The ANALYST

The Journal of the Society for Analytical Chemistry: a monthly publication dealing with all branches of analytical chemistry

Published for the Society by
W. HEFFER & SONS LTD., CAMBRIDGE

Volume 88

No. 1045, Pages 249-330

April 1963

THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

NOTICE TO SUBSCRIBERS

(Other than Members of the Society)

All subscriptions to *The Analyst*, including *Analytical Abstracts*, should be sent through a Bookseller direct to

W. HEFFER & SONS LTD., CAMBRIDGE, ENGAND

Subscriptions are NOT accepted for *The Analyst* alone

Subscriptions to *The Analyst*, together with *Analytical Abstracts* printed on one side of the paper (without Index) can be accepted at £8 15s. per annum

Subscriptions to *The Analyst*, together with *Analytical Abstracts* printed on one side of the paper (with index) can be accepted at £9 12s. 6d. per annum

N.B.—Members send their subscriptions to the Hon. Treasurer

Single copies Price 15s.

Subscription Rate, inclusive of Analytical Abstracts and Indexes, £8 8s. per annum, post free

Volume 88, No. 1045 April, 1963

THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETING

AN Ordinary Meeting of the Society was held at 3 p.m. on Tuesday, April 9th, 1963, at Chelsea College of Science and Technology, Manresa Road, London, S.W.3. The Chair was taken by the President, Dr. D. C. Garratt, Hon. M.P.S., F.R.I.C.

The meeting consisted of contributions from Research Workers in Universities and Colleges of Advanced Technology and the following papers were presented and discussed: "The Detection of Ions in Low Concentrations Using Ion-exchange Resins," by J. F. Harvey, B.Sc.; "The Determination of Molybdenum in Several Valence States," by I. L. Marr, B.Sc.; "The Determination of Alkaline Earth Metals by Flame Spectrophotometry," by D. Spincer, B.Sc.; "The Investigation of 8-Mercaptoquinoline as a Gravimetric Reagent for Palladium and Nickel," by J. A. W. Dalziel, Ph.D., D.I.C., A.R.C.S., F.R.I.C., and D. Kealey, Ph.D., M.Sc., D.I.C., A.R.C.S.; "The Volumetric Determination of Molybdenum and Tungsten in the Presence of Iron," by M. S. Taylor, A.R.I.C.; "The Nature of the Silver (I)—Rhodamine Reaction," by A. Townshend, B.Sc.

DEATHS

WE record with regret the deaths of

Frederick Mason Brewer
Alfred Henry Moore.

NORTH OF ENGLAND SECTION

A JOINT MEETING of the North of England Section with the North Lancashire Section of the Royal Institute of Chemistry was held at 7.30 p.m. on Friday, February 8th, 1963, at the Harris Technical College, Preston. The Chair was taken by the Chairman of the North Lancashire Section, Mr. T. E. V. Horsley, B.Sc., F.R.I.C.

A lecture on "Gas Chromatography" was given by A. T. James, B.Sc., Ph.D.

A JOINT Meeting of the North of England Section with the Leeds University Chemical Society was held at 6.30 p.m. on Friday, February 15th, 1963, in the Lecture Theatre, The University, Leeds. The Chair was taken by the Chairman of the North of England Section, Mr. C. J. House, B.Sc., A.R.C.S., F.R.I.C.

A lecture on "Dithizone and its Reactions" was given by Professor H. M. N. H. Irving, M.A., D.Phil., D.Sc., F.R.I.C., L.R.A.M.

A JOINT Meeting of the North of England Section with the Newcastle and North East Coast Section of the Royal Institute of Chemistry was held at 6.30 p.m. on Wednesday, March 6th, 1963, at the Lecture Theatre, Chemistry Department, King's College, Newcastle upon Tyne. The Chair was taken by the Chairman of the Newcastle and North East Coast Section, Dr. E. P. Hart, B.Sc., F.R.I.C.

The following paper was presented and discussed: "The Chemistry of Crime Detection," by H. J. Walls, B.Sc., Ph.D.

SCOTTISH SECTION

A JOINT Meeting of the Scottish Section with the Glasgow and West of Scotland Section of the Royal Institute of Chemistry was held at 7.15 p.m. on Friday, February 8th, 1963, in Room 24, Royal College of Science and Technology, George Street, Glasgow, C.1. The Chair was taken by the Chairman of the Glasgow and West of Scotland Section, Professor P. D. Ritchie, B.Sc., Ph.D., M.I.Chem.E., F.R.S.E.

The subject of the meeting was "Gas Liquid Chromatography" and the following papers were presented and discussed: "Recent Applications to the Study of Natural Products," by G. Eglinton, B.Sc., Ph.D.; "Applications to Reaction Kinetics," by J. H. Knox, B.Sc., Ph.D.

WESTERN SECTION

A JOINT Meeting of the Western Section and the Bristol and District Section of the Royal Institute of Chemistry was held at 7.30 p.m. on Tuesday, February 19th, 1963, in the Technical College, Gloucester. The Chair was taken by Dr. C. G. Silcocks, B.Sc., F.R.I.C.

A lecture on "Luminescence" was given by Professor G. F. Garlick, D.Sc., F.Inst.P.

MIDLANDS SECTION AND BIOLOGICAL METHODS GROUP

A JOINT Meeting of the Midlands Section with the Biological Methods Group and the Association of Clinical Biochemists (Midland Region) was held at 6.30 p.m. on Thursday, February 14th, 1963, in the Haworth Block, The University, Edgbaston, Birmingham, 15. The Chair was taken by the Chairman of the Association of Clinical Biochemists (Midland Region), Dr. B, E, Northam, B,Sc., A.R.I.C.

The subject of the meeting was "The Microbiological Assay of Vitamin B_{12} and Folic Acid" and the following papers were presented and discussed: "The Assay of Vitamin B_{12} in Body Fluids," by Miss M. E. Gregory, M.Sc.Tech., Ph.D.; "The Assay of Folate Compounds in Body Fluids and Tissues," by A. H. Waters, M.B., B.S.; "The Clinical Interpretation of the Results of the B_{12} and Folic Acid Assays," by D. L. Mollin, B.Sc., M.R.C.S., L.R.C.P.

