2·0 mg of phosphorus, respectively. The tests were carried out as described above, except that the solutions were not heated above 40° C until most of the hydrogen peroxide had been decomposed by the silver oxide. This minor modification was necessitated by the violence of the reaction at temperatures close to the boiling-point of the solution. The results obtained were—

| | |
|---|---|
| Sulphur present, mg .. .. | 0·875 |
| Sulphur found, mg .. .. | 0·89, 0·85, 0·90, 0·87, 0·86, 0·86, 0·87, 0·86 |
| Mean value, mg .. .. .. | 0·87 Precision (as standard deviation) ± 0·017 |

In these experiments it was expected that, if the decomposition of hydrogen peroxide proceeds according to the reaction equation—

$$Ag_2O + H_2O_2 \rightarrow 2Ag + H_2O + O_2,$$

the amount of silver oxide required would be much greater than in the absence of hydrogen peroxide, but in fact the amounts required were not widely different. The results of the experiments described below suggest that the decomposition does not proceed entirely in accordance with the above reaction, but is in part effected by the catalytic action of the finely divided silver resulting from it. In the first experiment, silver oxide was added in excess to a solution containing 336 mg of hydrogen peroxide. The insoluble matter was filtered off and washed with water and then with ammonia solution to remove excess of silver oxide. The residual metallic silver was dissolved in nitric acid and the amount of silver present determined gravimetrically as the chloride. The weight of silver found, expressed as mg of silver per mg of hydrogen peroxide, was 0·1 mg, whereas the amount expected, in accordance with the above reaction equation, was 6·35 mg per mg of hydrogen peroxide. The catalytic effect of the finely divided silver was demonstrated by repeating the first experiment up to the isolation of the metallic silver, and then adding a small amount of the finely divided metal to 5 ml of 20-volume hydrogen peroxide. As expected, vigorous decomposition of the peroxide was observed.

## METHOD

With the information provided by the investigation of the separation of phosphate as the silver salt, no difficulty was experienced in applying the method to the oxygen-flask combustion technique. The procedure finally adopted is described below.

Sulphur was determined in benzyl disulphide, in the presence of benzene phosphinic acid in an amount equivalent to 2 mg of phosphorus. The results, corrected for blank value of 0·05 ml of 0·01 N barium perchlorate, were—

| | |
|---|---|
| Sulphur present (calculated) % .. | 26·03 |
| Sulphur found, % .. .. | 26·0, 25·7, 25·8, 25·8, 25·9, 25·8, 26·0, 25·9, 25·7, 25·9, 25·7, 25·9 |

The mean value was 25·84 and the precision, as a standard deviation, was ±0·11.

In each of these analyses the weights of sulphur and phosphorus present were about 1 mg and 2 mg, respectively. The application of the procedure to the simultaneous determination of phosphorus and sulphur has not been investigated, but this might be achieved by colorimetric determination of the phosphorus in the mixture of silver phosphate and excess of silver oxide, after solution of the precipitate in sulphuric acid.

### REAGENTS—

*Hydrogen peroxide,* 100 *volume*—M.A.R. grade.

*Ethanol, absolute.*

*Barium perchlorate,* 0·01 N, *aqueous*—Adjust to about pH 3·0 by adding perchloric acid.

*Thorin indicator solution*—Dissolve 25 mg of thorin in 5 ml of distilled water.

*Methylene blue indicator solution*—Dissolve 15 mg of methylene blue in 50 ml of water.

*Silver oxide*—To a 3·0 per cent. solution of silver nitrate, add a small excess of 1·0 N sodium hydroxide, with continuous stirring. Wash the precipitate repeatedly by decantation with water, and store the product under water.

*Cation-exchange resin, Amberlite IR-*120(*H*)—Wash about 400 g of the analytical-grade resin with about 700 ml of 3·0 N hydrochloric acid, by the column method, and then wash with 4 or 5 litres of distilled water, and transfer the resin to a 1-litre flask. Remove most of the water by decantation, add about 100 ml of ethanol, and shake thoroughly for a few minutes. Decant the alcohol, and repeat the washing with two further 100-ml portions of ethanol.

Remove the alcohol by repeated washing with water by decantation. Add 400 ml of 0·5 N sodium hydroxide, shake vigorously for about 10 minutes, and decant the slightly turbid solution. Finally, wash the resin with water until all fine suspended particles have been removed, filter on a Buchner funnel, press the drained resin between filter-papers, and store the moist product in a glass stoppered bottle sealed with adhesive tape. One 25-g portion of resin containing about 45 per cent. of water suffices for at least twenty analyses.

### PROCEDURE—

Weigh out an amount of sample corresponding to about 1·0 mg of sulphur, and decompose it in an "oxygen flask" containing 5·0 ml of 20-volume hydrogen peroxide as absorbent.

Rinse the stopper and sample holder with not more than 10 ml of distilled water, and boil the solution to reduce its volume to about 10 ml. During this operation, further rinsing of the stopper can be effected by supporting it loosely in the neck of the flask. Cool the solution to about 40° C, and add small portions of an aqueous suspension of silver oxide until effervescence ceases. Heat the solution to near boiling-point, and, with occasional re-heating, add further portions of silver oxide until an excess is present. With a sintered-glass filter, provided with a thin layer of paper pulp, filter the cold solution into a conical flask containing about 25 g of resin previously rinsed with two 20-ml portions of ethanol. Rinse the sample flask with not more than 10 ml of water in successive small portions, and pass the washings through the filter. Repeat this operation with two 5-ml portions of alcohol, and then add about 4·0 ml of water to the filter. Allow the water to remain on the filter for several minutes before draining it off. Stopper the flask, shake it at frequent intervals during about 5 minutes, add 25 ml of ethanol, and decant the clear solution into a second conical flask. Wash the resin by decantation with three 25-ml portions of ethanol, and titrate the combined solutions with 0·01 N barium perchlorate solution, with 0·1 ml of thorin solution and 0·1 ml of methylene blue solution as indicator. Carry out a blank determination in the same manner, omitting only the sample. The blank value should not much exceed 0·05 ml.

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# The Determination of Thallium in Urine

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A method has been developed in which Brilliant green is used for the spectrophotometric determination of thallium in urine.

The thallium - Brilliant green complex, formed in the presence of the organic substrate, is extracted into toluene. The optimum conditions for the determination are described, and the sources of various interferences examined.

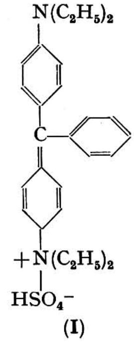
The method has been used to check thallium excretion in the urine of patients.

FOR an evaluation of the effectiveness of chemotherapy in treating thallium poisoning, a rapid method for determining the amount of thallium in the patient's urine is required.

In most existing methods the organic substrate is destroyed as a preliminary step to determining the thallium.[1 to 14] This approach increases the time required for each determination and entails considerably more work. In a few instances the determination of thallium without treatment with mineral acids is described; Stavinoha and Nash[15] extract thallium from urine with an organic solvent and determine it by flame spectrometry, and Reis[16] and Gorzelewska[17] determine extracted thallium spectrophotometrically.

A critical exploration of the approaches available for determining thallium in urine indicated that the method based on the formation of a coloured ion-association complex between a thallic halide anionic complex ($TlX_4^-$) and the dye Brilliant green (a triphenylmethane derivative) should prove useful.

Brilliant green (**I**) has colour index number 42040.[18] It is green in aqueous and alcoholic solutions ($\lambda_{max.} = 623$ m$\mu$) and yellow-red in hydrochloric acid solutions. The addition of sodium hydroxide to the aqueous solution results in the precipitation of the bright-green dye base. The reagent was first used by Kul'berg for detecting thallium.[19]



(**I**)

Reis[16] and Gorzelewska[17] have already used Brilliant green for determining thallium in urine; attempts to repeat their work have, however, been unsuccessful. It was therefore decided to investigate the method systematically, giving due regard to the many variables involved.

Thallium$^{III}$ in halogen acid media forms anionic complexes of the type $TlX_4^-$,[20] where $X$ indicates the halogen atom. The pertinent formation constants cited in the literature[21] are—

$$\log \beta\ TlBr_4^- = 20{\cdot}4$$

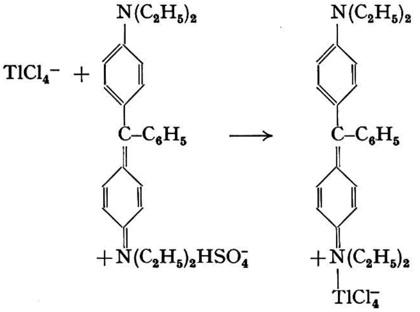
$$\log \beta\ TlCl_4^- = 15{\cdot}4$$

where $\beta$ is the cumulative constant for the reaction—

$$mM + nL \rightleftharpoons M_mL_n$$

$$\log \beta_{mn} = \frac{[M_mL_n]}{[M]^m[L]^n}$$

An inspection of the above values indicates that the bromide complex will be formed preferentially in media containing high concentrations of bromide. In media containing both chloride and bromide, with the concentration of the former far exceeding the latter, the formation of a chloride or a mixed complex can be expected; both, however, will form ion-association complexes with the dye, as shown below (**II**) for $TlCl_4^-$.



(**II**)

This reaction is selective for thallium; the complex formed is coloured and can be extracted with various organic solvents.

## EXPERIMENTAL

### THE THALLIUM - BRILLIANT GREEN COMPLEX—

A sample of "synthetic urine" to which 10·5 µg of $Tl^+$ had been added was oxidised with alkaline bromine solution and acidified with hydrochloric acid; Brilliant green dye solution was added and the coloured complex was extracted into toluene. (The "synthetic urine" was prepared by dissolving 38 g of disodium hydrogen orthophosphate, $Na_2HPO_4.12H_2O$, 30 g of sodium chloride and 9·25 g of ammonium chloride in 500 ml of water; 1 ml of this solution contains the average amount of salts present in 5 ml of urine.) The absorption spectrum of the extract is shown in Fig. 1. No change in the optical density of this coloured solution could be detected within 90 minutes of its formation. The optimum amount of reagent was determined by keeping all other variables constant and adding different amounts of reagent; an approximately thousand-fold excess of reagent (1 mg of Brilliant green for each µg of thallium) is necessary to ensure reproducible optical densities. With smaller amounts of the dye, lower optical-density values were obtained.

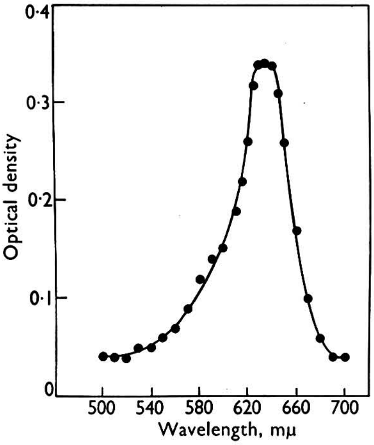


Fig. 1. Absorption spectrum of the thallium chloride - Brilliant green ion-association complex in the toluene extract

OXIDATION OF THALLOUS TO THALLIC—

The oxidation of thallium with sodium nitrite[16,17] did not yield quantitative results (this has also been reported by Campbell[12]); it was therefore decided to investigate the applicability of bromine as oxidant in this method. The presence of halide ions depresses considerably the oxidation potential of the $Tl^{3+}$ - $Tl^{+}$ couple,[22] so that the oxidation of thallous ions by bromine becomes feasible. The presence of excess of bromine must be avoided, since it causes bromination of the reagent, accompanied by the formation of coloured derivatives, some of which are extracted into the organic layer. The presence of appreciable amounts of urea in urine can be exploited for destroying the excess of bromine, especially in an alkaline medium. Twelve millilitres of alkaline bromine solution, prepared as described under "Reagents," were sufficient to effect quantitative oxidation of up to 16 µg of thallous ions present in 15 ml of urine or synthetic urine; for synthetic samples about 0·5 g of urea was added to destroy the excess of bromine, whereas in most samples of urine sufficient urea was present to make this addition unnecessary.

EXTRACTION OF THE COLOURED COMPLEX—

The number of extraction steps recommended was determined on prepared samples composed of 2 ml of synthetic urine, 0·6 g of urea and 8 ml of standard thallium sulphate solution containing 16·2 µg of $Tl^{+}$. These samples were treated with 5 ml of concentrated hydrochloric acid, 6 ml of alkaline bromine solution and 1 ml of 1 per cent. Brilliant green solution, and extracted with successive 2·5-ml portions of toluene. The organic layers were collected in a centrifuge tube, spun, and their optical densities measured at 640 mµ. Extraction of the ion-association complex formed was quantitative after four successive extractions with 2·5-ml portions of toluene (a further extraction did not appreciably increase the optical densities).

To determine the optimum shaking period, the optical density of the organic layer was measured after various shaking times in the range 30 seconds to 3 minutes. Thirty seconds were found to be sufficient both for samples of synthetic urine and for samples of urine to which thallous ions had been added.

ACIDITY OF THE MEDIUM—

The influence of the acidity of the medium at different stages in the determination was investigated by carrying out a series of blank experiments, with both synthetic urine samples and urine, at various concentrations of hydrochloric acid. Further experiments were made with samples to which known amounts of thallium had been added. It was found that, at acid concentrations up to 1·6 M in the aqueous phase, the dye was extracted into the toluene layer, even in the absence of thallium; at acid concentrations above 3·5 M, on the other hand, the extraction of the coloured thallium - dye complex was less efficient and lower optical densities were obtained. This last effect can be ascribed to competing equilibria, such as the depression of the dissociation of $HTlCl_4$ by the high concentration of hydrochloric acid present.[20,23]

## METHOD

REAGENTS—

*Standard thallium solution*—Dissolve an appropriate amount of thallous sulphate in dilute sulphuric acid and adjust the volume with distilled water. Dilute freshly as required to give solutions containing 4 to 16 p.p.m. of $Tl^{+}$.

*Alkaline bromine solution*—Mix 10 ml of saturated bromine water with 2 ml of 25 per cent. sodium hydroxide solution in a small, stoppered Erlenmeyer flask. To avoid introducing bromine droplets with the bromine water, which leads to the addition of an excessive amount of bromine, the saturated bromine water must be prepared, stored and dispensed from the apparatus shown in Fig. 2. Saturation of the bromine water and settling of the bromine droplets is achieved by preparing the bromine water at least 2 days before use. The alkaline bromine solution must be freshly prepared as needed.

*Brilliant green solution, 1 per cent.*—Dissolve 1 g of the dye in 100 ml of 75 per cent. ethanol, with gentle heating and stirring. This solution is stable for at least 1 month.

*Urea solution, 20 per cent., aqueous*—Dissolve analytical-reagent grade urea in water, and pass the solution through a column of Amberlite 120-H ion-exchange resin.

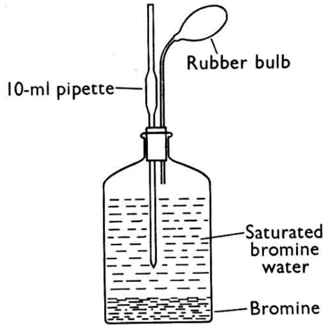


Fig. 2. Apparatus for storing and dispensing bromine water

*Toluene*—Analytical reagent grade.
*Hydrochloric acid, concentrated*—Analytical-reagent grade.

APPARATUS—

*Spectrophotometer*—A Unicam SP500 with 1-cm cells and a Bausch and Lomb Spectronic 20 with 1·17-cm cells were used in the work described here.

PROCEDURE—

Place 10 ml of urine containing not more than 16 µg of thallium (samples containing higher concentrations of thallium must be diluted appropriately) in a separating funnel, add 5 ml of distilled water and then 12 ml of freshly prepared alkaline bromine solution, and cautiously invert the funnel. Add 10 ml of concentrated hydrochloric acid, allow any gases evolved to escape, and again cautiously invert the funnel (it may be necessary at this stage to open the stopper to allow any further gas evolved to escape). Add 2 ml of 1 per cent. Brilliant green solution, and shake the funnel for 30 seconds. (The solution should now be reddish brown; if, however, the reagent has been brominated and the solution has become green, discard the test solution and begin again with a fresh portion of urine to which an appropriate volume of 20 per cent. urea solution—approximately 0·5 g of urea—has been added.)

Extract the coloured complex with four successive 2·5-ml portions of toluene, shaking at each extraction for 2 minutes. Transfer the aqueous layers to a second separating funnel, and collect the toluene layers in a centrifuge tube. When the four toluene layers have been combined, insert the stopper in the tube, and spin in a centrifuge for 3 to 4 minutes at 2000 to 3000 r.p.m. Transfer the clear toluene layer by pipette to a spectrophotometer cell, and measure its optical density at 640 mµ against a toluene blank. Determine the thallium content of the sample with the aid of calibration curves prepared by the same procedure with samples of thallium-free urine to which various amounts of standard thallium solution have been added.

## DISCUSSION OF THE METHOD

### ADHERENCE TO BEER'S LAW AND CALIBRATION CURVES—

Linear calibration graphs were obtained for samples of synthetic urine and urine to which various amounts of standard thallium solution had been added. These calibration curves, for samples containing up to 16 µg of thallium per 10 ml, agreed with each other within the limits of precision obtainable by this method (see Figs. **3** (*a*) and (*b*)). Each point on these calibration graphs represents the average of a number of parallel determinations; the coefficient of variation of the method was found to be ±10 per cent.

### INTERFERENCES—

The influence on the results of some substances, either originally present in the urine samples or introduced in the course of the determination, was investigated. The salt content of urine can differ considerably from sample to sample. Experiments with 10-ml samples prepared by dilution of 1 to 4 ml of the synthetic urine with distilled water showed the presence of a "salting-in" effect, *i.e.*, the optical densities for the higher concentrations exceeded those for the lower concentrations. The loss of precision in the determination of thallium caused by this variation is, fortunately, well within the over-all limits of precision attainable by the method.

The addition, during the determination, of urea to the urine or synthetic urine samples caused excessively high blank optical-density readings, owing to the presence of an unknown contaminant in the analytical-reagent grade urea. Efforts to identify the interfering substance by trace analysis and spot-test methods applied on large samples of urea failed; recrystallisation failed to remove it. Passing the urea solution through a cation-exchange resin was found to provide an adequate method for its purification.

Because of conflicting information[24 to 28] about the selectivity of the method for determining thallium, blank tests were carried out on synthetic urine samples to which various ions had been added. Of all the ions tested ($Fe^{2+}$, $Fe^{3+}$, $Sn^{2+}$, $Sn^{4+}$, $Sb^{3+}$, $Sb^{5+}$, $As^{3+}$, $As^{5+}$, $Hg^{2+}$, $Co^{2+}$, $Zn^{2+}$ and $Cu^{2+}$) only antimony, in amounts exceeding 1 p.p.m., and iron, in amounts exceeding 50 p.p.m., interfere.

Since the method is intended for use in clinical laboratories where the cleaning of glassware with detergents is prevalent, the influence of the presence of sulphonated detergents on the determination was tested. Blank determinations carried out with samples containing traces of these detergents resulted in coloured extracts having similar absorption spectra to those obtained in the presence of the thallium - Brilliant green complex. Glassware must therefore be scrupulously rinsed.