MICROCHEMISTRY GROUP

THE Nineteenth Annual General Meeting of the Group was held at 6.45 p.m. on Friday, February 22nd, 1963, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the Chairman of the Group, Mr. C. Whalley, B.Sc., F.R.I.C. The following Officers and Committee Members were elected for the forthcoming year: *Chairman*—Mr D. W. Wilson. *Vice-Chairman*—Mr. R. Goulden. *Honorary Secretary*—Mrs. D. E. Butterworth, National Chemical Laboratory, Teddington, Middlesex. *Honorary Treasurer*—Mr. G. Ingram. *Members of Committee*—Dr. Cora W. Ayers, Dr. Bella B. Bauminger, Dr. R. A. Chalmers, Mr. C. B. Dennis, Mr. T. R. F. W. Fennell and Mr. C. Whalley. Dr. L. H. N. Cooper and Mr. H. Childs were re-appointed as Honorary Auditors.

The Annual General Meeting was followed at 7 p.m. by an Ordinary Meeting of the Group, at which the retiring Chairman, Mr. C. Whalley, B.Sc., F.R.I.C., gave an Address on "Microchemistry and the Space Age." The Chair at this meeting was taken by the new Chairman of the Group, Mr. D. W. Wilson, M.Sc., F.R.I.C.

PHYSICAL METHODS GROUP

THE eighty-seventh Ordinary Meeting of the Group was held at 6 p.m. on Tuesday, February 26th, 1963, in the Cuthbert Wallace Lecture Room, Royal College of Surgeons of England, Lincoln's Inn Fields, London, W.C.2. The Chair was taken by the Chairman of the Group, Dr. W. Cule Davies, F.R.I.C.

The subject of the meeting was "Fractionation of High Molecular Weight Compounds" and the following papers were presented and discussed: "Gel Filtration as a Method of Polymer Fractionation," by M. F. Vaughan, B.Sc.; "Heterogeneity Studies on a Protein Hormone," by W. M. Hunter, B.Sc. (see summaries below).

The meeting was preceded at 2.30 p.m. by a visit to the Research Departments of the Royal College of Surgeons of England, Lincoln's Inn Fields and the Imperial Cancer Research Fund.

Gel Filtration as a Method of Polymer Fractionation

Mr. M. F. Vaughan said that gel fractionation was a type of molecular sieving that depended on the depth of penetration of different molecules into gel-like materials. Thus, when a solution of molecules of different sizes was eluted through a column of a gel, the smallest molecules penetrated further into the gel particles, differential retardation occurred and the molecules left the column in order of decreasing molecular weight.

Some of the earliest experiments on this phenomenon had been carried out by Lathe and Ruthven (*Biochem. J.*, 1956, **62**, 665), who used columns of starch swollen with water. They were able to show that the degree of penetration of molecules into the starch granules decreased with increasing molecular size and they had indicated how this effect might be used as a separation technique. Porath and Flodin (*Nature*, 1959, **183**, 1657) had developed this idea and had shown how columns of swollen crosslinked dextrans could be used to separate water-soluble molecules of different sizes. Further improvements had been made, and it was now claimed that, by using dextrans of different degrees of crosslinking, separations could be achieved over the molecular-weight range 4000 to 2,000,000.

Crosslinked polymer structures analogous to crosslinked dextrans were fairly easily prepared and such materials as (*i*) vulcanised rubber and (*ii*) copolymers of styrene and divinyl benzene had been used in adapting this technique to non-aqueous systems. Brewer (*Nature*, 1960, **188**, 984) had used pieces of vulcanised natural rubber latex for separating polymers from mineral oil, and crosslinked polystyrene beads had been used by Cortis-Jones (*Nature*, 1961, **191**, 272) as a separation method for low molecular-weight materials and by ourselves (*Nature*, 1960, **188**, 984) at Teddington as a method for fractionating polystyrene. However, certain experimental difficulties had been encountered because, in order to fractionate the largest molecules, very lightly crosslinked beads were required. These were difficult to prepare and when swollen were so soft that they tended to pack down giving very slow elution rates. An investigation had therefore been made into the possibility of using other materials (*Nature*, 1962, **195**, 801).

The name gel filtration appeared to imply that some swollen jelly-like material was required. On the other hand, if this phenomenon really was a type of molecular sieving, then it should have been possible to use more rigid microporous materials. Some fractionation had been obtained with polyethylene gels, cellulose gels, Linde type molecular sieves, porous glass, Johns Manville firebrick, etc., but best results had been obtained with certain highly expanded silica gels.

With the silica gel Santocel A (Monsanto Chemical Co. Ltd.) the fractionation efficiencies with polystyrene had been similar to those obtained by conventional fractionation techniques. Compared with these other methods, a gel filtration method had the advantages of simplicity of operation, speed and economy of solvents. In dealing with industrial polymers, therefore, a fractionation method of this type should have been very useful as a comparatively rapid way of evaluating molecular-weight distribution curves and possibly for the large-scale processing of polymers and copolymers.

Heterogeneity Studies on a Protein Hormone

Mr. W. M. Hunter said that most of the published evidence indicated that human growth hormone (HGH) prepared by the Raben procedure (*Science*, 1957, **125**, 883), from human pituitaries, was a homogeneous protein: sedimentation coefficient 2·5S, i.e.p. 4·9; M.W. 29,000. Hydrolysis yielded amino acids only. Investigation of the hormone in reactions with antibodies raised against the hormone in the rabbit largely confirmed the acceptance of the preparation as a single protein (Grumbach, M. M., and Kaplan, S. L., Ciba Coloquia on Endocrinology, 1962, **14**, 63). However, immunoelectrophoresis in agar gel indicated that the preparation consisted of a small family of proteins immunologically identical, but differing slightly in electrophoretic mobility.

The radio-immunoelectrophoretic method, used as an analytical tool, possessed great precision and good discrimination (Hunter, W. M., and Greenwood, F. C., *Biochem. J.*, 1962, **85**, 39P). This method had been used together with a bioassay method, the tibia line assay and amino acid analysis, to follow fractionation of HGH on starch grain electrophoresis and on columns of the crosslinked dextran 6200 Sephadex.

In free–boundary electrophoresis this preparation was homogeneous at pH 8·6 barbitone buffer (ionic strength 0·1). However, electrophoresis in starch grain resulted in partial separation of a slower peak and a faster more diffuse one (pH 8·4 barbitone sodium chloride ionic strength 0·11, 5 V per cm, 40 hours). Fractions comprising the trailing edge of the slow component and the leading edge of the faster component showed indistinguishable biological and immunological activities. Amino acid analyses (kindly carried out by Dr. J. E. Eastoe) were identical, except that the faster component had two fewer amide groups than had the slower one. The presence of two more free carboxyl groups in the faster component could account for the greater electrophoretic mobility. Either the hormone was present in the pituitary gland in an amido form and as a family of varying degrees of de-amido forms, or was produced by de-amidation during the initial extraction of the hormone in 85 per cent. acetic acid at 70° C.

G200 Sephadex was capable of distinguishing between proteins on the basis of molecular size over the approximate molecular-weight range of 20,000 to 500,000. Under three different conditions of pH, HGH was eluted as a single peak in the position expected for M.W. 29,000 (0·1 N acetic acid, 0·05 sodium phosphate, pH 7·5, carbonate - bicarbonate, pH 10·6, I = 0·1). However, in columns in borate buffers (pH 8·6, 0·05N sodium borate - 0·05N potassium chloride) a component amounting to some 20 per cent. of the starting material appeared unretarded by the dextran in a position expected for the 19S-macroglobulins. The main peak (65 per cent.) appears in the position expected for material of M.W. 29,000, with some trailing between the two. Identical distributions were achieved over the range 0·5 to 10 mg of HGH per ml applied to the column. Material from each of the two peaks re-chromatographed in borate appeared at its distinctive position.

In the analytical ultracentrifuge the same solution of HGH in borate - potassium chloride buffer yielded only a single peak of slow sedimenting material approximately S2·7. It appeared therefore that there was some unexplained interaction between the borate buffer potassium chloride, the dextran and a specific fraction of the Raben HGH. The two peaks, although they appeared to be identical in the ultracentrifuge, differed markedly when tested immunologically by a sensitive assay. It was not known whether the peaks differed in biological activity.

ATOMIC ABSORPTION SPECTROSCOPY DISCUSSION PANEL

THE second meeting of the Panel was held at 2 p.m. on Wednesday, February 13th, 1963, at the Department of Medical Physics, The General Infirmary, The University, Leeds, 1. The Chair was taken by the Chairman of the Panel, Mr. W. T. Elwell, F.R.I.C.

A discussion on "The Problems in Design and Construction of Atomic Absorption Apparatus" was opened by Dr. J. B. Dawson. The laboratories and workshops of the Department of Medical Physics were also open for inspection.

Analytical Methods Committee

REPORT PREPARED BY THE METALLIC IMPURITIES IN ORGANIC MATTER SUB-COMMITTEE

The Determination of Small Amounts of Copper in Organic Matter

THE Analytical Methods Committee has received the following report from its Metallic Impurities in Organic Matter Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The members of the Sub-Committee concerned with the preparation of this report were Mr. W. C. Johnson (Chairman), Mr. L. Brealey,* Miss E. M. Chatt,* Dr. J. C. Gage, Dr. T. T. Gorsuch, Mr. E. I. Johnson, Miss E. M. Johnson, Mr. T. McLachlan,* Dr. R. F. Milton, Mr. E. J. Newman, Mr. W. G. Sharples, Mr. G. B. Thackray and Dr. G. E. Willis,† with Miss A. M. Parry as Secretary.

INTRODUCTION

The copper reagents in the dithiocarbamate class had an obvious claim for consideration, but, before finally adopting one of this group, the Sub-Committee considered also the merits of the more recently introduced derivatives of 1,10-phenanthroline. Of these derivatives, neocuproine (2,9-dimethyl-1,10-phenanthroline) was investigated on a practical basis in individual and collaborative experiments. Neocuproine can be used under conditions that render it virtually specific for copper, but these conditions impose a rather narrow pH range in a region where, for example, certain salts of the alkaline-earth metals might be precipitated if present in moderate amounts. The literature also refers to precipitation that has been attributed to the reagent itself or to some compound of the reagent, and this problem does not appear to have been elucidated or resolved.

Compared with the diethyldithiocarbamates, neocuproine has only two-thirds the sensitivity towards copper, whereas 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline (bathocuproine) is said to be about twice as sensitive; the latter reagent has also been investigated in the form of its water-soluble disulphonic acid.[1] When more experience and information has accumulated on the use of these derivatives of 1,10-phenanthroline they may well be adaptable to methods for general application with some advantages over current practice. In the meantime, the Sub-Committee thought that the well established dithiocarbamates were adequate for present needs.

EXPERIMENTAL AND RESULTS

Of the several procedures in which the dithiocarbamates are used, that adopted by the International Union of Pure and Applied Chemistry[2] appeared the most acceptable. Many metals react with diethyldithiocarbamates, but, in the presence of ethylenediaminetetraacetic acid (EDTA) and citrate, all interference except that from bismuth and tellurium is overcome by the masking effects of these complexing agents.[3] Sodium diethyldithiocarbamate is used in the I.U.P.A.C. method. Having in mind the superior sensitivity of zinc dibenzyldithiocarbamate, the Sub-Committee investigated the possibility of substituting this reagent in the I.U.P.A.C. method. It was found, however, that in the test for the presence of bismuth and tellurium, potassium cyanide destroys only a small proportion of the copper complex.

A proposal to substitute diethylammonium diethyldithiocarbamate for the sodium salt used in the I.U.P.A.C. method was made, as this reagent offers certain advantages; the aqueous solution of the sodium salt is unstable, but the diethylammonium salt can be dissolved in

* Since resigned. † Since deceased.

carbon tetrachloride to provide a much more stable reagent solution. Instead of adding aqueous sodium diethyldithiocarbamate solution to the test solution and extracting the copper complex with carbon tetrachloride, the copper is now more simply extracted by shaking the test solution with the solution of diethylammonium salt (0·1 per cent.) in carbon tetrachloride. This was found to be more convenient both in the analytical procedure and in the purification of the reagent solution. Before this modification was adopted it was subjected to collaborative tests.