Cases of poisoning by heavy metals (and especially lead) have been treated with infusions of solutions of disodium-calcium ethylenediaminetetra-acetate ($CaNa_2$ - EDTA).[29] This treatment is based on the increase in the excretion of lead, owing to the efficient replacement of calcium by lead in the chelate (the formation constant of the lead complex greatly exceeds that of the calcium complex[30,31]). Attempts to treat cases of thallium poisoning by the same method have been made, despite the unfavourable value of the formation constant for thallium.[32,33] To test the efficiency of the proposed procedure in determining thallium in the urine of patients receiving the above treatment, considerable amounts of the disodium ethylenediaminetetra-acetate (1 to 4 ml of a 0·2 M solution) were added to urine samples containing 4 to 16 $\mu$g of thallium per 10 ml; these samples were subsequently treated by the proposed procedure, the optical densities were measured and a calibration curve was constructed (see Fig. 3 (*c*)). As could be predicted from the knowledge of the pertinent formation constants and the acidity of the sample solution prescribed in the method, no interference owing to the presence of the chelate could be detected, and the calibration graph obtained corresponds to the normal graph (see Figs. 3 (*a*) and (*b*)).

Clinical applications of the method showed that certain drugs, such as phenacetin, acetylsalicylic acid and antipyrine derivatives, interfered in the determination. Phenacetin and the antipyrines reacted competitively with thallium, thus suppressing the extraction

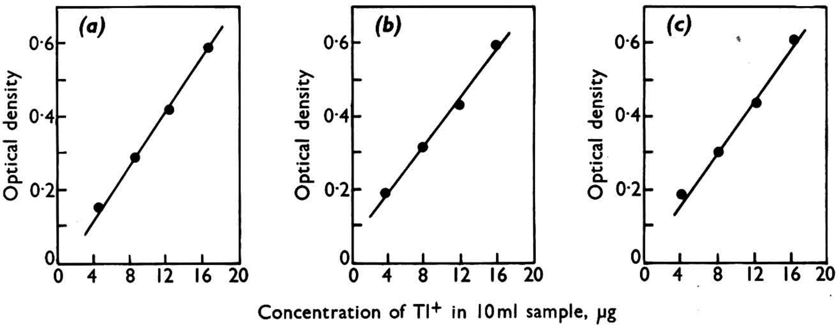


Fig. 3. Calibration curves: (**a**) 10-ml samples of "synthetic urine" containing 4 to 16 $\mu$g of $Tl^+$; (**b**) 10-ml samples of urine containg 4 to 16 $\mu$g of $Tl^+$; (**c**) 10-ml samples of urine containing 4 to 16 $\mu$g of $Tl^+$ to which had been added 4 ml of a 0·2 M solution of disodium ethylenediaminetetra-acetate before the start of the determination of thallium

of the thallium - Brilliant green complex; phenacetin and the acetylsalicylates passed into the organic layer and gave absorption spectra similar to that of the thallium - dye complex. When the patient had been treated with one or more of these drugs, thallium could not be determined in the urine by the direct application of the proposed method; the method could be applied only after removal of the interfering drugs by treating the sample with mineral acids.

CLINICAL TESTS—

The applicability of the method to routine clinical conditions was tested by using it to check the daily excretion of thallium in the urine of three patients. The reliability of the procedure was checked by the method of standard addition. After determining the thallium content of the sample by the direct method and reference to the appropriate calibration graph, a suitable portion of standard thallium stock solution was added to a fresh sample of the same urine, and the thallium content of this mixture was again determined.

The proposed method proved to be sufficiently rapid and sensitive, and its precision was satisfactory for clinical determinations; 0·4 µg of thallium per ml could be determined with a coefficient of variation not exceeding ±10 per cent.

This paper is part of a thesis presented by one of us (D.B.) to the Senate of the Israel Institute of Technology in partial fulfilment of the requirements for the M.Sc. degree.

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# Procedure for Routine Determination of Biphenyl and *o*-Phenylphenol on and in Citrus Fruit*

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An analytical procedure applicable to the routine determination in citrus fruits of the mould inhibitors biphenyl and *o*-phenylphenol is described. Both inhibitors are freed from the bulk of the fruit by steam-distillation before separation of the *o*-phenylphenol by solution in alkali. Biphenyl is determined by its selective ultraviolet absorption after rigorous clean-up; *o*-phenylphenol is determined colorimetrically after coupling with *p*-nitrobenzenediazonium fluoroborate.

THERE is need for an analytical procedure applicable to the routine determination of the widely used mould inhibitors biphenyl and *o*-phenylphenol on and in citrus fruit. Many papers have described analytical procedures for determining one or both of these materials. Colorimetric procedures described for determining biphenyl include the production of a blue colour by reaction with sulphuric acid, formaldehyde and ferric ion[1,2,3] or by reaction with freshly sublimed aluminium chloride[4,5] and production of a purple colour by nitration and then reduction and coupling with *N*-1-naphthylethylenediamine.[6] Instrumental methods for

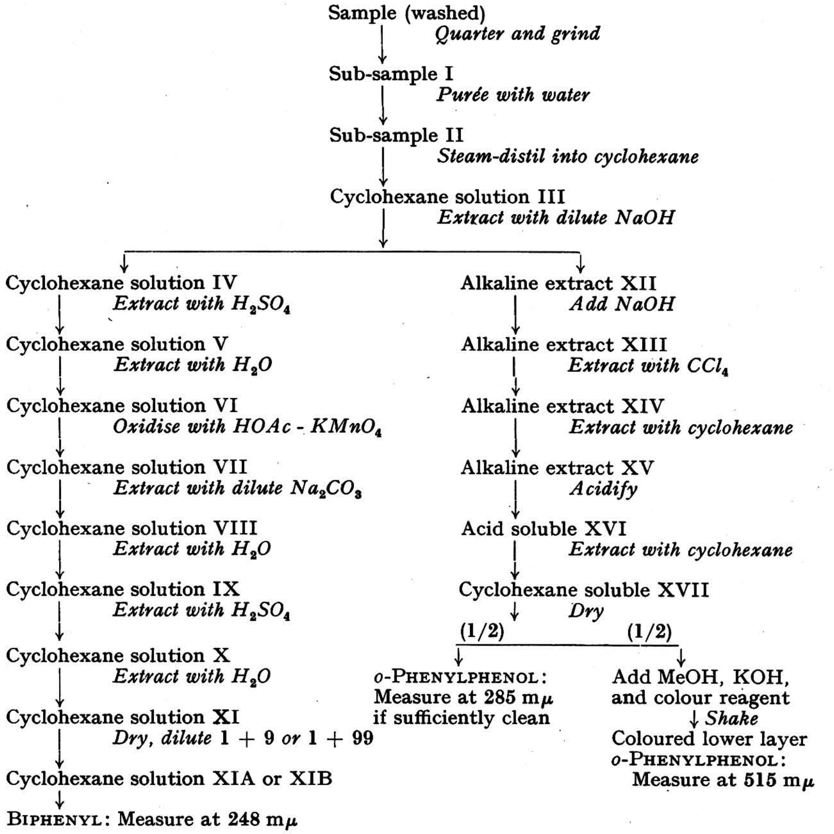

Fig. 1. Flow diagram summarising the operations involved in quantitatively determining both biphenyl and *o*-phenylphenol on a single sample of citrus fruits

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* University of California Citrus Research Center and Agricultural Experiment Station Paper No. 1389.

determining biphenyl include ultraviolet spectrophotometric measurement at the strong absorption peak at 248 mμ,[7 to 15] infrared spectrophotometric measurement in the absorption band at 14·34 μ[16,17] and gas chromatography with a 6-foot silicone-oil column at 160° C and an argon ionisation detector.[18] The use of "chromatostrips" of activated silicic acid with light petroleum for visual detection of biphenyl in ultraviolet light or for clean-up for ultraviolet spectrophotometric determination[12,13] and paper chromatography of biphenyl after exposure to bromine vapour with ultraviolet detection have also been described.[19] For determining *o*-phenylphenol the colorimetric procedures include the use of 4-aminoantipyrene and potassium ferricyanide[20,21,22,23] coupling with Brentamine fast red GG,[24,25] coupling with diazotised sulphanilic acid[5] and the use of sulphuric acid and titanium sulphate[26] to produce a red colour, the reaction of sulphuric acid and formaldehyde to produce a pink colour[27] and the production of a blue colour by reaction with 2,6-dibromo-*p*-benzoquinonechlorimine.[4,27] Instrumental methods for this compound include ultraviolet spectrophotometric determination in cyclohexane by absorption at 250 mμ[9] and at 284 mμ,[25] in sodium hydroxide solution by absorption at 315 mμ,[25] by the hypsochromic effect from changing pH of its solutions,[25] by measuring the fluorescence of its solution in sodium hydroxide with visual matching of sample with standard solutions[9,25] and by gas chromatography with a 6-foot silicone-oil column at 160° C and an argon detector.[18] Paper chromatography with dilute sodium hydroxide solution to develop the chromatogram and 2,6-dibromo-*p*-benzoquinonechlorimine for detection has also been used.[29]

The procedures described here combine selected features of many of the cited procedures and are designed to be adaptable to determining a large number of residues of both biphenyl and *o*-phenylphenol in combination and on a routine basis. In fact, these procedures have been used satisfactorily for this purpose in several regulatory laboratories for some years.[30,31] These procedures utilise ultraviolet spectrophotometry for the final determination of biphenyl and colorimetric coupling of *o*-phenylphenol with the readily available, stable and convenient *p*-nitrobenzenediazonium fluoroborate[32,33] for its determination at 515 mμ. The direct determination of *o*-phenylphenol in citrus extractives by ultraviolet spectrophotometry was unsatisfactory owing to the highly variable background contributions from this substrate. A flow diagram summarising the operations involved is shown in Fig. 1.

## METHOD

### APPARATUS—

All glassware and other equipment must be scrupulously clean. The recommended procedure is to flush thoroughly and successively with water, 95 per cent. ethanol or acetone and then water; the glassware is then rinsed with 95 per cent. ethanol and then water. Occasionally, charring will occur in the boiler flasks; if this happens, flush out all loose material and add about 20 g of crystalline trisodium phosphate and about 100 ml of water, and set aside overnight before cleaning in the usual manner. All stopcocks must be lightly lubricated with a high-vacuum-type silicone grease, including those in separating funnels.

*Meat grinder,* with 5-mm holes in grinding plate.
*Blender,* Waring-type, with 1000-ml jar.
*Clevenger trap,* modified (see Fig. 2).
*Heater,* giant cone with rheostat control, or equivalent high-capacity heater.
*Shaking machine.*
*Pipette,* 5·0 ml, with lower 1 cm of tip bent at 30° angle.
*Spectrophotometer,* suitable for measuring optical densities at 248 and 540 mμ.

### REAGENTS—

All reagents must be of analytical grade unless otherwise specified.
*Sulphuric acid, concentrated.*
*Antifoam agent*—Dow-Corning Antifoam A was used in this work.
*Cyclohexane*—Spectrophotometric grade.
*Sodium hydroxide solution, 0·5 per cent. w/v.*
*Potassium permanganate solution, 6 per cent. w/v.*
*Acetic acid, glacial*—Redistil at atmospheric pressure in all-glass apparatus before use.
*Sodium carbonate solution, 5 per cent. w/v.*
*Sodium sulphate, anhydrous, granular.*

*Sodium hydroxide pellets.*

*Carbon tetrachloride*—Redistilled at atmospheric pressure.

*Potassium hydroxide solution, methanolic*—Dissolve 0·34 g of potassium hydroxide in 1000 ml of methanol.

*Colour reagent*—Dissolve 0·10 g of *p*-nitrobenzenediazonium fluoroborate in 100 ml of methanol immediately before use. Use freshly prepared solution only.

*n-Hexane*—Redistil at atmospheric pressure.

*Hydrochloric acid*, 6 N.

PROCEDURE—

*Processing and steam-distillation*—Select fruits at random. For the minimum size of sample use 15 grapefruits, 40 lemons, 30 oranges or 50 tangerines.

Rinse the fruits in tap-water, drain them briefly, cut them into polar quarters, and grind alternate quarters in the meat grinder. Mix the ground material thoroughly, and sub-sample by quartering to two sub-samples each of 450 ± 1 g. Fill a 1000-ml graduated cylinder with water, and add about 200 ml of this water to the clean jar of the blender. Transfer one of the sub-samples quantitatively to the jar, stir the mixture for a few seconds at low speed, add more water from the graduated cylinder until the jar is about $\frac{3}{4}$ full, and then blend at high speed for 10 ± 1 minute. If the mixture in the jar is too concentrated, the final purée will become hot enough to evolve steam and thus lose some biphenyl and *o*-phenylphenol; if this occurs, divide future sub-samples between two blender jars, with consequently greater dilution in each.

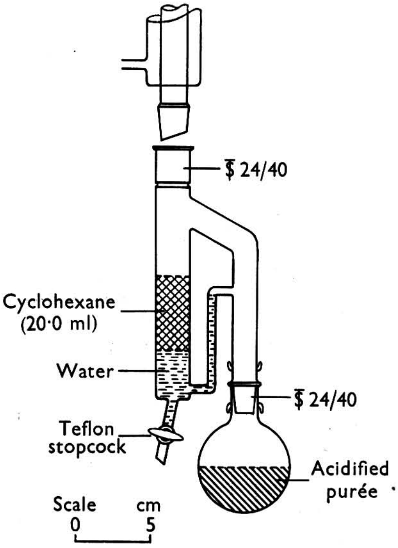

Fig. 2. Modified Clevenger trap

Transfer the resulting purée quantitatively to a clean tared 2-litre graduated cylinder with the remainder of the original litre of water, and weigh to the nearest 3 g to obtain the weight of diluted purée; volume measurements are invalidated by the presence of foam. To a clean round-bottomed 2-litre flask add 3 or 4 carborundum chips, 2 ± 0·2 ml of concentrated sulphuric acid and 0·5 ± 0·2 g of antifoam agent; weigh the flask to the nearest gram. Add to this flask $\frac{1}{3}$ by weight, to the nearest gram, of the thoroughly mixed diluted purée.

In the meantime, arrange the operating condenser, modified Clevenger trap, flask and heater on a ring stand so that the heater and flask can be removed without disturbing the condenser and trap.

Fill the clean trap with distilled water to the overflow point, and attach the flask containing the sub-sample. Carefully transfer by pipette 20·0 ml of spectrophotometrically pure cyclohexane through the upper trap opening on to the surface of the water in the trap. Move the pre-heated heater into position with a piece of wire gauze between it and the flask, and boil under reflux vigorously for 2 ± ¼ hour from the time boiling begins, care being taken to control excessive foaming. At the end of this period, remove the heater, and drain the contents of the trap from the drain cock into a 100- or 125-ml separating funnel. Rinse the trap through its upper opening with 5·0 ml of the pure cyclohexane by means of the special pipette, and put these rinsings in the separating funnel; discard the aqueous layer.

Each millilitre of this final cyclohexane solution now represents 6·00 ± 0·05 g of the parent sample of ground fruit. This solution may be stored awaiting further treatment.

*Separation of biphenyl from* o-*phenylphenol*—The cyclohexane solution contains biphenyl, *o*-phenylphenol, citrus oils, waxes, other citrus extractives and traces of emulsified water. The desired phenol is separated from this mixture by extraction into dilute alkali. Extract the cyclohexane solution from the steam-distillation with five approximately 10-ml portions of 0·5 per cent. sodium hydroxide solution. There will be considerable emulsification during the first and second extractions only; do not withdraw the emulsion with the aqueous layer. Combine the alkaline extracts from a given sample, and store them in a tightly capped bottle in a refrigerator to await the determination of *o*-phenylphenol.

*Ultraviolet spectrophotometric determination of biphenyl*—Repeatedly extract the phenol-free cyclohexane solution with approximately equal-volume portions of concentrated sulphuric acid until a fresh acid layer remains clear and colourless after having been shaken; from 5 to 8 extractions will be required. Wash the cyclohexane solution three times with approximately equal-volume portions of water, and transfer it through the neck of the separating funnel to a glass-stoppered 500-ml Erlenmeyer flask; do not rinse the funnel with further cyclohexane, as this would invalidate the weight - volume relationships. To the contents of this flask add approximately 25 ml of the potassium permanganate solution and approximately 25 ml of the glacial acetic acid. Insert the stopper in the flask, holding it tightly in place with a rubber band if necessary, and shake the flask vigorously for 30 ± 5 minutes.

Transfer this oxidation mixture to a clean separating funnel, and drain and discard the aqueous layer. Wash the remaining cyclohexane layer twice with approximately 10-ml portions of the sodium carbonate solution, and then once with approximately 25 ml of water. Repeatedly extract this washed cyclohexane solution with approximately 20-ml portions of concentrated sulphuric acid until a fresh acid layer remains clear and colourless after having been shaken; from 3 to 6 extractions will be required.

Wash the cyclohexane layer three times with approximately equal-volume portions of water, and decant it into a storage bottle containing approximately 3 g of the anhydrous sodium sulphate.

After at least 10 minutes, withdraw 1·0 ml of this solution, transfer to a 10-ml calibrated flask, and make up to the mark with the spectrophotometrically pure cyclohexane.

Rinse a silica spectrophotometer cell at least four times with about 1-ml portions of this diluted solution. Fill the cell, and measure the optical density or transmission at 248 mμ, with the pure cyclohexane in the reference cell. If the optical density is less than 0·046 (transmission more than 90 per cent.), use the undiluted solution; if the optical density is more than 0·743 (transmission less than 18 per cent.), use the diluted solution further diluted (1 + 9).

Determine the concentration of biphenyl (in p.p.m.) from a standard calibration graph for purified biphenyl in the pure cyclohexane, with correction for the final dilution. The original 25·0 ml of cyclohexane represents 150 g of ground fruit, or 6 g per ml. Dilution (1 + 9) gives 0·6 g per ml and further dilution of (1 + 9) gives 0·06 g per ml.

*Colorimetric determination of* o-*phenylphenol*—Add approximately 2 g of the sodium hydroxide pellets to the storage bottle containing the combined 0·5 per cent. alkaline extracts of the original cyclohexane trap solution from the steam-distillation. After the pellets have dissolved, transfer the solution quantitatively with the aid of a few millilitres of water to a clean separating funnel. Extract this solution thoroughly four times with approximately 10-ml portions of the carbon tetrachloride, discarding the lower layer. Extract the remaining aqueous solution once with approximately 10 ml of n-hexane; transfer the lower aqueous layer to a clean separating funnel, and discard the hexane solution. Carefully and slowly add 25 ml of the 6 N hydrochloric acid solution to the alkaline solution in the separating funnel, and then extract the resulting acidified solution with 20·0 ml of n-hexane; discard the lower aqueous solution.

Transfer 10·0 ml of the hexane solution to a clean dry separating funnel. Add 3·0 ml of the methanolic potassium hydroxide solution and 1·0 ml of the freshly prepared colour reagent; shake the resulting mixture thoroughly, and set it aside for about 10 minutes for the colour to develop. Transfer the clear red lower layer to a cell, cap the cell, and measure the optical density or transmission at 540 mμ, with the yellow reagent blank solution (prepared by the same procedure from 10·0 ml of n-hexane) in the reference cell. If the red lower layer is cloudy owing to emulsification, gentle warming of the solution will provide optical clarity. If the optical density is more than 0·743 (transmission less than 18 per cent.), use a smaller volume of solution to achieve an optical density reading of between 0·046 (90 per cent. transmission) and 0·743 (18 per cent. transmission). Dilution with a mixture of methanolic potassium hydroxide and colour reagent causes deviation from Beer's law.

Determine the concentration of *o*-phenylphenol (in p.p.m.) from a standard calibration graph prepared daily from a graded series of dilutions of *o*-phenylphenol in pure cyclohexane, treated as described above with the methanolic alkali and the colour reagent; this curve should cover the range 0·0 to about 10 p.p.m.

## Discussion of the method

### Biphenyl—

The ultraviolet absorption spectra for several concentrations of biphenyl in spectrophotometric-grade cyclohexane are shown in Fig. 3. Measurement was made at the strong absorption peak at 248 mμ. A calibration curve prepared from Fig. 3 for biphenyl in cyclohexane has a slope of 0·9 μg of biphenyl per ml of cyclohexane solution per 0·1 unit of optical density. Since 1 ml of cyclohexane solution represents 0·06 g of fruit after dilution 1 + 99, this represents 15·6 p.p.m. of biphenyl per 0·1 unit of optical density. This is adequately sensitive for regulatory work as can be seen by the several tolerances for biphenyl in citrus fruits shown in Table I.

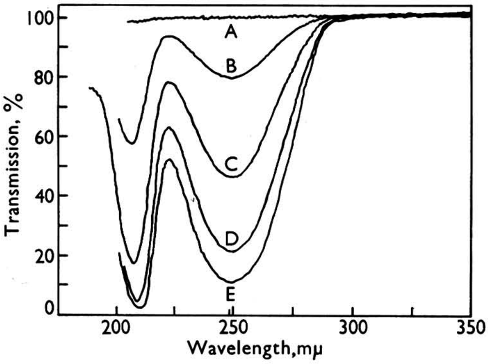

Fig. 3. Standard spectra for biphenyl in cyclohexane: curve A, no biphenyl; curve B, 0·9 μg per ml; curve C, 3·2 μg per ml; curve D, 6·3 μg per ml; curve E, 9·0 μg per ml

TABLE I

TOLERANCES FOR BIPHENYL AND *o*-PHENYLPHENOL IN CITRUS FRUITS

| Country | Biphenyl, p.p.m. | *o*-Phenylphenol, p.p.m. |
|---|---|---|
| Canada .. .. .. | 110 | 10 |
| England .. .. .. | 110* | |
| France .. .. .. | 70 | — |
| Germany .. .. .. | 70 | 10 |
| U.S.A. .. .. .. | 110 | 10 |

* Total for biphenyl *plus* *o*-phenylphenol.

In Table II, the background values are shown for apparent biphenyl found in untreated fruit and recoveries achieved from fortified fruit. As is evident from the results, recoveries are essentially quantitative. The ultraviolet spectrum for untreated citrus fruit is shown

TABLE II

BACKGROUND VALUES AND RECOVERIES FOR THE BIPHENYL PROCEDURE

| Fruit | Background, p.p.m. | Biphenyl added, p.p.m. | Recovery, % |
|---|---|---|---|
| Lemons .. .. .. | 1·82 ± 0·38 | 150 | 100 ± 1 |
| | | 41 | 99 ± 1 |
| Navels .. .. .. | 0·95 ± 0·20 | 150 | 99 ± 1 |
| | | 55 | 100 ± 1 |
| Valencias .. .. .. | 3·14 ± 0·88 | 150 | 100 ± 1 |
| | | 50 | 98 ± 1 |

in Fig. 4. While the magnitude of this background was negligible for regulatory purposes, the nature of ultraviolet absorbing material was investigated. By means of gas chromatographic isolations and subsequent comparative infrared, ultraviolet, and nuclear magnetic resonance spectrometry it was found that this background absorption was due to *p*-cymene.

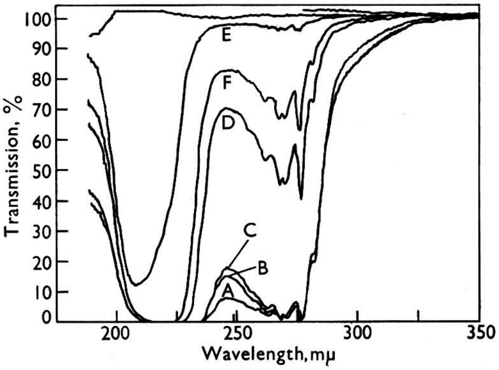

Fig. 4. Biphenyl background spectra for untreated lemons: curve A, sample 26; curve B, sample 27; curve C, sample 28; curve D, sample 28 diluted (1 + 4); curve E, sample 28 diluted (1 + 99); curve F, sample 28 diluted (1 + 9)

*o*-PHENYLPHENOL—

In Fig. 5 are shown the absorption spectra of the colour produced from several concentrations of *o*-phenylphenol by the colorimetric procedure described. Measurement is made at the strong absorption peak at 540 mμ. A calibration curve for *o*-phenyphenol,

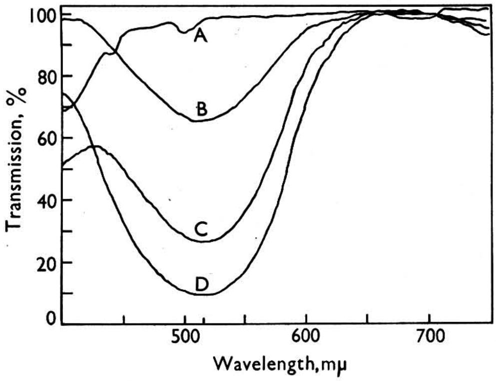

Fig. 5. Standard spectra for *o*-phenylphenol carried through the colour-developing reaction without dilution: curve A, no *o*-phenylphenol; curve B, 8·4 μg per 4 ml; curve C, 21·0 μg per 4 ml; curve D, 42·0 μg per 4 ml

prepared from Fig. 5, has a slope of 4·52 $\mu$g per 0·1 unit of optical density; or for a 150-g sample, 0·06 p.p.m. per 0·1 unit of optical density. The colour produced is adequately stable for from 5 to 40 minutes after colour development (apparent concentrations = +1·5 per cent. after 40 minutes).

*o*-Phenylphenol cannot be steam-distilled so readily by the proposed method and is more soluble in water than is biphenyl. The cyclohexane trapping solution will contain all the biphenyl present, but, when the citrus purée is steam-distilled under the recommended conditions, this trap solution will contain from 80 to 100 per cent. of any *o*-phenylphenol present, depending upon several factors difficult to control. More consistent yields are obtained by carrying out the distillation as directed, and then replacing the cyclohexane trapping solution by fresh cyclohexane for further steam-distillation with subsequent combination of the two distillates. Alternatively, the distillable citrus oils have been used as the sole trapping solvent with excellent recoveries of *o*-phenylphenol.[29] In Table III are shown the background values of apparent *o*-phenylphenol found in untreated fruit by the proposed procedure. Recoveries achieved ranged from 89 to 100 per cent. with from 0·5 to 10 p.p.m. of *o*-phenylphenol.

TABLE III

BACKGROUNDS FOR *o*-PHENYLPHENOL PROCEDURE

| Fruit | Background, p.p.m. |
|---|---|
| Lemons .. .. .. | 0·04 ± 0·01 |
| Navels .. .. .. | 0·03 ± 0·01 |
| Valencias .. .. .. | 0·04 ± 0·01 |

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# A Rapid Assay of Radioactive Caesium in Milk

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A rapid method for the assay of radioactive caesium in milk is described. This involves ashing the sample and separating caesium by allowing a solution of the ash in nitric acid to flow through a bed of ammonium 12-molybdophosphate. The assay is completed by beta-counting the bed of ammonium 12-molybdophosphate.

THE routine assay of radioactive nuclides in milk and other foodstuffs is a long and tedious process. Radioactive strontium is determined by a method based on precipitation (together with an isotopic carrier) by nitric acid, radiochemical purification, the establishment of secular equilibrium between strontium-90 and its daughter element yttrium-90, the separation of parent and daughter elements, and counting. Caesium-137, which is not considered to be so hazardous a nuclide as strontium-90, is determined either on the residues after the removal of strontium nitrate or by direct gamma-ray spectrometry on the original sample; however, this latter method requires expensive equipment and may yield poor counting statistics.

The cation-exchange properties of ammonium 12-molybdophosphate have lately received a great deal of attention from several authors.[1,2,3,4] A method has recently been proposed for the assay of caesium-137 in rain water by allowing the acidified sample to flow through a pre-formed precipitate of the compound supported in a small de-mountable Buchner funnel, and then beta-counting the bed of ammonium 12-molybdophosphate.[5] Since the caesium is so selectively removed from acid solution in this way, it appears feasible that the normal radiochemical assay procedure for milk can be reversed and the caesium-137 determined, before the separation of radioactive strontium, by means of a bed of ammonium 12-molybdophosphate. The method described below enables radioactive caesium to be assayed in 3 days or even less; radioactive strontium can be determined in the usual way after radioactive caesium has been removed from solution.

In an emergency, when many samples contaminated by fission products need to be processed quickly, a rapid assay of caesium-137 may prove useful as a preliminary "screen"; under such conditions, the much larger activities involved would permit a considerable reduction in sample size, more rapid ashing, and a still more rapid estimation to be made. Further, the information obtained may prove a more reliable guide to general fission product contamination than a gross beta-count (when possible) of a sample of ash.

## PROCEDURE—

Liquid milk will not readily pass through a bed of ammonium 12-molybdophosphate and, in any case, curdles when acidified with dilute nitric acid. It is therefore necessary to ash the sample. The method adopted for this is based on that of Murthy and Campbell[6]; milk is allowed to flow under gravity from a polythene reservoir, through a polythene tube cooled in ice-water (to prevent coagulation of the milk owing to heat) and to drip into a rotating silica dish heated by two Meker burners. By suitable adjustment of the flow, the rate of rotation of the dish (about 10 to 15 r.p.m. is suitable) and the burners, the milk is immediately evaporated and partly ashed. The temperature must be maintained below 450° C in order to avoid loss of caesium. Ashing is then completed in a muffle furnace at 400° to 450° C in about 48 hours.

Samples of dried milk are charred at 100° to 150° C, and ashing is completed at 400° to 450° C as for liquid milk.

The ash is then damped with a solution containing 1 mg of caesium cations and carriers for any other nuclides to be assayed, and sufficient concentrated nitric acid is added to dissolve the ash. Finely divided carbon is invariably present. Most of this is removed by filtering the solution through paper pulp; no significant retention of caesium-137 by this carbon has been detected.

After concentration of the filtered solution to about 60 ml and adjustment of the nitric acid concentration to between 2 and 8 M, caesium is removed by passage through a bed of ammonium 12-molybdophosphate prepared as described by Broadbank, Dhabanandana and Harding[5]; the filtrate may be used to determine radioactive strontium, or other radionuclides. The bed is sucked almost dry, the funnel carefully de-mounted and caesium-137 determined by counting the filter-paper and the bed of ammonium 12-molybdophosphate under an end-window counter.

COUNTING—

Beta-counting via a thin end-window Geiger counter is convenient and efficient. Unfortunately, however, recovery of caesium-137 from labelled milk samples is not complete. On the other hand, counting with a gamma-scintillation counter (under the photopeak, to reduce background) indicates quantitative recovery of caesium-137. This discrepancy is thought to be due to the presence of an extremely thin film of finely divided carbon on the surface of the bed of ammonium 12-molybdophosphate; the maximum beta energy of caesium-137 - barium-137*m* is comparatively low and even a small thickness of absorber is significant. Removal of carbon, following dissolution of the milk ash, by filtration through paper pulp is more rapid and complete than by filtration under reduced pressure through a filter-paper. This leads to higher apparent recoveries of caesium-137, as determined by beta-counting (see Table I). This apparent recovery is also dependent on the amount of sample taken. Presumably, the distribution of carbon particle sizes in the ash obtained under given conditions is approximately constant and, therefore, the thickness of the carbon film on the bed of ammonium 12-molybdophosphate is approximately proportional to the weight of ash taken (compare Tables I and II).

A further disadvantage of beta-counting is that careless handling of the bed of ammonium 12-molybdophosphate will cause it to break up; this introduces a variable amount of self-absorption by the source, since the caesium is originally present in the surface layers of the bed (see, for example, sample 3 in Table I).

TABLE I

THE RECOVERY OF CAESIUM-137 FROM LABELLED MILK ASH

| Sample number | Beta counts per minute | Gamma counts per minute | Recovery of caesium-137 by— beta-counting, % | gamma-counting, % |
|---|---|---|---|---|
| Reference | 1365 ± 11·8 | 397·3 ± 6·5 | — | — |
| 1 | 1163 ± 10·9 | 391·9 ± 6·4 | 85 | 99 |
| 2 | 1192 ± 11·0 | 406·9 ± 6·5 | 87 | 102 |
| 3 | 975 ± 10·0 | 393·2 ± 6·4 | 71* | 99 |
| 4† | 1113 ± 10·7 | 400·2 ± 6·5 | 81 | 101 |
| 5† | 1047 ± 10·2 | 381·3 ± 6·2 | 77 | 96 |

* Ammonium 12-molybdophosphate bed broken up.
† Carbon removed by filtration through Whatman No. 1 filter-paper instead of paper pulp.

TABLE II

THE RECOVERY OF CAESIUM-137 FROM LABELLED DRIED MILK

| Sample number | Beta counts per minute | Gamma counts per minute | Recovery of caesium-137 by— beta-counting, % | gamma-counting, % |
|---|---|---|---|---|
| Reference | 1455 ± 9·4 | 616 ± 9·0 | — | — |
| 1* | 1006 ± 7·8 | — | 69 | — |
| 2* | 988 ± 7·7 | 606 ± 9·0 | 67 | 98 |
| 3† | 952 ± 7·6 | 560 ± 8·6 | 65 | 91 |
| 4† | 1069 ± 8·0 | 588 ± 8·8 | 73 | 95 |

* 1 mg of caesium carrier added before ashing.
† 1 mg of caesium carrier added after ashing.

However, in spite of these disadvantages, beta-counting is generally preferred. The adoption of a standardised technique and sample weight yields consistent recoveries of caesium-137, and the high counting efficiency coupled with the low background (especially with a guard tube in anti-coincidence) of the Geiger counting assembly are particularly useful where low activities are to be measured. In an emergency, when relatively high activities are involved, the less efficient gamma-counting may be preferable.

## RESULTS

### LABELLED MILK ASH—

The results in Table I, for 10 g of labelled milk ash, were obtained by the procedure described below.

Beta-counting was carried out under a Mullard end-window Geiger counter, type MX123, with the sample covered with cellulose tape and on the second shelf of a square lead castle (ERD pattern). The counting efficiency of this arrangement, determined by counting a bed of ammonium 12-molybdophosphate through which a known amount of caesium-137 had been passed under conditions of complete retention, was approximately 6 per cent. Transferring the sample to the top shelf of the castle increased this figure to about 17 per cent., and omission of the cellulose tape increased it further to about 24 per cent.

Gamma-counting was carried out with the same sample on the top shelf of the same castle, under a sodium iodide crystal (1 inch × 1½ inch diameter) in conjunction with an Isotope Developments Limited scintillation head, type 653; a single-channel pulse height analyser was used to allow counting to be carried out under the 0·66-MeV photopeak of caesium-137 - barium-137*m*. The counting efficiency was determined, as described in the previous paragraph, to be about 2 per cent.

Completeness of recovery was judged by comparing count-rates of samples (each of which contained about $10^{-5}$ mC of caesium-137) with that of a bed of ammonium 12-molybdophosphate through which the same amount of caesium-137 had been passed under conditions of complete retention.

TABLE III

CAESIUM-137 IN UN-LABELLED DRIED MILK

| Sample | Caesium-137 content found by— | |
|---|---|---|
| | proposed method, pC per kg | direct gamma-ray spectrometry,* pC per kg |
| A | 574 ± 15 | 585 |
| B | 1073 ± 15 | 943 |
| C | 657 ± 28 | 778 |

* We are grateful to the Agricultural Research Council Radiobiological Laboratory for these samples. Comparable errors are not quoted for the gamma-ray spectrometric measurements.

All counting results have been corrected for background (about 14 c.p.m. for the Gieger counter and 10 c.p.m. for the scintillation counter).

### LABELLED DRIED MILK—

The results in Table II, for 500 g of labelled dried milk, were obtained by the procedure described below.

One millilitre of caesium-137, with or without caesium carrier, was thoroughly mixed with a cream prepared from 500 g of dried milk and distilled water. After the cream had been dried, it was ashed at 400° to 450° C for 48 hours, caesium carrier was added, if this had been omitted initially, and the 25 to 30 g of ash were treated as described above for labelled milk ash.

The loss of caesium, as determined by gamma-counting, is small, but the lower apparent recovery of caesium by beta-counting, as compared with the smaller ash samples of Table I, is apparent. Whether the caesium carrier is added before or after ashing appears to have little influence on the results.

### UN-LABELLED DRIED MILK—

The results in Table III were obtained with 500-g samples of dried milk, whose caesium-137 content had already been determined by direct gamma-ray spectrometry (we are indebted to The Agricultural Research Council Radiobiological Laboratory, through Dr. J. D. Burton, for these samples). In all tests, the final beds of ammonium 12-molybdophosphate were counted, uncovered, under a Mullard Geiger counter, type MX123, on the top shelf of a square lead castle (ERD pattern). Sample *plus* background and background alone were counted alternately for $10^3$ seconds until at least ten readings for each had been obtained. The standard deviations quoted in Table III are based on the standard deviations of the difference between the means of the two sets of count-rates.

For evaluation of activities from count-rates, a known volume of a reference solution of caesium-137 was used and the percentage recovery was taken as 65 per cent., as seemed reasonable from a consideration of Table II.

## INTERFERENCE FROM POTASSIUM

The isotope potassium-40, which occurs to the extent of 0·0118 per cent. in natural potassium, is radioactive. Since ordinary milk contains about 1·4 g of potassium per litre, its radioactivity due to potassium-40 is far greater than that normally due to caesium-137. Owing to the similarity in the chemical behaviour of the two elements, it is of interest to examine the possibility of interference by potassium in the assay of radioactive caesium.

A solution of 2 g of potassium nitrate in 2 to 8 M nitric acid was passed through a bed of ammonium 12-molybdophosphate, which was then sucked dry and counted under an end-window Geiger counter, the background of which had been reduced to 1·4 c.p.m. by an anti-coincidence arrangement. A count rate of less than 0·5 c.p.m. above background was recorded over a counting period of 4½ hours.

If the whole of the ammonium ions in the ammonium 12-molybdophosphate were replaced by potassium, then the molybdophosphate bed would contain a little more than 7 mg of potassium and the potassium-40 content would give about 12 disintegrations per minute. This corresponds, at a counting efficiency of 20 per cent., to about 2·5 c.p.m., which represents an amount of caesium-137 smaller than the standard deviations reported in Table III.

## CONCLUSIONS

The procedure described allows the assay of caesium-137 in milk and dried milk to be carried out rapidly and with simple and inexpensive counting equipment. The accuracy and precision of the results are adequate for most purposes.