To 5-g portions of white wheaten flour of breadmaking grade were added known amounts of copper as measured volumes of a standard solution, the samples were then decomposed with nitric and sulphuric acids and the added copper was determined by the method described in the Appendix, p. 256. The flour was found to contain originally about 2 p.p.m. of copper, and corrections for the original content are incorporated in the recovery figures shown in Table I.

TABLE I

RECOVERY OF COPPER

All results were obtained on the whole of the residue from the wet decomposition with the exception of those for Laboratory B, where the residue after wet decomposition was diluted to 100 ml and a 25-ml aliquot was taken

Laboratory	Copper added, μg	Copper recovered, μg
A	10·0	10·0
	10·0	9·8
	40·0	39·0
	40·0	39·5
B	10·0	12·0
	10·0	10·0
	40·0	42·0
	40·0	38·0
C	10·0	9·5
	10·0	10·0
	40·0	41·0
	40·0	40·5
D	10·0	10·7
	40·0	41·8
E	10·0	10·2
	10·0	11·5
	10·0	11·5
	10·0	9·5
	40·0	38·0
	40·0	40·0

TABLE II

RECOVERY OF COPPER IN PRESENCE OF BISMUTH

All results were obtained on the whole of the residue from the wet decomposition with the exception of those for Laboratory B, where the residue after wet decomposition was diluted to 100 ml and a 25-ml aliquot was taken

Laboratory	Copper added, μg	Bismuth added, μg	Copper recovered, μg
A	10·0	250	9·5
	10·0	250	9·8
B	10·0	250	12·0
	10·0	250	14·0
	10·0	250	10·0
C	10·0	250	10·5
	10·0	250	10·0
D	10·0	250	9·5
E	10·0	250	11·0
	10·0	250	11·5

Copper diethyldithiocarbamate is destroyed by cyanide, but bismuth diethyldithiocarbamate is not.[4] Tellurium diethyldithiocarbamate is partly destroyed by cyanide. If, therefore, the carbon tetrachloride layer containing the copper complex is shaken with cyanide and does not turn colourless, bismuth or tellurium, or both, are present. A further series of experiments was conducted with 5-g samples of the same flour to which known amounts of bismuth were added, and the special procedure for separating bismuth and tellurium described in the Appendix was followed. Copper diethyldithiocarbamate is stable during this treatment, but the bismuth and tellurium complexes are destroyed.[5]

Recovery figures for copper from these experiments are shown in Table II.

It was noted that, if the decomposition of the flour with nitric and sulphuric acids was completed rapidly, recovery of copper was variable. This was overcome by a final treatment of the residue with a little perchloric acid.

In Laboratory A, recovery experiments were carried out on a mixture of inorganic salts simulating the products from the wet decomposition of 5 g of full-cream milk powder. The solution prepared was—

Ferric alum	.. ..	0·05 g
Calcium carbonate	.. ..	2·0 g
Magnesium sulphate heptahydrate	.. ..	0·84 g
Potassium dihydrogen orthophosphate	.. ..	2·60 g
Hydrochloric acid, concentrated	.. .. ..	50 ml
Water	.. .. to	500 ml

The solution was extracted with diethylammonium diethyldithiocarbamate solution in carbon tetrachloride to remove any copper that may have been introduced with the chemicals.

To 25-ml aliquots known amounts of copper (see Table III) or of copper and bismuth (see Table IV) were added, and the solutions were heated with 5 ml of concentrated sulphuric acid until fumes were evolved. Each solution was cooled and diluted to 25 ml, and was then treated as described under "Determination of Copper," p. 257. Recovery figures for copper from these experiments are shown in Tables III and IV.

TABLE III

RECOVERY OF COPPER FROM SIMULATED PRODUCTS OF WET DECOMPOSITION OF MILK POWDER

Copper added, μg	10·0	10·0	10·0	40·0	40·0	40·0
Copper recovered, μg	10·3	10·0	10·4	40·3	40·6	40·4

TABLE IV

RECOVERY OF COPPER IN PRESENCE OF BISMUTH FROM SIMULATED PRODUCTS OF WET DECOMPOSITION OF MILK POWDER

Copper added, μg	Bismuth added, μg	Copper recovered, μg
10·0	20·0	9·7
10·0	20·0	10·1
10·0	20·0	9·8
40·0	20·0	39·5
40·0	20·0	39·0
40·0	20·0	40·0
10·0	40·0	10·7
10·0	40·0	9·7
10·0	40·0	9·7
40·0	40·0	39·5
40·0	40·0	39·0
40·0	40·0	39·5

PRECAUTIONS AGAINST FADING—

In the extraction stage it was noted that the colour of the carbon tetrachloride solution of the metal complex can fade unless the organic layer is removed from contact with the aqueous layer as soon as it has separated. A similar effect was also observed in determining

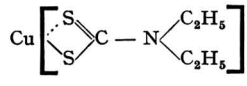
copper in the presence of bismuth when the latter was removed from the carbon tetrachloride extract by shaking with N sodium hydroxide. It was found necessary to remove the carbon tetrachloride from contact with the aqueous layer as quickly as possible and to wash the organic layer immediately with water, otherwise fading occurred. Thus, after 20 minutes in contact with the sodium hydroxide solution the optical densities of two copper-containing extracts were reduced from 0·140 and 0·544 to 0·108 and 0·492, respectively. Cautionary wording against these possible sources of error has been introduced into the recommended method.

Appendix

RECOMMENDED METHOD FOR THE DETERMINATION OF COPPER

PRINCIPLE OF METHOD—

The organic matter in the sample is destroyed by a suitable method of wet oxidation. The residue is diluted with water, ethylenediaminetetra-acetate (EDTA) and citrate are added, and the pH is adjusted to 8·5 with dilute ammonia solution. The solution is then shaken with a solution of diethylammonium diethyldithiocarbamate in carbon tetrachloride. The golden-brown copper complex—



is formed and is extracted, together with those of bismuth and tellurium, if present, from the aqueous layer into the carbon tetrachloride. The organic layer is separated, and its optical density is measured at 436 mμ.