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We also thank Mr. E. R. Mercer and Dr. J. D. Burton of the Agricultural Research Council Radiobiological Laboratory for their co-operation.

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# A Liquid-scintillation Method for determining Tritium in the Tissues of Animals dosed with Tritium-labelled Vitamin A

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A liquid-scintillation counting method is described for determining tritium as tritium-labelled vitamin A in suspensions of tissue, blood and faeces.

Some problems of the method are examined, *e.g.*, the influence on the measurements of the composition of the scintillator and the auxiliary compound, anhydrous sodium sulphate. The use of an internal standard is described, and the range of error and the evaluation of the results are discussed.

Finally, the procedure is described in detail and results are reported that show good agreement between this method and the conventional method for determining vitamin A.

THIS paper describes the development of an extremely sensitive method for determining tritium as vitamin A and its decomposition products in tissue and faeces. These investigations are a continuation of earlier absorption experiments with vitamin A.[1,2]

Vitamin A may be labelled with carbon-14 or tritium, the radioactive isotope of hydrogen. The labelling of organic compounds containing hydrogen can be carried out fairly easily[3]; further, in biological experiments it is possible to use this isotope in high activities, because of its weak irradiation. For this reason tritium-labelled vitamin A was used in these experiments. Previously, the determination of tritium was considered difficult because of its weak irradiation. To-day these difficulties have been overcome, as tritium can be determined by means of liquid-scintillation counting. By this method the radioisotope is added to a solution containing an organic compound, "the liquid scintillator," which will, under the influence of the ionising radiation from tritium, emit light photons. The light is absorbed by a photocell (photomultiplier). Each light quantum gives rise to an electric pulse, which, after amplification, is counted.

In the liquid-scintillation method it is not always necessary to isolate the tritium-labelled compound, as it is possible to measure the tritium activity directly in the tissues. Tissue can be dissolved in formamide by heating. A turbid solution is thus formed and measurements are made after dispersing it in a solution of liquid scintillator.[4,5,6,7] Passmann, Radin and Cooper[8] introduced the high molecular-weight quaternary ammonium base, hyamine, as a solvent for carbon dioxide; this compound has since been used successfully as a solvent for tissue. Agranoff[9] briefly states that freeze-dried tissue powdered and dissolved in hyamine exhibits a slight colour. Herberg,[10] however, mentions that coloured materials must be removed with hydrogen peroxide and that phosphorescence must be overcome by acidifying with concentrated hydrochloric acid, which may cause some precipitation. The preparation procedures are often long and time-consuming.

## EXPERIMENTAL

The soft $\beta$-radiation from tritium has been measured in tissue and faeces as described below. The minced tissue is ground with anhydrous sodium sulphate, and the resulting powder is suspended in a thixotropic gel containing the scintillator. The suspension is measured in a liquid-scintillation counter, with attention to quenching, light-absorption and phosphorescence.

### SOME PROBLEMS OF THE METHOD—

*The liquid scintillator*—The liquid scintillator fluid should be able to count all the different fractions, *i.e.*, aqueous solutions, as well as organic fluids and tissue particles; a gel can be used for the tissue particles. White and Helf[11] have described a thixotropic gel formed by adding Thixcin (Baker Castor Oil Company) to an organic fluid. Helf[12] states that at a

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concentration of 25 g of Thixcin per litre of fluid, 22 ml of gel will keep 1 g of material suspended. Helf further mentions that the addition of Thixcin to ordinary scintillators decreases the optical transmission by up to 10 per cent. when carbon-14 is being measured. Furst, Kallmann and Brown[13] mention that the counting efficiency with a mixture of dioxan and water is lower than that with the scintillator in toluene alone; the original efficiency can, however, be obtained by the addition of naphthalene.

As it is often necessary to assay aqueous solutions, naphthalene was added to the scintillator solution for optimal efficiency. To ascertain whether naphthalene could compensate for the small decrease in efficiency caused by the presence of Thixcin, the efficiencies of the scintillators for counting a tritium standard were determined; the results are shown in Table I.

TABLE I

EFFICIENCY OF COUNTING A TRITIUM STANDARD IN A ONE-CHANNEL LIQUID-SCINTILLATION APPARATUS (NUCLEAR ENTERPRISES (GB) LTD.) WITH DIFFERENT LIQUID SCINTILLATORS

| Liquid scintillator | Counting efficiency (background 60 c.p.m.) | |
|---|---|---|
| | not de-oxygenated, % | de-oxygenated for 5 minutes with argon, % |
| I. 4 g of PPO (2,5-diphenyloxazole), 0·1 g of POPOP (1,4-di-[2-(5-phenyloxazoyl)] benzene) and 70 g of naphthalene per litre dissolved in toluene .. | 14·1 | 16·0 |
| II. 4 g of PPO, 0·1 g of POPOP, 70 g of naphthalene and 25 g of Thixcin per litre dissolved in toluene .. | 13·9 | 15·9 |
| III. 4 g of PPO, 0·1 g of POPOP and 25 g of Thixcin per litre dissolved in toluene .. .. .. .. | 13·5 | 15·6 |

It can be seen from Table I that the addition of naphthalene to the Thixcin gel results in the formation of a transparent gel that has the same efficiency as the liquid scintillator. Liquid scintillator I can be used for water and organic fluids and liquid scintillator II for the counting of particles—by applying simple corrections it is possible to achieve the same counting efficiency for both.

*Anhydrous sodium sulphate*—The tissue must be dehydrated before dispersion in the Thixcin gel for radioassay. This may be done by freeze-drying or by the use of anhydrous sodium sulphate. Since freeze-drying requires extensive laboratory equipment, which was not available, the tissues were dried with sodium sulphate. It was therefore necessary to investigate the influence of the latter on the scintillator solution. Its quenching effect should be minimal to avoid extensive corrections. This was examined in an experiment with anhydrous sodium sulphate, anhydrous sodium sulphate *plus* water (4 + 1) and anhydrous sodium sulphate *plus* water (2 + 1). In each test 10 ml of scintillator gel and 50 $\mu$l of a tritium standard solution with different amounts of the sodium sulphate mixtures were counted. From Table II it can be seen that the quenching effect or light absorption is negligible in all mixtures—in concentrations up to 200 mg per 10 ml of liquid scintillator. The sodium sulphate becomes nearly transparent in the gel.

TABLE II

TRITIUM QUENCHING VALUES FOR SODIUM SULPHATE

| Amount of sodium sulphate added to 10 ml of liquid scintillator II, mg | Relative counting rate | | |
|---|---|---|---|
| | No water | 20% of water | 40% of water |
| 0 | 100 | 100 | 100 |
| 25 | 101 | 101 | 101 |
| 50 | 101 | 98 | 101 |
| 75 | 100 | 100 | 99 |
| 100 | 101 | 96 | 97 |
| 125 | 100 | 98 | 97 |
| 150 | 97 | 98 | 97 |
| 200 | 99 | 91 | 95 |

The sodium sulphate gel, however, has a phosphorescence that takes much longer to fade than the 10 minutes required for the phosphorescence of an ordinary liquid scintillator to reach the background value.[14] The decline in phosphorescence of a suspension of 100 mg of anhydrous sodium sulphate dispersed in 10 ml of scintillator gel is shown in Fig. 1.

The phosphorescence decreases to the normal background value within approximately 2 hours. The phosphorescence of dried sodium sulphate *plus* water, in the proportions (2 + 1) and (4 + 1), decreases in a similar way.

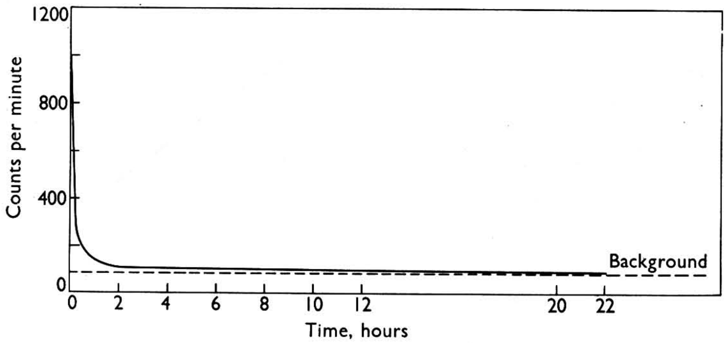

Fig. 1. Decline of phosphorescence in 100 mg of anhydrous sodium sulphate suspended in 10 ml of liquid scintillator II

*Corrections for quenching and colour of solution*—On measuring ionising radiations in tissue in a liquid-scintillation apparatus several factors influence the results, *e.g.*, ordinary quenching and the colouring matter in the tissue. Correction for these factors can be made by the introduction of an internal standard. The correction factor, k, is obtained by comparing the count rate for the standard solution in the liquid scintillator alone with that for the standard in the liquid scintillator containing the tissue suspension. Thus the correction factor is given by the equation—

$$\mathrm{k} = \frac{s}{x - c}$$

where $s$ = the count of the standard,
$x$ = the count of the tissue *plus* standard and
$c$ = the count of the tissue alone.

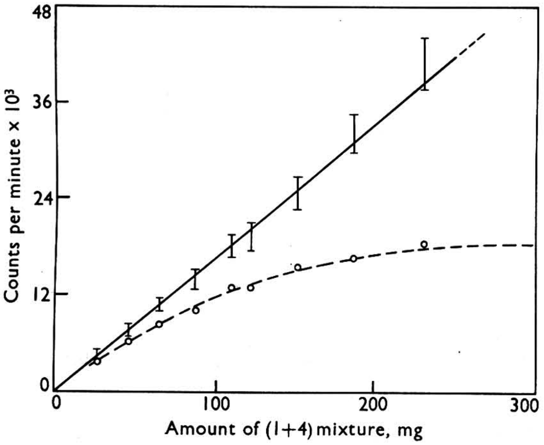

Fig. 2. Relationship between corrected (full line) and uncorrected (broken line) values, in counts per minute, for tritium-labelled vitamin A in liver tissue by the internal standard method

The true count for the tissue is then obtained by multiplying the observed count of the tissue fraction by the correction factor. Fig. 2 shows the relation between uncorrected and corrected values for tritium-labelled vitamin A in liver tissue. The latter values show a direct relationship with the amounts of tissue taken for assay. The tissue was ground with 4 parts of dried sodium sulphate, because it was found that the correction factor for this mixture was approximately one third of that for the (1 + 2) mixture.

### PREPARATION OF SAMPLE—

The weighed tissue sample is homogenised in a Griffith tube and then ground with 4 parts of anhydrous sodium sulphate (Pharmacopoea Danica 1948). To ensure uniform mixing, the powder is moistened with light petroleum, which does not quench the light emitted from the liquid scintillator and in which vitamin A is soluble. The mixture is stirred until it is again dry. Material with a high connective tissue content, *e.g.*, lung and muscle tissue, is initially treated with trypsin for one hour at 37° C. Trypsin does not influence the measurements. Faeces are not treated in a Griffith tube, since vitamin A and its decomposition products are free in the faeces and not enclosed by cells.

### RANGE OF ERROR, EVALUATION, ETC.—

The coefficient of variation for the radioassay of liver tissue is about 6 per cent. (see Table III) and this variation also applies to other tissues and faeces as well as to the background. As the final result obtained is the difference between two measurements (tissue *minus* background) both with a coefficient of variation of less than 6 per cent., the coefficient of variation of the final result is 8·5 per cent.

As the vitamin-A activity is concentrated in certain tissues, other tissues contain little or no activity. If the count rate for a 0·1-g mixture of a tissue sample is within ±6 per cent. of the value for corresponding tissue from a control animal, it is presumed that the tissue does not contain radioactivity.

Faeces have a high background count as well as a high quenching factor. Daily measurements on faeces are therefore taken during the period of excretion of tritium, and the results are then compared with background results from the period before the animal was dosed with tritium or with the average of the measurements lying within ±6 per cent. of the level found in the faeces after tritium excretion had ceased. The results are corrected for daily variation by means of the daily background count when this count is above or below the average count for the period by ±6 per cent.

TABLE III

STANDARD DEVIATION OF RESULTS ON LIVER TISSUE

| Weight of tissue, mg | Count rate, c.p.m. | k | $C_k$ | $C_k$ per 20 mg | $X - \bar{x}$ | $(X - \bar{x})^2$ |
|---|---|---|---|---|---|---|
| 19·7 | 284 | 1·60 | 455 | 463 | +27 | 729 |
| 20·4 | 297 | 1·55 | 460 | 450 | +14 | 196 |
| 20·9 | 324 | 1·48 | 480 | 459 | +23 | 529 |
| 20·0 | 277 | 1·45 | 402 | 402 | −34 | 1156 |
| 20·0 | 280 | 1·45 | 406 | 406 | −30 | 900 |
| 21·0 | 305 | 1·48 | 451 | 430 | − 6 | 36 |
| 20·2 | 292 | 1·51 | 442 | 437 | + 1 | 1 |
| 20·7 | 291 | 1·68 | 488 | 471 | +35 | 1225 |
| 20·1 | 280 | 1·57 | 439 | 436 | 0 | 0 |
| 20·8 | 292 | 1·45 | 424 | 408 | −28 | 784 |
| | | | Total .. | 4362 | Total .. | 5556 |

$$\text{Standard deviation} = \sqrt{\frac{5556}{9}} = 24{\cdot}8$$

A comparison of the sodium sulphate method with the standard method for determining vitamin A (saponification and shaking with an organic liquid) was carried out on certain tissues taken from animals dosed with 10 μC of tritium-labelled vitamin A by measuring the tritium contents. This preparation was examined by paper chromatography to decide whether or not the activity originated from vitamin A. The tissues and the blood were chromatographed on paper in accordance with the technique described by Christensen, Engelund, Handler and Terp.[15] Only the homogenates of the tissue showed a small labelled zone with an $R_F$ value of 0·66 (the $R_F$ value of vitamin A). The results of the comparison are shown in Table IV.

The sodium sulphate method has also been used in other experiments in which the rats were dosed with only 2 $\mu$C of tritium-labelled vitamin A per rat.

PROCEDURE FOR SOFT TISSUES—

The organ is removed and weighed, and then ground in a Griffith tube. This tube is normally used for mincing tuberculous materials. The ground tissue is weighed, and mixed with 4 parts of anhydrous sodium sulphate; the sodium sulphate is added gradually. The mixture is then moistened with light petroleum, and stirred until dry; the moistening and stirring are repeated. An aliquot is taken and is moistened with light petroleum and stirred until dry.

A 0·100-g ($\pm$10 per cent.) portion of the resulting dry powder is weighed in a measuring cell, 10 ml of liquid scintillator II are added, and the mixture is shaken vigorously for 15 seconds only, care being taken to avoid incorporating air in the suspension, otherwise the suspension may become white and opaque.

## TABLE IV

### COMPARISON OF THE SODIUM SULPHATE AND THE STANDARD METHODS, BY MEASURING THE TRITIUM ACTIVITY OF CERTAIN TISSUES*

| | Liver | | Muscle | | Blood | |
|---|---|---|---|---|---|---|
| | Count rate, c.p.m. per g | Average, c.p.m. per g | Count rate, c.p.m. per g | Average, c.p.m. per g | Count rate, c.p.m. per g | Average, c.p.m. per g |
| Assay on unsaponifiable matter | 863,000,<br>835,000 | 849,000 | 349,000,<br>343,000 | 346,000 | 17,970,<br>16,910 | 17,440 |
| Sodium sulphate method | 820,000,<br>856,000 | 838,000 | 354,500,<br>350,000 | 352,250 | 17,100,<br>18,300 | 17,700 |

* It was ascertained by paper chromatography that the activity originated from vitamin A.

## TABLE V

### MEASUREMENT OF TRITIUM ACTIVITY IN MUSCLE

Weight of muscle = 4·70 g; $s$ = 13,000 c.p.m.

| | Test | | | Control | | |
|---|---|---|---|---|---|---|
| | 906·9<br>801·8 | 832·3<br>731·6 | 274·1<br>173·6 | 586·8<br>484·8 | 435·5<br>334·0 | 364·0<br>263·8 |
| Weight of mixture, mg | 105·1 | 100·7 | 100·5 | 102·0 | 101·5 | 100·2 |
| Weight of tissue corresponding to weight of mixture, mg | 21·0 | 20·1 | 20·1 | 20·4 | 20·3 | 20·0 |
| $c$ Counts | $10^4$ | $10^4$ | $10^4$ | 3430 | 3380 | 3492 |
| $c$ Minutes | 6·80 | 7·09 | 6·90 | 20·00 | 20·00 | 20·00 |
| $c$ c.p.m. | 1470 | 1410 | 1448 | 171 | 169 | 175 |
| $x$ Counts | $2 \times 10^4$ | $2 \times 10^4$ | $2 \times 10^4$ | $2 \times 10^4$ | $2 \times 10^4$ | $2 \times 10^4$ |
| $x$ Minutes | 1·60 | 1·63 | 1·56 | 2·00 | 1·85 | 1·81 |
| $x$ c.p.m. | 12,500 | 12,250 | 12,800 | 10,000 | 10,800 | 11,000 |
| $k = \frac{s}{x - c}$ | 1·23 | 1·25 | 1·19 | 1·37 | 1·27 | 1·25 |
| k × $c$ | 1810 | 1760 | 1720 | 234 | 214 | 219 |
| k × $c$ per 20 mg | 1720 | 1750 | 1710 | 230 | 211 | 219 |
| Average c.p.m. per 20 mg | | 1725 | | | 220 | |

$$\text{Tritium activity in the muscle} = \frac{(1725 - 220) \times 4{\cdot}70}{0{\cdot}02} = 354{,}000 \text{ c.p.m.}$$

The sample is left in darkness until the next day, and then placed in the apparatus in darkness for 10 minutes and then counted ($c$ counts per minute). When this count is completed, 25 $\mu$l of a tritium standard solution ($s$ counts per minute) are added in red light. The cell is shaken for 15 seconds, and then left in darkness for approximately 2 hours. A further count is then carried out as before ($x$ counts per minute). The results are treated as shown in Tables V and VI, and the correction factor is calculated. The count rate for the powder ($c$) is corrected for quenching by multiplying it by the correction factor k (k × $c$), and the result is then converted to a corrected count for exactly 20·0 mg of tissue. The average of several measurements is calculated. The final result—the total activity in the tissue—is calculated by multiplying the difference between the count rates for the sample and control by the weight of the organ in grams and dividing the result by 0·02.

### PROCEDURE FOR BLOOD—

Blood is treated in the same manner as described above for soft tissues.

TABLE VI

MEASUREMENT OF TRITIUM ACTIVITY IN LIVER

Weight of liver = 6·20 g; $s$ = 13,000 c.p.m.

| | Test | | | Control | | |
|---|---|---|---|---|---|---|
| | 904·4<br>803·0 | 837·9<br>731·5 | 375·1<br>274·4 | 586·8<br>486·6 | 437·4<br>335·2 | 367·5<br>264·6 |
| Weight of mixture, mg .. .. | 101·4 | 106·4 | 100·7 | 100·2 | 102·2 | 102·9 |
| Weight of tissue corresponding to weight of mixture, mg .. | 20·3 | 21·3 | 20·1 | 20·0 | 20·4 | 20·6 |
| $c$ Counts .. .. .. | 4018 | 4245 | 4878 | 4358 | 4178 | 4400 |
| Minutes .. .. .. | 20·00 | 20·00 | 20·00 | 20·00 | 20·00 | 20·00 |
| c.p.m. .. .. .. | 201 | 212 | 244 | 218 | 209 | 220 |
| $x$ Counts .. .. .. | $2 \times 10^4$ | $2 \times 10^4$ | $2 \times 10^4$ | $2 \times 10^4$ | $2 \times 10^4$ | $2 \times 10^4$ |
| Minutes .. .. .. | 2·48 | 2·53 | 2·43 | 2·54 | 2·48 | 2·43 |
| c.p.m. .. .. .. | 8060 | 7912 | 8200 | 7880 | 8060 | 8200 |
| $k = \dfrac{s}{x - c}$ .. .. .. | 1·67 | 1·71 | 1·65 | 1·72 | 1·68 | 1·65 |
| k × $c$ .. .. .. .. | 336 | 362 | 405 | 375 | 354 | 364 |
| k × $c$ per 20 mg .. .. .. | 331 | 340 | 400 | 375 | 345 | 354 |
| Average c.p.m. per 20 mg .. | | 357* | | | 358 | |

* Since 357 c.p.m. is within the limits of 358 ± 21 c.p.m., it is assumed that there is no tritium activity in the liver.

### PROCEDURE FOR ORGANS RICH IN CONNECTIVE TISSUE—

The organ is cut into small pieces, placed in a Griffith tube, and then treated with trypsin (approximately 5 mg per g of tissue) at 37° C for 1 hour. The procedure is continued as described for soft tissues. Table VII shows the result of measurements on different tissues.

### PROCEDURE FOR FAECES—

Faeces are weighed and ground in a mortar with 4 parts of anhydrous sodium sulphate. The procedure is continued as described above.

The final result—the activity of faeces over a long period—is obtained from the daily count for 20·0 mg of faeces corrected for daily variation, *e.g.*, of the apparatus, by means of the daily background count when it is above or below the average background count of the period by ±6 per cent. (see Table VIII).

The activity of the total daily faecal output is obtained by multiplying the difference between the daily corrected count and the average background count for the period by the total weight of faeces in grams, and dividing the result by 0·02.

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TABLE VII

DETERMINATION OF TRITIUM ACTIVITY, AS TRITIUM-LABELLED VITAMIN A, IN VARIOUS TISSUES

| Tissue | Weight of tissue, mg | Count rate, c.p.m. | k | c.p.m. × k | $\frac{\text{c.p.m.} \times \text{k}}{\text{per 20 mg}} = C_k$ | $C_k - C_b$ | Weight of organ, g | Total c.p.m. |
|---|---|---|---|---|---|---|---|---|
| *Rat* 2 (*test*)— | | | | | | | | |
| Liver .. .. .. | 20·4 | 209 | 1·57 | 328 | 322 | 129 | 6·7 | $\frac{129 \times 6{\cdot}7}{0{\cdot}02} = 43{,}200$ |
| Adrenal gland.. .. | 20·9 | 244 | 1·69 | 412 | 394 | 0 | 0·1 | 0 |
| Lung .. .. .. | 20·7 | 77 | 2·63 | 202 | 196 | 0 | 1·4 | 0 |
| Kidney .. .. | 20·8 | 113 | 1·67 | 188 | 181 | 0 | 1·4 | 0 |
| Muscle .. .. .. | 21·1 | 107 | 1·31 | 141 | 134 | 0 | 8·6 | 0 |
| Blood .. .. .. | 19·4 | 56 | 3·69 | 206 | 213 | 21 | 1·0 | $\frac{21 \times 1{\cdot}0}{0{\cdot}02} = 1050$ (per g) |
| *Rat* 3 (*control*)— | | | | | | | | |
| Liver .. .. .. | 20·4 | 123 | 1·60 | 196 | 193* | | | |
| Adrenal gland.. .. | 20·0 | 237 | 1·65 | 392 | 392* | | | |
| Lung .. .. .. | 19·3 | 78 | 2·69 | 207 | 215* | | | |
| Kidney .. .. | 20·2 | 116 | 1·56 | 181 | 180* | | | |
| Muscle .. .. .. | 20·7 | 114 | 1·21 | 138 | 134* | | | |
| Blood .. .. .. | 20·6 | 54 | 3·66 | 198 | 192* | | | |

* These are the values of $C_b$.

## TABLE VIII

MEASUREMENT OF TRITIUM-ACTIVITY IN FAECES (AN EXTRACT FROM A RECORD BOOK FOR 5 DAYS OF A PERIOD OF 16 DAYS)

| Date | Weight of faeces, mg | Count rate, c.p.m. | k | c.p.m. × k | c.p.m. × k per 20 mg | f* | $I = \frac{\text{c.p.m.} \times \text{k}}{\text{per 20 mg}} \times \text{f*}$ | I − g′ | Weight of faeces, g | Total c.p.m. |
|---|---|---|---|---|---|---|---|---|---|---|
| *Rat* 2 (*test*)— | | | | | | | | | | |
| 8/11 | 20·3 | 394 | 5·32 | 2090 | 2060 | — | 2060 | 1295 | 0·13 | $\frac{1295 \times 0·13}{0·02} = 8400$ |
| 9/11 | 20·0 | 294 | 4·27 | 1255 | 1255 | — | 1255 | 490 | 0·58 | $\frac{490 \times 0·58}{0·02} = 14,200$ |
| 10/11 | 20·3 | 187 | 3·84 | 725 | 712 | — | 712 | 0 | 0·55 | 0 |
| 11/11 | 20·6 | 244 | 4·40 | 1070 | 1040 | — | 832 | 67 | 0·70 | $\frac{67 \times 0·70}{0·02} = 2345$ |
| 12/11 | 20·4 | 216 | 3·80 | 821 | 805 | — | 805 | 0 | 1·00 | 0 |
| | | | | | | | $g' = 765 \pm 46$ | | | |
| *Rat* 3 (*control*)— | | | | | | | | | | |
| 8/11 | 19·8 | 204 | 4·29 | 875 | 884 | 1·0 | | | | |
| 9/11 | 21·3 | 210 | 4·55 | 955 | 896 | 1·0 | | | | |
| 10/11 | 20·3 | 146 | 6·66 | 971 | 958 | 1·0 | | | | |
| 11/11 | 20·3 | 200 | 5·88 | 1176 | 1158 | $\frac{905}{1158} = 0·8$ | | | | |
| 12/11 | 19·9 | 208 | 3·93 | 906 | 911 | 1·0 | | | | |
| | | | | | $g'' = 905 \pm 54$ | | | | | |

* f = correction factor for daily variation. g′ = average for the entire period *minus* first 5 days. g″ = average for the entire period.

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# The Determination of Strontium in Tap-water by X-ray Fluorescence Spectrometry

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A method is described for determining natural strontium in tap-water by X-ray fluorescence analysis as a preliminary step to the determination of the strontium-90 content. Calcium and strontium are co-precipitated as oxalates and converted to nitrates. A known amount of rubidium is added as internal standard and the ratio of the intensities of the strontium K$\alpha$ and rubidium K$\alpha$ lines measured. The rubidium internal standard compensates for the absorption effect of the varying concentration of calcium present in tap-waters, and a linear relationship exists between the intensity ratio of the strontium and rubidium K$\alpha$ radiation and the weight ratio of strontium to rubidium in the prepared sample.

IN determining the radioactive-strontium content of tap-water it is necessary to know the amount of natural strontium present in the sample as this is often comparable with that of the added carrier. The distribution of this element in tap-water usually varies between 0·02 and 0·4 mg per litre, according to locality; examples are shown in Table I. The calcium content is considerably higher and, expressed as a calcium - strontium ratio, varies from 200 to 600. Until recently the total strontium was determined by a flame spectrophotometric method usually involving preliminary removal of calcium. Experiments in which an X-ray fluorescence technique was used suggested that a simpler method could be employed, with rubidium as internal standard, which compensated for the absorption effect due to the variable concentration of calcium in water samples.

## EXPERIMENTAL

### APPARATUS—

The X-ray fluorescence instrument used was the Research and Control Instruments Ltd. total-vacuum spectrometer. In this equipment the specimen is normally carried in an aluminium cup that has a Mylar-film base through which the primary beam of X-rays irradiates the material. In the proposed method of sample preparation drops of solution are evaporated on to the surface of the Mylar exposed directly to radiation, producing a thin coherent film of residue extending over the whole area irradiated by the primary X-ray beam. This reduces the effect of matrix absorption and increases the ratio of peak to background intensity. The film of residue should be centrally placed in order to obtain good reproducibility. Under these conditions, 0·5 $\mu$g of strontium on the film can be measured.

Operating conditions for the instrument are a tungsten target, lithium fluoride crystal analyser and scintillation counter detector. The target potential is 40 kV and the beam current is 20 mA. Both sample and spectrometer chambers are evacuated. Strontium K$\alpha$ and rubidium K$\alpha$ radiations have wavelengths of 0·875 Å and 0·926 Å, respectively, the theoretical goniometer settings being 25·09° and 26·58°; these are determined experimentally at the beginning of the analysis of a batch of samples. The background is level, and only one background measurement is made at 25·6°. There are no interfering lines.

### PROCEDURE—

An aliquot of the tap-water sample, preferably containing about 20 to 30 $\mu$g of strontium, is concentrated to 100 ml. Twenty millilitres of 8 per cent. w/v oxalic acid are added and the solution is adjusted to pH 4 by adding ammonia solution. After the solution has been set aside at 60° C for 1 hour, the precipitate of strontium and calcium oxalates is collected with the aid of a filter stick, and the paper is ignited in a silica dish at 500° C. The resulting carbonates are dissolved in 30 per cent. v/v nitric acid, and the solution is evaporated to dryness.

The residue of nitrates is dissolved in 1 ml of rubidium chloride solution containing 200 $\mu$g of rubidium, and two drops of 1/60,000 solution of an anionic wetting agent are added to reduce surface tension. Two drops of the prepared sample solution are then placed on the Mylar-film base of an upturned specimen container, care being taken to ensure that the drops are centrally placed and do not spread to the edge of the film. The cups are placed, still upside down, in a vacuum desiccator. The round spot of liquid immediately freezes and then slowly evaporates over a period of 20 minutes. All the sample material should be within the area irradiated by X-rays, and this can be achieved by the control of the amount of wetting agent added and the number of drops of sample solution taken. After they have been dried, the specimen holders are placed in the instrument, which is then evacuated; this keeps the residue dry and reduces the background radiation due to air scatter.

Standards are prepared by adding suitable volumes of standard strontium nitrate solution to 1 ml of rubidium chloride solution containing 200 $\mu$g of rubidium, evaporating to 1 ml, and treating as described in the preceding paragraph.

TABLE I

DISTRIBUTION OF STRONTIUM IN TAP-WATER

| Locality | Strontium content, mg per litre | Calcium content, mg per litre | Ratio of calcium to strontium |
|---|---|---|---|
| Belfast .. .. .. .. | 0·005 | 1·1 | 220 |
| Loch Lomond (surface water) .. | 0·032 | 4·8 | 150 |
| Exeter .. .. .. .. | 0·049 | 25·3 | 516 |
| Norwich .. .. .. .. | 0·420 | 97·4 | 232 |

TABLE II

DETERMINATION OF STRONTIUM IN WATERS

| Strontium added, $\mu$g | Strontium found, $\mu$g | Ratio of calcium to strontium |
|---|---|---|
| *Simulated tap-water—* | | |
| 5 | 4·5 | 50 |
| 20 | 18·3 | 50 |
| 30 | 32·0 | 50 |
| 63 | 64·0 | 260 |
| 83 | 81·2 | 400 |
| *Tap-water with added strontium—* | | |
| 20 | 21·4 | 580 |
| 5 | 4·6 | 500 |

TABLE III

DETERMINATION OF LOW LEVELS OF STRONTIUM

| Strontium content, $\mu$g per ml | Rubidium content, $\mu$g per ml | Background rate, pulses per second | Net Rb $K\alpha$ rate (A), pulses per second | Net Sr $K\alpha$ rate (B), pulses per second | Ratio $\frac{B}{A}$ | Strontium found (calculated by comparison with standard), $\mu$g |
|---|---|---|---|---|---|---|
| 1·7 | 200 | 42·4 | 1850 | 15·4 | 0·0083 | 1·8 |
| 0·8 | 200 | 38·1 | 1948 | 6·76 | 0·0035 | 0·8 |
| 0·4* | 200 | 35·8 | 1638 | 2·05 | 0·0013 | 0·3 |
| 83·0 | 200 | 65·3 | 321·3 | 119·3 | 0·371 | — |

* The coefficient of variation, calculated from the number of pulses counted, was 15 per cent.

The intensities of the background and of the $K\alpha$ radiations of strontium and rubidium are measured by counting over duplicate periods of 100 seconds at each angular position. Although the calibration curve is linear, it is the practice in this laboratory to include one standard with each daily batch of tap-water samples, the strontium concentrations being calculated from this standard. This compensates for any slight changes in instrumental sensitivity at the goniometer settings used, and provides a check on the functioning of the scaling and timing electronics. It has been found experimentally that a change of 1 per cent. in the X-ray tube potential causes a change of 0·8 per cent. in peak intensity ratio. During several months of intermittent work the calibration constants have not significantly altered.

## RESULTS

The net peak ratio of strontium K$\alpha$ to rubidium K$\alpha$ has been determined for a number of solutions of different strontium and calcium concentrations. A linear relation has been found to exist between the peak ratio and strontium concentrations from 50 to 1000 $\mu$g per ml with calcium - strontium ratios ranging from 0 to 400. The relation for this instrument can be expressed as—

$$\frac{\text{Sr K}\alpha}{\text{Rb K}\alpha} = 0{\cdot}96 \times \frac{\text{Concentration of strontium}}{\text{Concentration of rubidium}}$$

A number of simulated tap-waters containing known amounts of strontium and also natural waters to which strontium had been added have been analysed; some of these results are shown in Table II. It is possible to determine 5 $\mu$g of strontium in a sample with reasonable precision. In such instances, the 1 ml of solution, obtained after adding the rubidium chloride, is concentrated to about 0·3 ml before the spot on the film is prepared. The ultimate sensitivity of the method depends on the ratio of peak to background and, in order to determine the lower limits of measurement, standards were prepared containing 1·6, 0·8 and 0·4 $\mu$g of strontium, each with a calcium - strontium ratio of 400. These were concentrated to about 0·2 ml and 4 drops were taken; thus almost all the strontium was placed on the film. In Table III are shown the count rates and ratios obtained over extended counting periods of 800 seconds. The peak ratios are compared with that for the high level standard to determine the amount of strontium. The results indicate that the determination of 0·5 $\mu$g is feasible by this method. In these conditions a useful reduction of background can be achieved by lining the inside of the specimen holder with lead to reduce the scatter of white radiation from the aluminium walls.

The precision depends on counting statistics and the reproducibility of the sample preparation technique. With counting periods of 100 seconds, a coefficient of variation of better than 5 per cent. at the 40 $\mu$g level was obtained, which improved to 1·8 per cent. at 275 $\mu$g of strontium.

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# The Micro-determination of Hydrazine Salts and Certain Derivatives

By M. Z. BARAKAT AND MONIER SHAKER

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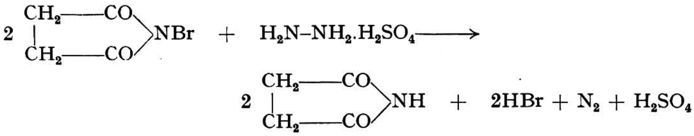
In the presence of dilute sulphuric acid, *N*-bromosuccinimide readily and quantitatively oxidises aqueous solutions of hydrazine salts and certain derivatives. The reaction takes place at room temperature, and *N*-bromosuccinimide is irreversibly reduced to succinimide. The mechanism of the reaction is discussed. The determination of hydrazine sulphate, phenylhydrazine hydrochloride and phenylhydrazine-*p*-sulphonic acid by titration with standard *N*-bromosuccinimide solution is described; the experimental error does not exceed ±2 per cent. Results are reported for comparative analysis of hydrazine sulphate and phenylhydrazine hydrochloride by the proposed method and by the generally accepted potassium iodate method.

VARIOUS titrimetric,[1,2] colorimetric[3] and potentiometric[4] methods have been described for determining hydrazine salts, and the oxidation of hydrazine[5,6,7] has been widely used as the basis of techniques for its measurement.

The titrimetric methods suffer from certain defects, *e.g.*, oxidation of hydrazine in alkaline solution with potassium permanganate[8] gives inaccurate results in the absence of barium and telluric acid. The end-point in the chloramine B method[9] is indistinct; in the presence of an excess of potassium bromide the end-point is sharp and accurate results can be obtained. There is some doubt about the reliability of the potassium iodate method.[5,10]

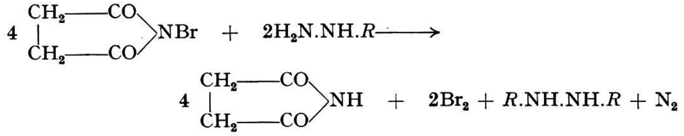
The investigation described here deals with the micro-determination of hydrazine salts, *e.g.*, hydrazine sulphate and certain derivatives such as phenylhydrazine hydrochloride and phenylhydrazine-*p*-sulphonic acid, by the use of *N*-bromosuccinimide. The method is based on the fact that *N*-bromosuccinimide[11] readily and quantitatively oxidises an aqueous solution of hydrazine sulphate and is itself reduced to succinimide, with the evolution of nitrogen and the formation of hydrogen bromide, as represented by the equation—



$$2\left.\begin{array}{l}\mathrm{CH_2{-}{-}CO}\\ |\\ \mathrm{CH_2{-}{-}CO}\end{array}\right\rangle\mathrm{NBr} + \mathrm{H_2N{-}NH_2.H_2SO_4} \longrightarrow 2\left.\begin{array}{l}\mathrm{CH_2{-}{-}CO}\\ |\\ \mathrm{CH_2{-}{-}CO}\end{array}\right\rangle\mathrm{NH} + 2\mathrm{HBr} + \mathrm{N_2} + \mathrm{H_2SO_4}$$

The reaction proceeds quantitatively in aqueous solution at room temperature in the ratio of two molecules of *N*-bromosuccinimide to one molecule of hydrazine sulphate. Effervescence owing to evolution of nitrogen is perceptible. Succinimide has been isolated from the reaction mixture and identified by melting-point and mixed melting-point determinations. The presence of hydrogen bromide and sulphuric acid in the reaction mixture has been confirmed.

Similarly, *N*-bromosuccinimide is quantitatively and rapidly reduced to succinimide by phenylhydrazine[12] or phenylhydrazine-*p*-sulphonic acid at room temperature, in accordance with the equation—



$$4\left.\begin{array}{l}\mathrm{CH_2{-}{-}CO}\\ |\\ \mathrm{CH_2{-}{-}CO}\end{array}\right\rangle\mathrm{NBr} + 2\mathrm{H_2N.NH}.R \longrightarrow 4\left.\begin{array}{l}\mathrm{CH_2{-}{-}CO}\\ |\\ \mathrm{CH_2{-}{-}CO}\end{array}\right\rangle\mathrm{NH} + 2\mathrm{Br_2} + R.\mathrm{NH.NH}.R + \mathrm{N_2}$$

where $R$ can be $C_6H_5-$ or $HO_3S.C_6H_4-$.