The carbon tetrachloride extract containing the copper complex is tested for bismuth and tellurium by shaking with aqueous potassium cyanide solution. If bismuth or tellurium is shown to be present, another portion of the sample solution is extracted in the usual way, but the solution of the diethyldithiocarbamates in carbon tetrachloride is washed with sodium hydroxide solution to decompose the bismuth and tellurium complexes, and the stable copper complex is determined absorptiometrically as before.

APPLICABILITY—

The method is of general applicability, but bismuth and tellurium interfere. A procedure is described that overcomes interference from these two metals.

RANGE—

The aliquot of the sample solution taken should not contain more than 50 μg of copper. The lower limit of measurement is about 2 μg, but depends on the magnitude of the reagent blank value and the type of spectrophotometric equipment available.

APPARATUS—

All glassware should be of borosilicate glass and must be thoroughly cleaned with sulphuric and nitric acids and then thoroughly washed with distilled water immediately before use.

REAGENTS—

All reagents should be of analytical-reagent grade. Reagents for the destruction of organic matter are specified in the general methods referred to under "Procedure."

Water—Use de-ionised water or water re-distilled in an all-glass apparatus for the preparation of reagents and throughout the procedure.

Ammonia solution, 6 N—Purify by extraction with 15-ml portions of diethylammonium diethyldithiocarbamate solution until no more colour is extracted. Alternatively, concentrated ammonia solution "for foodstuff analysis" may be diluted with water.

Diethylammonium diethyldithiocarbamate solution—A 0·1 per cent. w/v solution in carbon tetrachloride.

EDTA - citrate solution—Dissolve 20 g of ammonium citrate and 5 g of disodium dihydrogen ethylenediaminetetra-acetate in water, and dilute to 100 ml with water. Purify by extraction with 15-ml portions of diethylammonium diethyldithiocarbamate solution until no more colour is extracted.

Potassium cyanide solution—A 5 per cent. w/v solution in water.

Sodium hydroxide solution, approximately N—Purify by extraction with 15-ml portions of diethylammonium diethyldithiocarbamate solution until no more colour is extracted.

Standard copper solution—Dissolve 0·393 g of copper sulphate, $CuSO_4.5H_2O$, in 2 N sulphuric acid, and dilute to 1 litre with 2 N sulphuric acid.

$$1 \text{ ml} \equiv 100 \ \mu\text{g of copper.}$$

Dilute this solution with 2 N sulphuric acid shortly before use to give a working standard solution containing 2 μg of copper per ml.

Sulphuric acid, 2 N.

Thymol blue indicator solution—Warm 0·1 g of thymol blue with 4·3 ml of 0·05 N sodium hydroxide and 5 ml of 90 per cent. v/v ethanol until dissolved; dilute with 20 per cent. v/v ethanol to 250 ml.

Procedure

Reagent blank value—

Carry out a blank test, by the entire procedure, on the precise amounts of reagents used in the test, omitting only the sample.

Destruction of organic matter—

General methods for the destruction of organic matter appear in a separate report[6] and, of these, Methods of Wet Decomposition (I)A, (I)B, (I)C and (I)D, with continuation (I)(*b*)1, are suitable for use in the determination of copper, the particular method being chosen to suit the type of sample to be analysed. Dry decomposition may be preferred in some circumstances for the destruction of organic matter in the sample; the recommended method can easily be adapted to such procedures, and it is not intended to preclude such methods. The possibility of losses under some conditions of dry decomposition must, however, be kept in mind; Gorsuch[7] has demonstrated and discussed the extent of these losses.

Cool the residue from the wet decomposition, and dilute it with water. If any insoluble matter is present, filter the solution through an acid-washed filter-paper, and wash the filter-paper with water.

Dilute the solution with water to any convenient volume.

Determination of copper—

Transfer 10 ml of EDTA - citrate solution and a 25-ml aliquot of the solution prepared from the decomposition residue to a separating funnel. Add 0·25 ml (5 drops) of thymol blue indicator solution and 6 N ammonia solution until the solution is green or bluish green; cool the solution before the final adjustment.

Shake the solution vigorously for 2 minutes with 15 ml of diethylammonium diethyldithiocarbamate solution, and allow the layers to separate. Place a piece of cotton-wool in the stem of the separating funnel, and, without delay, run the carbon tetrachloride layer into a 1-cm spectrophotometer cell. Take care to avoid undue exposure of the extract to light, otherwise fading may occur. Delay in separating the two layers after the extraction may also lead to fading.

Measure the optical density immediately against the reagent blank solution at 436 mμ. Convert the optical density obtained to micrograms of copper by reference to a calibration graph prepared as described under "Calibration" below.

Qualitative test for bismuth and tellurium—Transfer the carbon tetrachloride extract from the cell to a 25-ml test-tube or to a stoppered separating funnel. Add 10 ml of potassium cyanide solution, and shake. If the carbon tetrachloride layer turns colourless, bismuth and tellurium are absent.

Determination of copper in presence of bismuth or tellurium—Repeat the determination on another 25-ml aliquot of the sample solution. Transfer the carbon tetrachloride extract to a stoppered separating funnel, add 10 ml of N sodium hydroxide, and shake. After separation has taken place, remove the aqueous layer as completely as possible, repeat the washing with another 10-ml portion of N sodium hydroxide, and finally wash the carbon tetrachloride extract with 10 ml of water. Filter the extract through a cotton-wool plug inserted in the stem of the funnel. The aqueous layer should not be allowed to remain in contact with the organic layer any longer than is necessary, otherwise fading of the colour may occur; care should also be taken to avoid undue exposure of the organic layer to light.

Measure the optical density due to the copper diethyldithiocarbamate in the organic layer.