With hydrazine derivatives, *e.g.*, phenylhydrazine, bromine is formed, not hydrogen bromide as is formed with hydrazine sulphate.

*N*-Bromosuccinimide is a highly selective oxidising agent[13,14] and can decolorise methyl red in aqueous acid medium, but it oxidises hydrazine salts or derivatives preferentially. Until all the hydrazine salt or derivative present has been oxidised, the red colour of the indicator does not disappear. The slightest excess of *N*-bromosuccinimide added after all the hydrazine salt or derivative has been oxidised decolorises the methyl red. Methyl red has been used previously as an indicator in titrations involving the use of bromine[15] and *N*-bromosuccinimide.[16]

Comparative determinations of hydrazine salts have been carried out by the proposed method and the most generally accepted potassium iodate method. *N*-Bromosuccinimide is analogous to potassium iodate; it acts as an oxidising agent, but is better than iodate in that it can be used for determining extremely small amounts of hydrazine salts or derivatives. The potassium iodate method cannot be used for determining amounts of hydrazine salts less than 30 mg, whereas the proposed method will determine amounts as low as 500 µg.

## EXPERIMENTAL

### REACTION BETWEEN *N*-BROMOSUCCINIMIDE AND HYDRAZINE SULPHATE—

A 1·3013-g portion of hydrazine sulphate (0·01 mole) was dissolved in 20 ml of distilled water, and 3·56 g of *N*-bromosuccinimide (0·02 mole) were dissolved in 200 ml of hot distilled water. When the *N*-bromosuccinimide solution was cool, it was added gradually, with shaking, to the cold hydrazine sulphate solution. During the addition a strong effervescence was observed owing to evolution of nitrogen. When the bubbles of gas had ceased, the reaction mixture was a colourless solution.

The presence of hydrobromic acid was established by treating 10 ml of the colourless solution with nitric acid and 10 per cent. silver nitrate solution; a yellowish white precipitate of silver bromide was deposited.

The presence of sulphuric acid was confirmed by treating 10 ml of the colourless solution with hydrochloric acid and 10 per cent. barium chloride solution; a white precipitate of barium sulphate was observed.

Succinimide was isolated by distilling *in vacuo* the remaining 200 ml of the colourless solution and crystallising the solid residue from benzene. Melting-point and mixed melting-point determinations with an authentic sample showed that the colourless crystals obtained were succinimide.

### VALIDITY OF THE REACTION FOR QUANTITATIVE DETERMINATION—

Before applying the reaction to the determination of hydrazine salts or derivatives in test solutions, we decided to verify quantitatively the reaction between *N*-bromosuccinimide and hydrazine sulphate. An accurately measured volume of solution containing 0·13013 g (1 millimole) of hydrazine sulphate per 100 ml was placed in a 25-ml conical flask, and an equal volume of 10 per cent. v/v sulphuric acid and 2 drops of 0·04 per cent. methyl red indicator solution in 95 per cent. ethanol were added. The mixture was titrated with 0·1 per cent. w/v *N*-bromosuccinimide solution, added dropwise from a microburette, with continuous shaking after each addition. When the red colour of the indicator faded, another drop was added, and the titration was continued until the red colour completely disappeared. A similar series of experiments was carried out with *N*-bromosuccinimide solution containing twice the number of molecules of solute as in the first solution. It was found that the reaction was stoicheiometric in the presence of dilute sulphuric acid at room temperature; the results were—

| Volume of hydrazine sulphate solution (1 mmole per 100 ml), ml | 10 | 5 | 4 | 3 | 2 | 1 |
|---|---|---|---|---|---|---|
| Titre of *N*-bromosuccinimide (1 mmole per 100 ml), ml .. .. | 20·25 | 10·15 | 8·12 | 6·18 | 4·11 | 2·06 |
| Titre of *N*-bromosuccinimide (2 mmole per 100 ml), ml .. .. | 10·13 | 5·10 | 4·09 | 3·08 | 2·05 | 1·02 |

The equimolecular solutions of hydrazine sulphate and *N*-bromosuccinimide (freshly prepared) were then diluted ten-fold with distilled water in 100-ml calibrated flasks. The diluted solutions were titrated as before and gave reproducible results.

## METHOD

### REAGENTS—

*Methyl red solution,* 0·04 *per cent. w/v in* 95 *per cent. ethanol.*

*Dilute sulphuric acid,* 10 *per cent. v/v.*

*Dilute hydrochloric acid,* 10 *per cent. v/v.*

N-*Bromosuccinimide solution,* 0·1 *per cent. w/v, aqueous*—This solution should be freshly prepared before use.

### PROCEDURE—

To an accurately measured volume, *e.g.*, 5 ml, of the hydrazine sulphate solution in a 50-ml conical flask add an equal volume of dilute sulphuric acid and 5 drops of methyl red indicator solution. Titrate the mixture with 0·1 per cent. w/v *N*-bromosuccinimide solution, added dropwise from a microburette graduated at 0·01-ml intervals, with continuous shaking. The end-point is reached when the last drop of titrant discharges the red colour. Calculate the hydrazine sulphate content of the sample solution from the expression—

**Hydrazine sulphate present, mg or $\mu$g = 130·13 $VC/356$**

where $V$ is the titre of *N*-bromosuccinimide solution in millilitres and $C$ is the concentration of the *N*-bromosuccinimide solution in mg or $\mu$g per ml.

## APPLICATIONS OF THE METHOD

### DETERMINATION OF HYDRAZINE SULPHATE—

A 0·5 per cent. stock solution of hydrazine sulphate was prepared by dissolving 0·5 g of pure hydrazine sulphate, obtained from E. Merck A.G., Darmstadt, Germany, in distilled water, and the volume was adjusted to 100 ml with distilled water in a calibrated flask. Solutions containing 0·4, 0·3, 0·2 and 0·1 per cent. of hydrazine sulphate were prepared by suitable dilutions of portions of this solution. The hydrazine sulphate contents of these solutions were then determined by the proposed method; the results are shown in Table I.

TABLE I

RECOVERY OF HYDRAZINE SULPHATE BY THE PROPOSED METHOD

| Concentration of hydrazine sulphate solution, % w/v | Volume of solution used, ml | Hydrazine sulphate content, mg | Titre of 0·1 per cent. w/v *N*-bromosuccinimide solution, ml | Hydrazine sulphate found, mg | Error, % |
|---|---|---|---|---|---|
| 0·5 | 1 | 5 | 13·55 | 4·95 | 1·00 |
| | 2 | 10 | 27·00 | 9·87 | 1·30 |
| | 3 | 15 | 40·50 | 14·80 | 1·33 |
| 0·4 | 1 | 4 | 11·05 | 4·04 | 1·00 |
| | 2 | 8 | 22·25 | 8·13 | 1·63 |
| 0·3 | 1 | 3 | 8·30 | 3·03 | 1·00 |
| | 2 | 6 | 16·55 | 6·05 | 0·83 |
| 0·2 | 1 | 2 | 5·55 | 2·03 | 1·50 |
| | 2 | 4 | 11·05 | 4·04 | 1·00 |
| 0·1 | 1 | 1 | 2·70 | 0·99 | 1·00 |
| | 2 | 2 | 5·47 | 2·00 | — |
| | 3 | 3 | 8·34 | 3·05 | 1·66 |
| | 4 | 4 | 11·05 | 4·04 | 1·00 |
| | 5 | 5 | 13·80 | 5·04 | 0·80 |
| | 6 | 6 | 16·25 | 5·94 | 1·00 |
| | 7 | 7 | 18·95 | 6·93 | 1·00 |
| | 8 | 8 | 21·70 | 7·93 | 0·88 |
| | 9 | 9 | 24·45 | 8·94 | 0·75 |
| | 10 | 10 | 27·10 | 9·91 | 0·90 |

The 0·1 per cent. hydrazine sulphate solution was then diluted ten-fold with distilled water in a 100-ml calibrated flask and the diluted solution (0·01 per cent.) was titrated with 0·05 per cent. w/v *N*-bromosuccinimide solution; the results are shown in Table II.

TABLE II

MICRO-DETERMINATION OF HYDRAZINE SULPHATE BY THE PROPOSED METHOD

| Volume of hydrazine sulphate solution used, ml | Hydrazine sulphate present, $\mu$g | Titre of 0·05 per cent. *N*-bromosuccinimide solution, ml | Hydrazine sulphate found, $\mu$g | Error, % |
|---|---|---|---|---|
| 10 | 1000 | 5·45 | 996·26 | 0·37 |
| 9 | 900 | 4·90 | 895·72 | 0·47 |
| 8 | 800 | 4·35 | 795·18 | 0·60 |
| 7 | 700 | 3·80 | 694·64 | 0·77 |
| 6 | 600 | 3·30 | 603·24 | 0·52 |
| 5 | 500 | 2·70 | 493·56 | 1·29 |

The proposed method was compared with the iodate method by titrating a 1 per cent. hydrazine sulphate solution with 0·5 per cent. *N*-bromosuccinimide solution and with 0·025 M potassium iodate; the results are shown in Table III.

TABLE III

COMPARISON OF RESULTS BY PROPOSED AND IODATE METHODS

1 ml of 0·5 per cent. *N*-bromosuccinimide ≡ 1·8275 mg of hydrazine sulphate
1 ml of 0·025 M potassium iodate ≡ 3·253 mg of hydrazine sulphate

| | | With 0·5 per cent. *N*-bromosuccinimide as titrant | | | With 0·025 M potassium iodate as titrant | | |
|---|---|---|---|---|---|---|---|
| Volume of 1 per cent. w/v hydrazine sulphate solution taken, ml | Hydrazine sulphate content, mg | Titre, ml | Hydrazine sulphate found, mg | Error, % | Titre, ml | Hydrazine sulphate found mg | Error, % |
| 10 | 100 | 55·40 | 101·24 | 1·24 | 30·55 | 99·38 | 0·62 |
| 9 | 90 | 50·10 | 91·56 | 1·73 | 27·60 | 89·78 | 0·24 |
| 8 | 80 | 44·10 | 80·59 | 0·74 | 24·50 | 79·70 | 0·43 |
| 7 | 70 | 38·50 | 70·36 | 0·51 | 21·55 | 70·10 | 0·14 |
| 6 | 60 | 33·10 | 60·49 | 0·82 | 18·40 | 59·86 | 0·23 |
| 5 | 50 | 27·60 | 50·44 | 0·88 | 15·20 | 49·45 | 1·10 |
| 4 | 40 | 22·10 | 40·39 | 0·98 | 12·45 | 40·50 | 1·25 |
| 3 | 30 | 16·60 | 30·34 | 1·13 | 9·35 | 30·42 | 1·40 |
| 2 | 20 | 10·90 | 19·92 | 0·40 | 6·30 | 20·49 | 2·45 |
| 1 | 10 | 5·50 | 10·05 | 0·50 | 2·95 | 9·60 | 4·00 |
| 0·5 | 5 | 2·76 | 5·04 | 0·80 | 1·70 | 5·53 | 10·60 |

### DETERMINATION OF PHENYLHYDRAZINE HYDROCHLORIDE—

Before the proposed method was applied to determining phenylhydrazine hydrochloride, it was decided to verify quantitatively the reaction between *N*-bromosuccinimide and phenylhydrazine hydrochloride. It was assumed that one molecule of phenylhydrazine hydrochloride was oxidised by two molecules of *N*-bromosuccinimide. Accordingly, a solution containing 0·1445 g (1 millimole) of phenyhydrazine hydrochloride per 100 ml was titrated with solutions containing 0·178 and 0·356 g (1 and 2 millimoles, respectively) of *N*-bromosuccinimide. It was found that the reaction between phenylhydrazine hydrochloride and *N*-bromosuccinimide was quantitative in the molecular concentrations expected; the results were—

| | | | | | | |
|---|---|---|---|---|---|---|
| Volume of phenylhydrazine hydrochloride (1 mmole per 100 ml), ml | 10 | 5 | 4 | 3 | 2 | 1 |
| Titre of *N*-bromosuccinimide solution (1 mmole per 100 ml), ml .. | 19·95 | 9·95 | 8·05 | 6·05 | 3·95 | 2·05 |
| Titre of *N*-bromosuccinimide solution (2 mmoles per 100 ml), ml .. | 10 | 5 | 4 | 3 | 2 | 1 |

A series of experiments was carried out in which a 0·1 per cent. w/v solution of phenylhydrazine hydrochloride was titrated by the proposed method with 0·1 per cent.*N*-bromosuccinimide; the results are shown in Table IV.

TABLE IV

RECOVERY OF PHENYLHYDRAZINE HYDROCHLORIDE BY PROPOSED AND IODATE METHODS

1 ml of 0·1 per cent. *N*-bromosuccinimide ≡ 0·4059 mg of phenylhydrazine hydrochloride
1 ml of 0·025 M potassium iodate ≡ 3·612 mg of phenylhydrazine hydrochloride

| | With 0·1 per cent. *N*-bromosuccinimide as titrant | | | With 0·025 M potassium iodate as titrant | | |
|---|---|---|---|---|---|---|
| Phenylhydrazine hydrochloride present, mg | Titre, ml | Phenylhydrazine hydrochloride found, mg | Error, % | Titre, ml | Phenylhydrazine hydrochloride found, mg | Error, % |
| 10 | 24·45 | 9·92 | 0·80 | 2·45 | 8·85 | 11·50 |
| 9 | 22·00 | 8·93 | 0·78 | 2·15 | 7·77 | 13·67 |
| 8 | 19·65 | 7·98 | 0·25 | 1·90 | 6·86 | 14·25 |
| 7 | 17·03 | 6·91 | 1·29 | 1·70 | 6·14 | 12·29 |
| 6 | 14·60 | 5·93 | 1·17 | 1·55 | 5·60 | 6·67 |
| 5 | 12·13 | 4·92 | 1·60 | 1·25 | 4·52 | 9·60 |

DETERMINATION OF PHENYLHYDRAZINE-*p*-SULPHONIC ACID—

It was ascertained quantitatively that one molecule of phenylhydrazine-*p*-sulphonic acid was oxidised by two moles of *N*-bromosuccinimide; the results obtained were—

| | | | | | | |
|---|---|---|---|---|---|---|
| Volume of phenylhydrazine-*p*-sulphonic acid solution (1 mmole per 100 ml), ml | 10 | 5 | 4 | 3 | 2 | 1 |
| Titre of *N*-bromosuccinimide solution (1 mmole per 100 ml), ml | 20·08 | 10·05 | 8·04 | 6·02 | 4·05 | 2·05 |

A 0·1 per cent. solution of phenylhydrazine-*p*-sulphonic acid was titrated by the proposed method with 0·1 per cent. *N*-bromosuccinimide solution; the results are shown in Table V.

TABLE V

DETERMINATION OF PHENYLHYDRAZINE-*p*-SULPHONIC ACID BY THE PROPOSED METHOD

1 ml of 0·1 per cent. *N*-bromosuccinimide ≡ 0·5284 mg of phenylhydrazine-*p*-sulphonic acid

| Volume of phenylhydrazine-*p*-sulphonic acid solution, ml | Phenylhydrazine-*p*-sulphonic acid present, mg | Titre of 0·1 per cent. w/v *N*-bromosuccinimide, ml | Phenylhydrazine-*p*-sulphonic acid found, mg | Error, % |
|---|---|---|---|---|
| 10 | 10 | 18·85 | 9·96 | 0·40 |
| 9 | 9 | 17·00 | 8·98 | 0·22 |
| 8 | 8 | 15·20 | 8·03 | 0·38 |
| 7 | 7 | 13·15 | 6·95 | 0·71 |
| 6 | 6 | 11·35 | 6·00 | — |
| 5 | 5 | 9·40 | 4·97 | 0·60 |
| 4 | 4 | 7·65 | 4·04 | 1·00 |
| 3 | 3 | 5·70 | 3·01 | 0·33 |
| 2 | 2 | 3·75 | 1·98 | 1·00 |
| 1 | 1 | 1·86 | 0·98 | 2·00 |

## DISCUSSION OF RESULTS

The accuracy of the generally accepted potassium iodate method depends mainly on the concentration of hydrazine sulphate in the sample solution.

Results show that the experimental error appears to increase as the concentration of hydrazine sulphate in the sample solution decreases. Moreover, the potassium iodate method cannot be used for determining amounts of hydrazine sulphate less than 30 mg (see Table III); further, it is difficult to detect the end-point when the iodate method is applied to low concentrations of hydrazine salts.

The results in Tables III and IV show that the proposed method is superior to the potassium iodate method in accuracy and sensitivity.

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# SHORT PAPERS

## Rapid Determination of Iodine-131 in Milk

BY A. L. BONI

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EXISTING methods for determining the concentrations of iodine-131 in milk involve either complex chemical separations or lengthy gamma-spectrometric analyses of large-volume samples.

Direct absorption of iodine-131 on anion-exchange resin and subsequent gamma-spectrometric analysis of the resin was found to be quantitative as well as time and labour saving in the routine determination of this radionuclide in milk. The method requires less than 2 hours per 1-gallon sample and a counting time of only 10 minutes to achieve a minimum detection limit of $3{\cdot}5 \times 10^{-15}$ curie per ml. The anion-exchange concentration step completely eliminates the interference of all other fission-product contaminants found in milk except ruthenium-103, ruthenium-106 and zirconium - niobium-95. Routine laboratory experience with the method during a period of $1\frac{1}{2}$ years showed an average recovery of 98 per cent.

### METHOD

PREPARATION OF ION-EXCHANGE COLUMN—

Loosely place a small plug of glass-wool in the bottom of a $1\frac{1}{2}$-inch × 4-inch Pyrex-glass column (column with cap is illustrated in Fig. 1). Fill the column with 90-ml of a water slurry of previously washed Dowex 1 × 8 (20 to 50 mesh) anion-exchange resin. After any water present has drained to the level of the resin, place a thin film of glass-wool over the bed. Fit the glass cap lubricated with stopcock grease on to the top of the column. With the bottom stopcock closed, carefully add water through the stopcock in the column cap until the entire space above the resin, including the stopcock, is full. Close the top stopcock. Connect an inverted 1-gallon polythene bottle, with the bottom removed, to the top of the column, and then connect a 4-litre filtering flask to the bottom of the column by means of a glass tube (see Fig. 2).

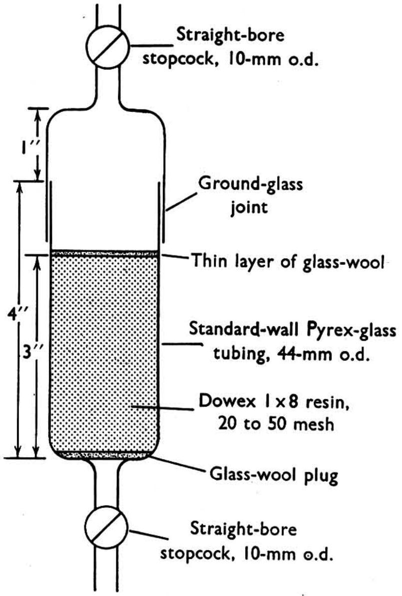

Fig. 1. Design of ion-exchange column

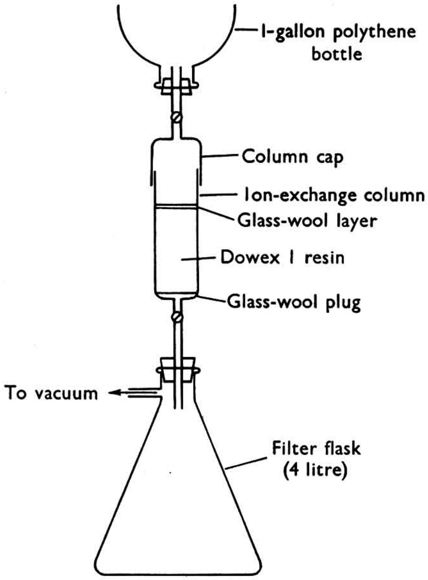

Fig. 2. Design of apparatus

PROCEDURE—

Put a measured ½ gallon of well-mixed fresh milk in the polythene container, and open the top stopcock. Evacuate the filter flask, and then open the bottom stopcock to obtain a flow rate of 30 to 40 ml per minute. Experience has shown that a vacuum is needed only with non-homogenised milk that is several days old or milk containing large amounts of cream. Before the first ½ gallon has drained completely from the polythene container, add a second measured ½ gallon (total volume of milk, 3785 ml). Before the second ½ gallon has drained completely from the polythene container, rinse the container with distilled water, and let the entire liquid volume drain to the level of the resin. (If for any reason the flow through the column has to be stopped during the analysis, always close the bottom stopcock, and never allow the milk to stand over the resin longer than is absolutely necessary, in order to avoid clotting.) Remove the cap from the column, and pass 30 to 40 ml of distilled water through the resin bed. Discard all effluents.

Disconnect the column from the system, invert, and with a stream of air force the resin from the column into a 500-ml 3-inch diameter polythene bottle from which the narrow mouth has been removed. With distilled water, remove any traces of resin remaining in the column, and add the resin and water to the contents of the polythene bottle. Make a slurry of the resin in the bottle with more distilled water, if necessary, in order to mix the resin evenly. Seal the top of the bottle.

COUNTING AND CALCULATIONS—

Place the resin container on top of a thallium-activated 3-inch × 3-inch sodium iodide crystal coupled to a multi-channel gamma spectrometer, and take a 10-minute count. The total count per minute under the iodine-131 0·36-MeV peak must be corrected for background and multiplied by the appropriate conversion factor ($6 \times 10^{-12}$ curie per count per minute). The conversion factor is obtained by counting a known iodine-131 standard in the same geometry as the samples. This figure, divided by the sample volume in millilitres and corrected for recovery by the procedure (98 per cent.), will give the concentration of iodine-131 in curies per millilitre.

$$\text{Concentration of } {}^{131}\text{I},\ 10^{-15}\ \text{C per ml} = \text{Conversion factor} \times \text{total count in 10 minutes} \times \frac{1}{3785} \times \frac{1}{0{\cdot}98}.$$

Milk has a high decontamination factor for ruthenium-103, ruthenium-106 and zirconium - niobium-95, but, if detectable amounts of these radionuclides are nevertheless present, the total count under the iodine-131 0·36-MeV peak and the total counts under the peak of these other radionuclides are tabulated from the gamma-ray spectrum. These results, together with the counting time, sample volume and iodine decay factor, are programmed into a computer and solved for the iodine-131 content in curies per millilitre of milk by Compton scatter correction.

## DISCUSSION OF THE METHOD

ION EXCHANGE—

Iodine-131, with other anions in milk, is directly absorbed on Dowex 1 × 8 anion-exchange resin.[1] Although some anion-complex formation of ruthenium-103, ruthenium-106 and zirconium - niobium-95 results in their slight concentration, if present, on the resin, the ion-exchange concentration step quantitatively separates iodine-131 from all other principal radionuclides found in milk. Analysis of the resin effluent substantiated that the iodine-131 in milk is free and not bound. Other than iodine-131, the principal radionuclides in milk, $^{89}$Sr, $^{90}$Sr, $^{137}$Cs, $^{140}$Ba and $^{40}$K, are cations. Since they do not form anion complexes in the milk, they are not concentrated by the anion resin. The various radionuclides reported in milk and their percentage absorption on the anion resin are: $^{131}$I, >99 per cent.; $^{137}$Cs, $^{89}$Sr, $^{90}$Sr, Ba - $^{140}$La, Ce - $^{144}$Pr and $^{40}$K, <1 per cent.; $^{103}$Ru and $^{106}$Ru, 16 per cent.; Zr - $^{95}$Nb, 12 per cent.

The concentration and essentially complete separation of iodine-131 from these other contaminating radionuclides results in a rapid and accurate determination of low levels of the radionuclide in milk.

A column operation is used instead of batchwise mixing, since a much more favourable separation is achieved. Fresh milk and a fast rate of flow are essential, because milk will clog the resin bed if it is allowed to turn sour. Preservatives such as formaldehyde should be avoided, since they complex the iodine and inhibit its absorption on the resin. A flow rate of 30 to 40 ml per minute (*i.e.*, 1 gallon in 1·5 hours) is sufficient. A faster flow rate will cause incomplete absorption of the iodine-131, because insufficient time would be allowed for equilibrium to be reached between the radionuclide and the resin.

The capacity of the column for various amounts of milk can be increased or decreased by changing the length of the resin column proportionately.

COUNTING AND CALCULATIONS—

Because a constant geometry is needed in order to calculate the iodine content from sample to sample on a relative basis, the resin is transferred with water to a 500-ml polythene bottle. The volume of water used to transfer the resin is not critical, since the iodine is not extracted into the water and the resin settles to the bottom of the bottle.

One of the generally accepted methods used for determining quantitatively the concentration of particular isotopes from a gamma-ray spectrum is to subtract the Compton scatter contributions due to other radionuclides from the total count under the photo-peak of the radionuclide of interest.[2] Quantitative analysis by gamma-ray spectrometry becomes more difficult as the number of radionuclides in the sample increases.

Since iodine-131 is completely separated from all other radionuclides except ruthenium-103, ruthenium-106 and zirconium - niobium-95 (which are rarely observed in milk), the counting rate under its main photo-peak (0·36 MeV) is not affected by other radionuclides. The direct application of a conversion factor to this counting rate gives the total amount of iodine-131 present in the volume of milk sampled.

RECOVERY AND DETECTION LIMIT—

The method has yielded an average recovery of 98 per cent. with a standard of activity of 120 × $10^{-15}$ C per ml over a period of 1½ years; the precision was 17 per cent. (90 per cent. confidence limits). Statistics on the recovery procedure are shown in Fig. 3. The detection limit of the method is limited by the volume of milk (3785 ml), the efficiency and resolution of the sodium iodide crystal detector and the background count.

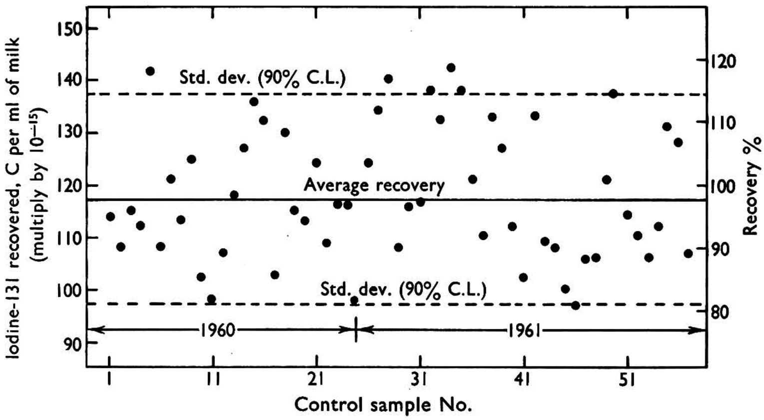

Fig. 3. Recovery statistics (iodine-131 added = 120 × $10^{-15}$ C per ml of milk)

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# The Determination of Lead in Carbon and Low-alloy Steel according to British Standard 1121: Part 41: 1960

By A. CLAASSEN and L. BASTINGS

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In the determination of lead in carbon and low-alloy steel as described in the British Standard,[1,2] lead is first precipitated as lead sulphide from a solution of the steel in hydrochloric acid, after the excess of acid has been removed by evaporation "almost to dryness." In this procedure, therefore, no provision is made for a more accurate adjustment of pH before hydrogen sulphide is passed through the solution. The American A.S.T.M. standard,[3] describing practically the same method, specifies the adjustment of pH to 2·0 to 2·5, and states that below pH 1·8 the precipitation of lead sulphide is incomplete and above pH 3·0 co-precipitation of iron occurs.

This led us to investigate the pH values of solutions obtained by carrying out the procedure described in the British Standard. Solutions of 10 g of pure iron (Fisher Combax accelerator) in hydrochloric acid to which 5·0 mg of lead (as a solution of lead chloride) had been added, were treated as described in section 5 of this standard. Before the hydrogen sulphide was passed through the solution, the pH was determined by means of a glass electrode and was found to be between 0·9 and 1·6. Within this range, the pH was dependent on the degree to which the solution had been evaporated; evaporation such that the hot residue was syrupy resulted in the lowest pH value being obtained, and evaporation such that the hot residue was just dry gave the highest pH value. Solutions of pH 0·9 were clear, whereas solutions of pH 1·6 were turbid when cold and hydrolysed appreciably when heated. In the latter solutions, addition of 2 ml of hydrochloric acid, sp.gr. 1·18, as recommended in Note 1 of the British Standard, lowered the pH by about 0·4 unit.

Precipitation of lead in these solutions took place slowly. After hydrogen sulphide had been passed for 20 minutes, as specified, the solutions were filtered, and the amount of lead in the filtrates was determined photometrically with dithizone. The solutions of pH 0·9 contained about 1·2 mg of unprecipitated lead, those of pH 1·3 about 0·25 mg and those of pH 1·8 about 0·06 mg.

When the passage of hydrogen sulphide was continued for 60 minutes instead of the specified 20 minutes, the amount of unprecipitated lead remaining in the solution was smaller; 0·2 mg at pH 0·9 and 0·07 mg at pH 1·6.

It is clear, therefore, that under the conditions of the procedure described in the British Standard, precipitation of lead is more or less incomplete. This is probably the reason that the B.I.S.R.A. Methods of Analysis Committee,[2] in investigating the lower limit of this method, found erratic and low results with amounts of lead below 5 mg.

Obviously it is necessary to precipitate the lead at a higher pH. Increasing the pH by adding ammonia solution incurs difficulties, however, as lumps of ferric hydroxide are formed that redissolve only slowly. With an extremely dilute solution of ammonia this difficulty is minimised, but neutralisation takes a longer time. The formation of lumps of ferric hydroxide was caused by the presence of considerable amounts of tervalent iron formed by aerial oxidation of the iron during evaporation of the solution to dryness. It proved easy to reduce the tervalent iron with ascorbic acid, which can be added in small portions as a powder while the solution is being stirred. The progress of the reduction is indicated by the change in the colour of the solution to light green. In our experiments about 200 mg of ascorbic acid were adequate for complete reduction. Separate experiments showed that even a large excess (1 g) of ascorbic acid does not interfere with the quantitative precipitation of lead, as sulphide, in the pH region 2 to 3. Adjustment of pH can now easily be made, the reduced solution remaining practically clear during the addition of ammonia solution, since ferrous hydroxide only begins to precipitate above pH 5. The measurement of pH with a narrow-range indicator paper is sufficiently accurate.

When hydrogen sulphide is passed into these reduced solutions adjusted to a pH between 2·0 and 2·8, lead sulphide precipitates extremely quickly and soon settles. The filtrates of solutions treated in this way contained less than 10 $\mu$g of lead after hydrogen sulphide had been passed through them for 20 minutes.

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Received *July 18th*, 1962

# A Compact Unit for the Determination of Carbon and Hydrogen by the Večeřa Method

BY D. R. CLIFFORD AND E. A. FRIBBINS
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THE Večeřa[1] method for the micro-determination of carbon and hydrogen has been in use in this laboratory for some time. Knobloch, Knobloch and Mai[2] have published details of an apparatus for the automatic combustion of samples by this method. This Note describes a simple, compact manually-operated apparatus.

The complete unit is shown in Fig. 1. It consists of a base, a metal box containing electrical components, a wooden panel holding the flowmeter, etc., and a chain-driven bunsen burner for burning the sample.

The small electrical furnace for heating the combustion-tube filling is made from a standard 600-watt Nichrome electric-fire element embedded in a cylindrical block of Sindanyo. To accept the element, holes are drilled on a suitable pitch circle so that they "break through" into a 0·44-inch diameter central hole that fits the combustion tube. The whole furnace is mounted in a fabricated stainless-steel pipe "tee." The completed unit is 2·2 inches long and has a diameteer of 1·4 inches. It is supported alongside the top of the instrument case by a Sindanyo panel that serves as a heat insulator. A current setting of 1·1 amps permits the catalyst layer to be maintained at 600° C.

The instrument case houses a 0- to 240-volt Variac transformer, ammeter, fuses and warning light. A subsidiary heater for warming the end of the water-absorption tube is attached to the top of the case, and a slot is cut in the top to accommodate the Večeřa-type[1] carbon dioxide absorption tube.

The wooden panel is bolted to the metal base of the apparatus. It holds a White - Wright type flowmeter,[3] a scavenging U-tube, a flow regulator and a precision pinch cock.

The burner unit consists of a bunsen burner mounted on a carriage that can be moved by operating the chain drive by means of a knob on the front of the apparatus.

Oxygen is supplied from a cylinder via a pressure regulator located at the back of the wooden panel.

The over-all dimensions of the apparatus are: length 23 inches, width 5½ inches, total height 15 inches.

Six to eight determinations per working day are easily achieved by one operator.

We thank Dr. D. Woodcock for his interest and encouragement, Mr. R. C. Perkins for constructing the glassware and Messrs. R. H. Davis and T. Rogers for valuable technical assistance.

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Received *August 10th*, 1962

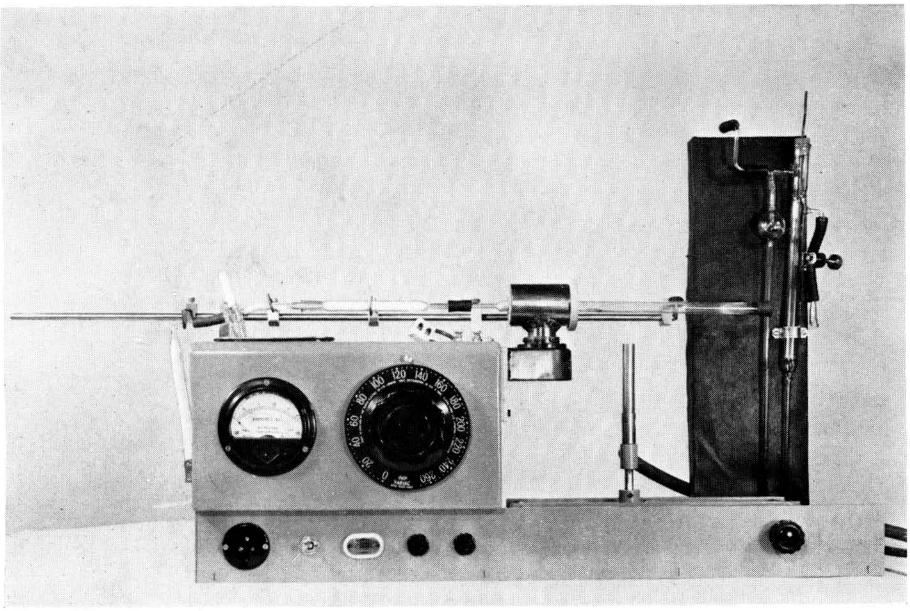

Fig. 1. Complete apparatus

[To face page 68

# Book Reviews

THE DITHIOCARBAMATES AND RELATED COMPOUNDS. By G. D. THORN and R. A. LUDWIG. Pp. viii + 298. Amsterdam and New York: Elsevier Publishing Company. 1962. Price Dfl. 20.

The authors describe their work as a "digest of the extensive literature on the dithiocarbamates . . ." and it must be remarked that a vast amount of information has been digested into a readily assimilable form. Although it has the cursory character of a review, each chapter is followed by such a comprehensive list of literature references that the reader may readily trace the details of his own particular interests. These references amount to over 1000, dated up to December, 1960, and the title of each paper is included. The authors have contributed extensively from their own original work, some of which has not been previously published.

The purely chemical aspects of the subject matter are taken in conventional sequence. An introduction on historical lines is followed by a chapter on the preparation of the dithiocarbamates; their properties are discussed from a physico-chemical viewpoint and a chapter on their chemical reactions introduces a number of important derivatives, such as the thiuram sulphides and the isothiocyanates.

The heading "Analysis of dithiocarbamates and thiuram sulphides" is the somewhat misleading title of the chapter on the determination of these substances in mixtures. The assay of the "pure" dialkyldithiocarbamates by direct titration with standard iodine is not mentioned. Six pages are devoted to a discussion of the uses of dithiocarbamates in inorganic analysis. Twelve differently substituted dithiocarbamates have found use in the determination of twenty-six metals, and a useful Table provides literature references for the analytical methods that have been employed for these purposes.

The rest of the book, comprising nearly half its bulk, deals with the biological reactions and applications of these substances. Individual chapters cover biochemical investigations, applications in clinical medicine, fungicidal action and agricultural applications. The authors, who are with the Canada Department of Agriculture, have a particular interest in the last two subjects. The important applications of these sulphur compounds in the rubber industry are not considered, although it is made clear that much of the purely chemical information made available originated from research related to rubber technology.

Painstaking authorship and excellent book production have gone hand in hand to produce a volume that will prove invaluable to all who have an interest in this class of compounds.

W. C. JOHNSON

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LABORATORY ORGANIZATION AND ADMINISTRATION. By K. GUY, F.I.S.T. Pp. xiv + 386. London: Macmillan & Co. Ltd.; New York: St. Martin's Press. 1962. Price 50s.

The author of this book is the Chief Laboratory Technician in the Department of Chemistry and Chemical Engineering in the University of Natal. He received his early training in London and at one time was Chief Technician in the Chemistry Department of the Sir John Cass College.

With this background therefore it is not surprising that he has written a useful and extremely practical book on the management, design and maintenance of laboratories, and in particular, of chemical laboratories.

He writes, in a common sense way, about the designing of laboratories and about the precautions that must be taken in the installation of laboratory equipment, *e.g.*, balances. His chapter on stores management could be read with advantage by laboratory stewards in many industrial, university, college and school laboratories.

In 70 pages of text the author provides the reader with a great deal of information on safety precautions in laboratories, including, *e.g.*, protective measures against radiation. It is obvious that this chapter has been written from the point of view of one who has spent a great deal of his working life "near the bench" and with the right philosophy.

Projection methods useful in the lecture theatre are described and there is a great deal of sound information on the filing of laboratory records and technical information. Some attention is also paid to the organisation of exhibitions and demonstrations.

There is no doubt that this book will be welcome in many chemical laboratories whether industrial or academic. Our approval, however, must be qualified. In a general book of this kind, a great deal of the information on such specialised topics as biological reagents should have been omitted; the descriptions of chemical reagents and solutions could then have been extended with great profit.