Calibration—Transfer to a series of separating funnels 10-ml portions of EDTA - citrate solution and the amounts of working standard copper solution and 2 N sulphuric acid shown below—

Working standard copper solution (1 ml ≡ 2 μg of copper), ml	0	1	2·5	5	10	15	20	25
2 N sulphuric acid, ml	25	24	22·5	20	15	10	5	0

Apply the procedure described under "Determination of Copper" to each mixture; measure the optical density of each solution against carbon tetrachloride, and construct a graph relating optical density at 436 mμ to micrograms of copper.

References

1. Blair, D., and Diehl, H., *Talanta*, 1961, **7**, 163.
2. International Union of Pure and Applied Chemistry, "Determination of Copper Content of Foodstuffs: Photometric Method," Butterworths Scientific Publications, London, 1959.
3. Sedivec, V., and Vasak, V., *Coll. Czech. Chem. Comm.*, 1950, **15**, 260.
4. Allport, N. L., and Garratt, D. C., *J. Soc. Chem. Ind.*, 1948, **67**, 382.
5. Jenkins, E. N., *Analyst*, 1954, **79**, 209.
6. Analytical Methods Committee, *Ibid.*, 1960, **85**, 643.
7. Gorsuch, T. T., *Ibid.*, 1959, **84**, 135.

The Determination of Magnesium in Aluminium Alloys by Atomic-absorption Spectroscopy*

By F. J. WALLACE

(*Foseco International Limited, Long Acre, Nechells, Birmingham 7*)

Aluminium seriously interferes in the atomic-absorption determination of magnesium in aluminium alloys, and a procedure is described in which 8-hydroxyquinoline is used to overcome this interference. With this reagent, interferences observed when sulphuric acid or nitric acid is present are also eliminated.

In the examination of alloys having high silicon contents a suitable compensating solution must be prepared.

It has been shown by previous workers[1,2,3,4] that the determination of magnesium by atomic-absorption spectroscopy is subject to interference, particularly from aluminium and silicon. Magnesium is, however, an important element in aluminium alloys, and its rapid determination is therefore of interest.

Various ways of avoiding the interference or of counteracting it have been suggested; Elwell and Gidley[1] use strontium, David[2] uses strontium and sulphate ions, Leithe and Hofer[3] use calcium and sulphate ions in an oxy-acetylene flame and Andrew[4] uses nickel. The amount of the reagent added to the magnesium solution is critical in most of the procedures mentioned above, and not all of them can be easily fitted into a routine composite analytical scheme for aluminium alloys. The work described in this paper represents an attempt to overcome some of these disadvantages.

It appeared, from an examination of the literature, that little work had been done to investigate the effect of organic reagents on the interference problems in atomic absorption, although Baker[5,6] demonstrated that sucrose has a strong effect on the absorption of calcium and he also looked into the effect of ethylenediaminetetra-acetic acid. Much work has been carried out on flame emission; in particular, Debras-Guédon and Voinovitch[7] investigated the action of 8-hydroxyquinoline on the emission of calcium and aluminium in the presence of fluoride and phosphate. The work described here extends the oxine technique of Debras-Guédon into the atomic-absorption field, and the findings suggest a method for determining magnesium in aluminium alloys.

Details of apparatus—

The equipment used in this work was a standard Hilger and Watts Uvispek, together with the Hilger atomic-absorption attachment modified to use an Evans Electroselenium atomiser. Throughout this work the normal potentiometric measuring circuit was used, no auxiliary equipment being attached.

The atomiser air-supply came from an Edwards RB.4 compressor with a stabilising tank of approximately 20-litres capacity in the air line.

Because of variations in the normal town-gas supply pressure, propane was used as fuel gas, the pressure being controlled through a single-stage regulator.

Operating conditions—

Preliminary work with solutions of pure magnesium established the optimum conditions listed below—

Lamp current	24 mA
Line wavelength	2582 Å
Slit width	0·1 mm
Air pressure	12 lb per sq. inch
Propane pressure	1 lb per sq. inch

It was found that the highest sensitivity was obtained when the propane pressure was 1 lb per sq. inch and the hollow-cathode lamp was so positioned that the radiation from it

* Presented at the meeting of the Midlands Section of the Society on Tuesday, October 9th, 1962. This paper received the Elwell Award for 1962.

passed through the base of the flame just above the tips of the blue cones. It had been observed that the sensitivity increased as the propane pressure was reduced from 6 lb per sq. inch to the finally chosen value of 1 lb per sq. inch, *i.e.*, the lowest pressure for which the regulator was calibrated.

Experimental

Initially, a calibration curve was prepared for pure magnesium over the range 0 to 20 p.p.m. (see Fig. 1, curve A). This shows good sensitivity, 0·1 p.p.m. of magnesium being easily detectable. A second calibration curve was prepared over the same range, but

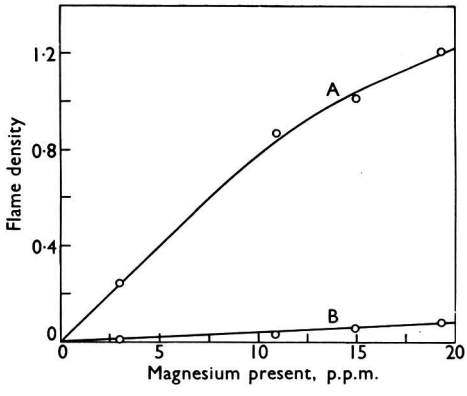


Fig. 1. Calibration curve for magnesium by atomic absorption: curve A, pure magnesium; curve B, magnesium *plus* 200 p.p.m. of aluminium.

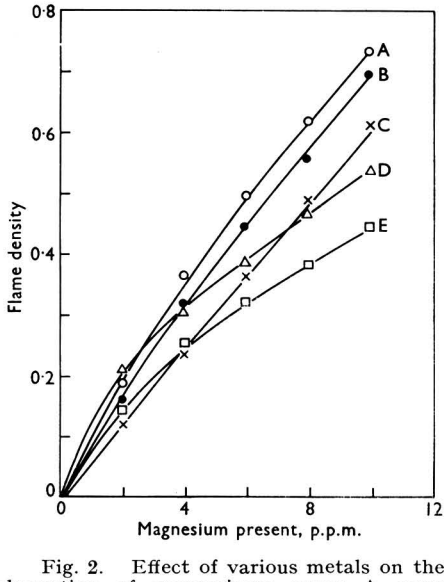


Fig. 2. Effect of various metals on the absorption of magnesium: curve A, pure magnesium; curve B, magnesium *plus* 200 p.p.m. of lead; curve C, magnesium *plus* 200 p.p.m. of copper; curve D, magnesium *plus* 200 p.p.m. of nickel; curve E, magnesium *plus* 200 p.p.m. of manganese

200 p.p.m. of aluminium were introduced into each standard magnesium solution. The resulting curve (Fig. 1, curve B) shows that 20 p.p.m. of magnesium in the presence of 200 p.p.m. of aluminium has a lower absorption than 1 p.p.m. of magnesium alone.

Fig. 2 (curves A to E) shows the effect of copper, nickel, iron, manganese and lead at similar concentrations to those quoted above.

Elwell and Gidley[1] have shown that both sulphuric and nitric acids depress the magnesium absorption, whereas hydrochloric acid has a slight enhancing effect.

To discover the effect of 8-hydroxyquinoline (oxine) on the absorption of magnesium in an aluminium - magnesium mixture, a solution was prepared so that 1 litre contained 10 g of this reagent in dilute acetic acid. This solution was then added in increasing amounts to a series of solutions that finally contained 14 p.p.m. of magnesium and 186 p.p.m. of aluminium in the presence of 10 per cent. v/v of hydrochloric acid. It was calculated that approximately 33 ml of the oxine solution would be sufficient just to complex all the metal present. The volume of solution required was not calculated more accurately because of the difficulties in preparing a reliable volumetric solution of oxine.

Additions of the solution increasing by 5-ml steps to 30 ml and then by 1-ml steps to 35 ml were made to the aluminium - magnesium solutions, which were finally diluted to 100 ml.

The resulting solutions, each containing similar amounts of magnesium and aluminium, were then sprayed, and the flame densities noted. The results of these measurements are shown in Fig. 3 (*a*) and (*b*).

To establish whether or not the increases in density shown in Fig. 3 were due to a definite effect on the mechanism of the interference or merely to the effect frequently termed "organic enhancement," a second experiment was carried out. Four series of solutions were prepared, each consisting of three solutions containing 4, 10 or 20 p.p.m. of magnesium. Nothing was added to the first series of solutions; to the second were added 10 ml of concentrated hydrochloric acid; to the third were added 10 ml of concentrated hydrochloric acid and 25 ml of 20 per cent. w/v oxine solution; to the fourth were added 10 ml of the hydrochloric acid and 50 ml of the oxine solution. The first three series of solutions were used to investigate the effects of hydrochloric acid alone and of oxine on magnesium. The fourth set of solutions containing double amounts of oxine, was used to investigate how critical was the amount of oxine added, the 50-ml addition of oxine solution corresponding to a 100-ml addition to the solution used in the preparation of the graph shown in Fig. 3. The solutions were sprayed and the flame densities measured. The resulting curves are shown in Fig. 4.

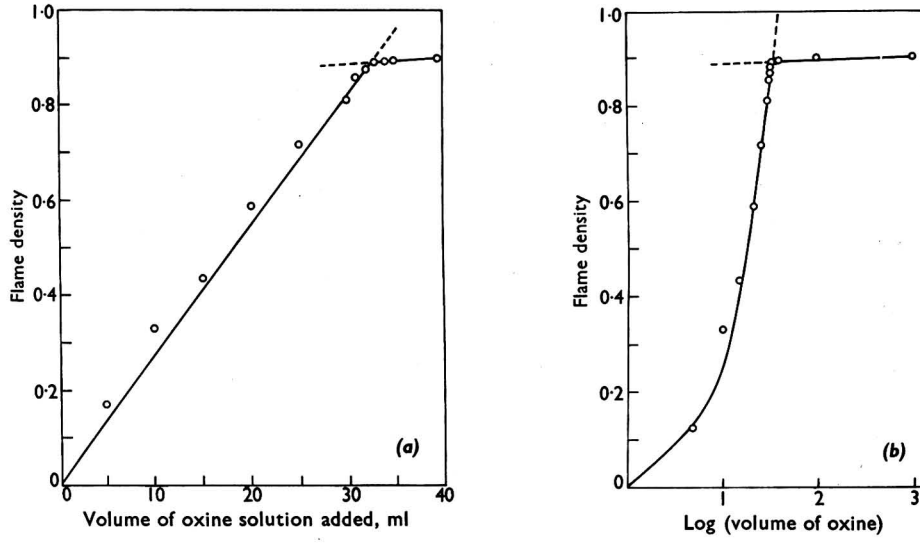


Fig. 3. Effect of oxine on magnesium in the presence of aluminium

Further investigation with solutions of pure metal indicated that all the interference shown in Fig. 2, with the exception of that by manganese, could be avoided by the use of oxine. Further, by using this technique, the effect of sulphuric and nitric acids reported by Elwell and Gidley is overcome.

Silicon was known to interfere, but it was thought that its effect could be best assessed during test analyses of alloy samples; it was considered unlikely that oxine would have any effect on the interference by silicon.