Finally, and one says this with a certain amount of regret, there are parts of the book that contain rather too many sentences with subjects in the singular and verbs in the plural. Incidentally, on page 147, the volume of sea water should be given.

J. HASLAM

KOMPLEXONE IN DER CHEMISCHEN ANALYSE. By RUDOLF PŘIBIL. Pp. xxii + 474. Berlin: Veb Deutscher Verlag der Wissenschaften. 1961. Price DM 56.

There can be no doubt that Dr. Přibil is, more than most others, responsible for the present high status in inorganic analysis of complexometric titration with ethylenediaminetetra-acetic acid. Those who have been privileged to hear and see his elegant lecture/demonstrations on his all too infrequent visits to the English-speaking countries will be familiar with his mastery of the topic, and certainly the author's name is known and at least some of his procedures and indicators are used in every laboratory where the EDTA bottle is on the shelf.

The Czech text of this book was first published in 1953 and the second edition appeared in 1956. The latter has now been translated into German, and a small appendix, dated 1960, has been added that cites some new indicators produced in the author's own laboratories or those of his associates, and a few applications to gravimetry, colorimetry, polarography, etc.

The text is introduced by a review of the nature of complexes and then proceeds to examine the basic aspects of the reactions between complexones and metal-ions in aqueous solution. The main part of the text on analytical applications comes next, with separate chapters on the masking action of EDTA in gravimetry, titrimetry (non-complexometric), colorimetry, polarography, chromatography, electrophoresis and qualitative inorganic analysis. A short chapter deals with the determination of EDTA itself, and the last two chapters, which occupy about half of the text, deal with direct titrimetric applications of EDTA. The first of these giant chapters discusses the determination of metals by EDTA under the heading of the complexometric indicator used, *e.g.*, murexide for copper, nickel, cobalt, calcium, etc. It also takes into account physico-chemical methods of end-point detection, and considers masking agents. The final chapter deals with practical applications of complexometric methods to the determination of selected metal ions in technical materials such as alloys, electroplating baths, pharmaceuticals, plant substances and paper. Lastly, there is a short appendix added to the 1956 Czech edition and the slightly larger appendix mentioned above.

This brief review of the subject matter of the text rather conceals the extensive treatment afforded to each topic and even the scope of the book. Beyond doubt, this book by Přibil is the most detailed and well-informed text-book on this topic, less encyclopaedic than some perhaps, but only just so, and full of skilful assessment of the techniques and procedures. It is a most outstanding book and should make a great impact.

The chronology of this text has already been cited and in this, to my mind, lies its only fault. Thus, though it was published in 1961, up to p. 440 of the text, there are practically no references beyond 1955, and the author's appendix gives a rather incomplete coverage up to 1958. This, however, is no reflection on the book, which paints an accurate picture of the present situation in EDTA titrimetry, with only some of the more recent advances, in which new complexones and more specific indicators, etc., are used, not present. However, this eventuality is bound to be found in any book dealing with a topic that expands still with explosion-like rapidity. It is to be hoped that any further translation into a world-wide language of a new edition will not have to wait so long in future.

For all who are concerned with complexometric analysis this book must be regarded as absolutely indispensable. Přibil in print in indisputably the equal of Přibil at the laboratory bench.

T. S. WEST

METHODS OF ORGANIC ELEMENTAL MICROANALYSIS. By G. INGRAM, A.R.I.C. Pp. xvi + 511. London: Chapman & Hall Ltd. 1962. Price 75s.

There are many who would argue that microchemistry has no valid claim to be regarded as a subject in its own right; certainly the term has defied any agreed definition, for the boundaries of the subject may be set as wide, or as narrow, as the individual requires. But the wider the limits are set the greater the controversy is likely to rage; there were, for example, subjects discussed at Birmingham in 1958 that some were surprised to find classified as microchemistry. With the contents of Ingram's "Organic Elemental Microanalysis," however, there can surely be little dispute. Nor can it be denied that many of the methods the author describes represent a technique so specialised that there is full justification to classify them as microanalysis, separate and distinct from the routine working of the general analyst.

Having made a case for regarding microanalysis as a specialist branch of analytical chemistry let it be said that in few other divisions of the analyst's work is there quite so much need for reliance on the manipulative skill and craftsmanship of the individual. In Part I of his book, which deals with the determinations of carbon, hydrogen, oxygen, nitrogen, sulphur and the halogens, the author reveals himself as the complete master of his craft. The selected methods that he gives, in several instances inspired by his own researches, are described with the scrupulous attention to detail that is so helpful to the newcomer to this field of work. Not all established practitioners will accept every point the author makes, for each laboratory develops its own traditions and preferences and, indeed, its own myths. The defence of the use of a selenium - mercury catalyst in Kjeldahl digestions will find its opponents; so will the use of a boric acid solution in ammonia distillations. Sometimes, too, the author has had to rely on the literature without, one would imagine, the benefit of having tested the method for himself, for example, McCutchan and Roth's procedure for decomposition of nitro-compounds with thiosalicylic acid. This is understandable, however, for with so large a field to cover no analyst could be expected to have tried every variant in the extensive literature. What is clear, however, is that those methods that the author gives in detail are distilled from his many years of practical experience.

Part 2 of the book deals with the determination of metals and non-metals and Part 3 with determinations on the microgram scale. It will be noted that no attempt has been made to describe the determination of functional groups; in his decision to confine himself to the determination of the elements the author has, one feels, made a wise decision. The size of just over 500 pages is a convenient one, well-suited to the laboratory handbook that this is intended to be and that it will surely become.

The book is well produced in a pleasing modern style, although some confusion may arise from the weight given to various sub-headings within a chapter—this seems particularly to be so in Chapter 4 on the Determination of Halogens. But this in no way affects the general conclusion —if you are concerned with organic elemental microanalysis, buy this book. The consistently high quality of the many diagrams is alone worth the money. C. A. JOHNSON

ABSORPTIONSSPEKTROSCOPIE IM ULTRAVIOLETTEN UND SICHTBAREN SPEKTRALBEREICH. By BRUNO HAMPEL. Pp. vi + 109. Braunschweig, Germany: Friedr. Vieweg & Sohn. 1962. Price DM. 16.80.

After 16 pages of introductory matter the author briefly discusses photographic methods, because, as he says, they are becoming obsolete. He then describes photo-electric spectrophotometry in general terms and three commercial instruments (Zeiss PMQ II, Unicam SP500, Beckman DK2) in more detail. This is followed by an account of technical methods and procedures. Finally, there is a full chapter on applications of absorption spectroscopy, covering the relationships between constitution and light absorption, chromophoric and auxochromic groups, some examples of constitutional problems elucidated by spectroscopy and some quantitative analytical problems.

This is an orthodox presentation, and for its size the book covers a lot of ground very well. It is presumably intended to provide an introduction to the subject that German-speaking students can afford to buy. The book is well printed, and the strong paper cover should give it a reasonable life. R. A. MORTON

STRAHLUNGSMESSUNG IM OPTISCHEN SPEKTRALBEREICH: MESSUNG ELEKTROMAGNETISCHER STRAHLUNG VOM ULTRAVIOLETT BIS ZUM ULTRAROT By GEORG BAUER. Pp. viii + 181. Braunschweig, Germany: Friedr. Vieweg & Sohn. 1962. Price DM 16.80.

This is Volume 16 in a series entitled "*Verfahrens- und Messkunde der Naturwissenschaft.*" It contains a good deal of physical optics and of fundamental theory. There is a good account of light sources of various kinds currently in use. Methods of dispersing radiation are described, and chapters on thermal and photo-electric detectors follow. The use of monochromators and of light filters is described, and the sensitivity of various detecting devices is discussed in detail.

This is a paper-back volume, well printed and illustrated and reasonably priced for the nature of its contents. It is scholarly, thorough and up to date wherever I have been able to test it.

R. A. MORTON

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SPECTROSCOPY. Volume 2. ULTRA-VIOLET, VISIBLE, INFRA-RED AND RAMAN SPECTROSCOPY. By S. WALKER, M.A., B.Sc., D.Phil., F.R.I.C., and H. STRAW, B.Sc. Pp. xxvi + 386. London: Chapman & Hall Ltd. 1962. Price 70s.

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*Reprinted from* Pure and Applied Chemisty, Vol. 5, Nos. 3-4.

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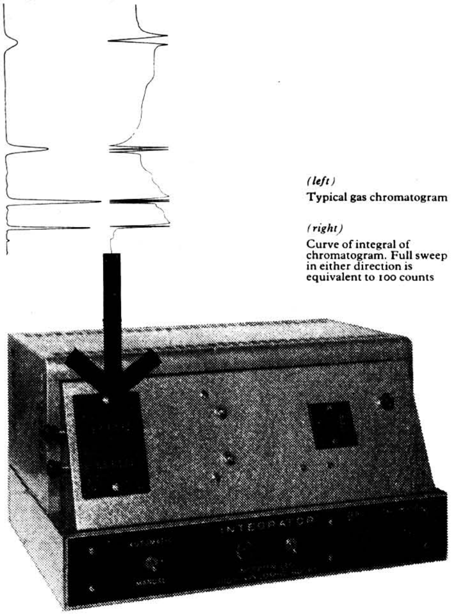

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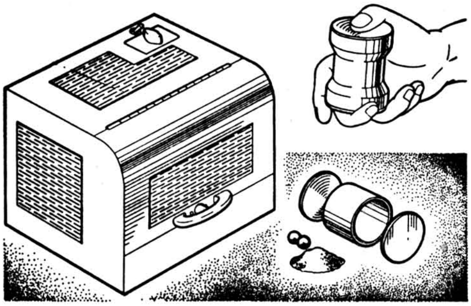

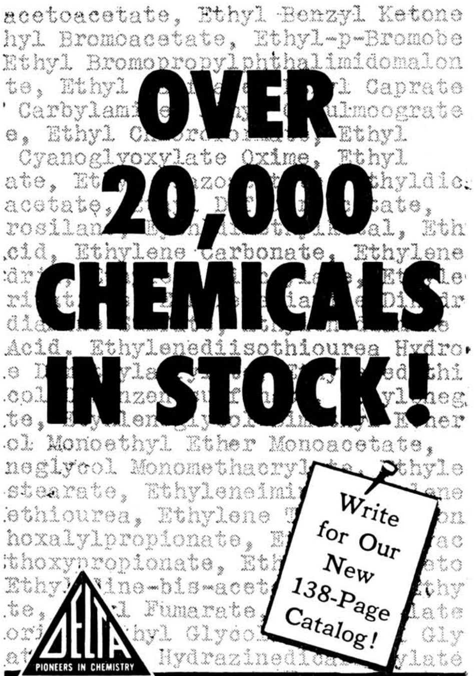
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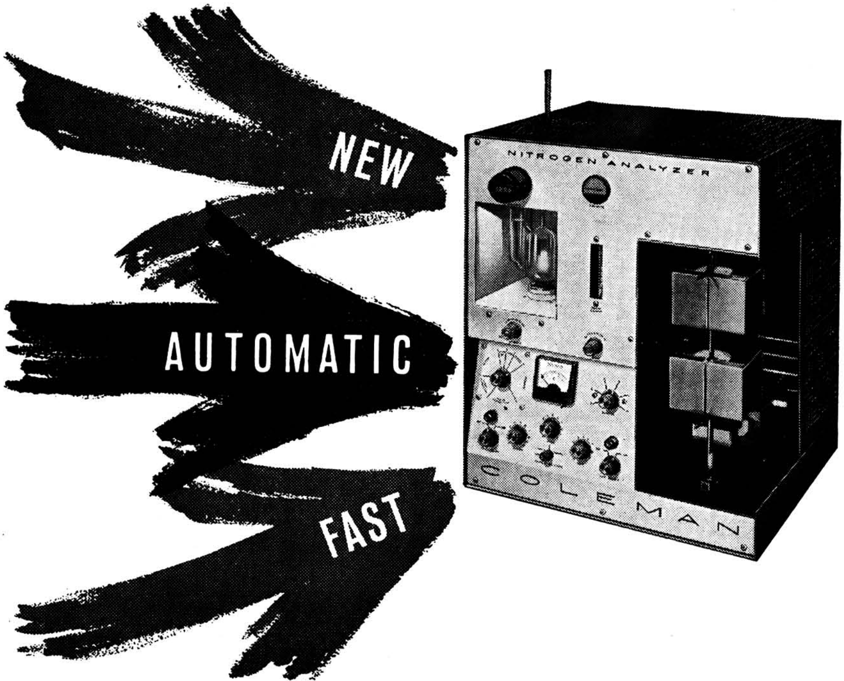

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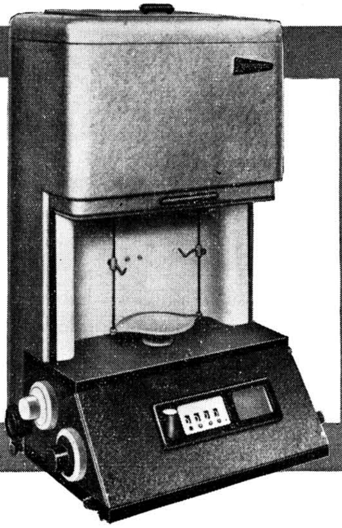

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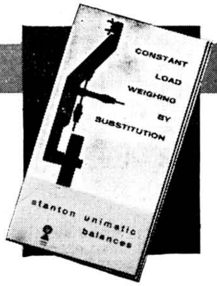

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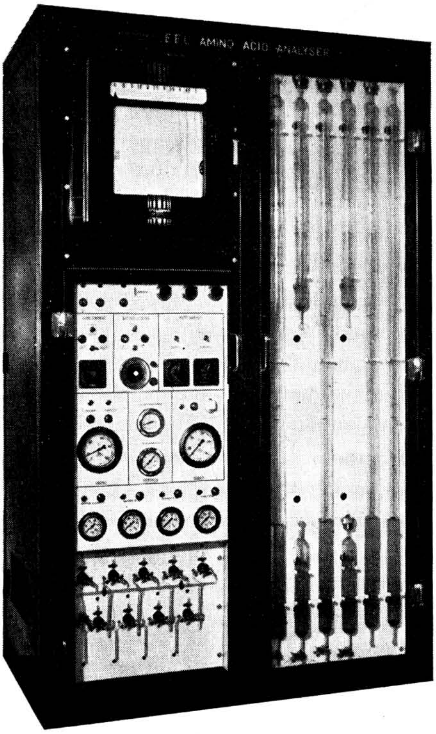
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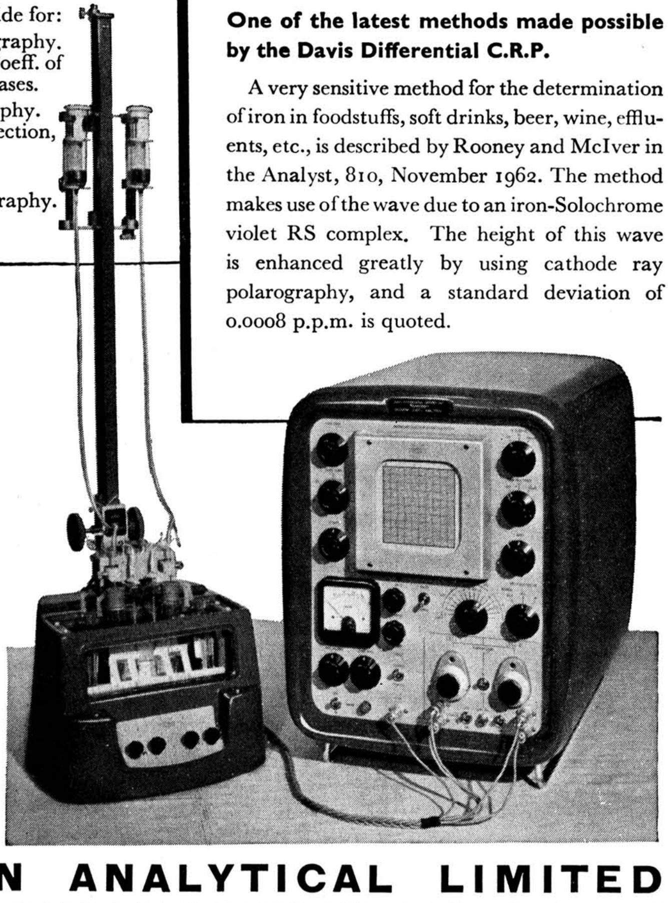

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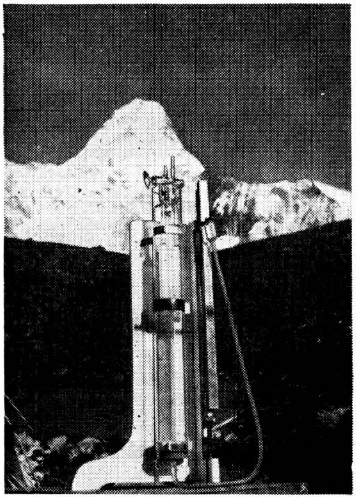

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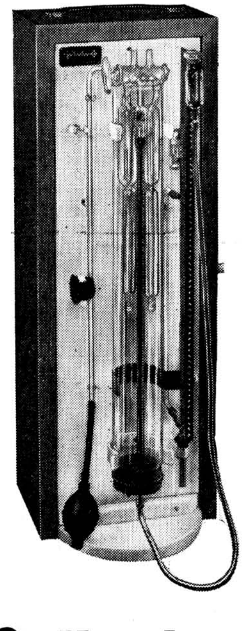

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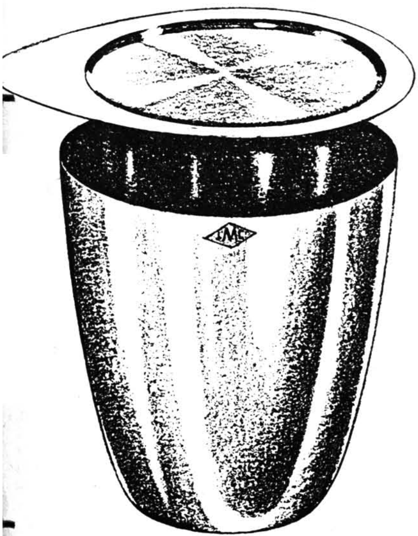

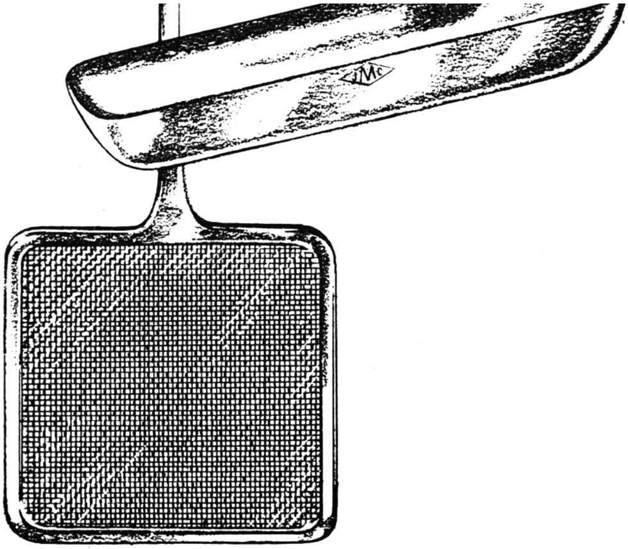

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