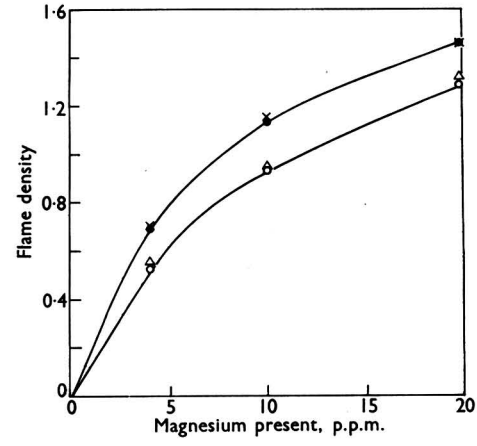


Fig. 4. Effect of oxine on pure magnesium: ○, pure magnesium; △, magnesium *plus* 10 per cent. hydrochloric acid; ●, magnesium *plus* 25 ml of oxine solution; ×, magnesium *plus* 50 ml of oxine solution

PREPARATION OF CALIBRATION CURVES—

Samples (1 g) of a series of British Chemical Standard alloys were dissolved in 40 ml of 20 per cent. w/v sodium hydroxide solution. The solution was acidified with 50 ml of 50 per cent. v/v nitric acid and boiled to dissolve precipitated hydroxides. The solution was then cooled, and diluted to 250 ml; a series of 5-ml portions was taken and additions of standard magnesium solution were made. Ten millilitres of concentrated hydrochloric acid and 25 ml of oxine solution were added, and the solution was diluted to 100 ml. The solutions were sprayed, and the magnesium absorption was measured. The resulting curves are shown in Fig 5.

It can be seen that two distinct curves are obtained. The main difference is that the standards used in the preparation of curve B had a high silicon content (11 per cent.), so that the observation made above that oxine would not counteract interference by silicon appears to be true. Curve A was prepared from alloys having silicon contents of less than 5 per cent.

ANALYSIS OF UNKNOWN SAMPLES—

Samples of aluminium alloys were analysed by the procedure described above.

For samples with magnesium contents of 4 to 9 per cent., 5-ml portions of solution were taken, and 50-ml portions were taken for samples with magnesium contents below 4 per cent. In each instance, the size of the portion used for the preparation of the calibration graph was similar to that used for the samples. It was necessary to spray standards every time analyses were carried out, because the position of the calibration curve varied slightly from day to day. This movement in the position of the curve was thought to be due to long-term variations in the atomiser and in the relative positions of the hollow-cathode lamp and the flame. The results are shown in Table I.

TABLE I

DETERMINATION OF MAGNESIUM

Sample No.	Alloy	Magnesium found by— proposed procedure, %	Magnesium found by— chemical method, %
1	Duralumin-type alloy (4% of copper, <0·7% of silicon, 0·3% of iron and 0·6% of manganese)	0·60, 0·59	0·62
2		0·65, 0·66	0·66
3	B.S.S. 1490 LM 6	0·11, 0·12	0·14
4	Aluminium - magnesium - silicon alloy (<0·1% of copper, 0·7% of silicon and 0·3% of iron)	0·69, 0·70	0·64
179	Magnesium - aluminium binary alloy (B.S.S. 1490 LM 5)	4·29	4·24
182		4·52	4·50
211		4·30	4·30
218		4·20	4·20
223		3·62	3·52
314*		3·00	2·96, 2·98, 2·95, 2·96
315*		3·00	2·95, 2·91, 2·99, 3·05
316*		3·00	3·00, 3·01, 3·00, 2·86
317*		3·15	3·03, 3·05, 3·02, 3·03

* Samples analysed chemically by four different methods, including one analysis in an independent referee laboratory.

One sample (sample 321) was analysed 14 times over a period of 3 days to evaluate the reproducibility of the method. The results were 2·65, 2·60, 2·58, 2·69, 2·57, 2·59, 2·56, 2·62, 2·63, 2·58, 2·59, 2·60, 2·57 and 2·67 per cent. of magnesium, average result 2·607 per cent.; the standard deviation was 0·038 per cent. A referee laboratory obtained a result of 2·60 per cent. The result when a sulphide separation and phosphate finish were employed was 2·61 per cent. The sample was taken from a magnesium - aluminium alloy containing <0·1 per cent. of copper, <0·6 per cent. of silicon, <0·7 per cent. of iron, <0·5 per cent. of manganese and <0·1 per cent. of zinc.

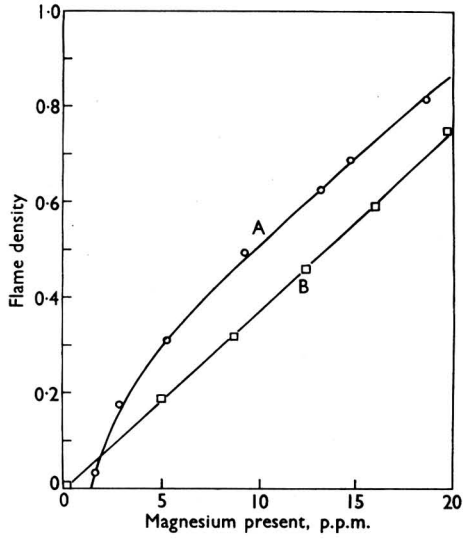


Fig. 5. Calibration curves for magnesium in aluminium alloys: curve A, low silicon alloys; curve B, high silicon alloys

METHOD

REAGENTS—

8-Hydroxyquinoline (oxine) solution—Dissolve 200 g of 8-hydroxyquinoline in 286 ml of glacial acetic acid and 200 ml of water, and dilute to 1 litre.

Sodium hydroxide solution, 20 per cent. w/v—Dissolve 200 g of sodium hydroxide in 500 ml of water, and dilute to 1 litre.

Nitric acid, 50 per cent. v/v—Dilute 500 ml of concentrated nitric acid to 1 litre.

Hydrochloric acid, concentrated.

PROCEDURE—

Dissolve a 1-g sample of aluminium alloy in 40 ml of 20 per cent. w/v sodium hydroxide solution, and, when the attack has ceased, add cautiously 50 ml of 50 per cent. v/v nitric acid. Boil the solution until all precipitated hydroxides have redissolved. Filter the solution, and dilute to 250 ml in a calibrated flask. Transfer, by pipette, a suitable portion (for magnesium contents of 4 to 9 per cent. use 5 ml, and 50 ml for magnesium contents of 0 to 4 per cent.) to a 100-ml calibrated flask, add 10 ml of concentrated hydrochloric acid and 25 ml of oxine solution, and dilute to the mark with water. Spray, and measure the flame densities three times under the conditions described under "Operating Conditions," p. 259. Calculate the average flame density.

The calibration curve should be prepared from similar aluminium alloys of known magnesium content in order to allow for the effect of silicon.

DISCUSSION OF RESULTS

The investigation shows that the addition of oxine solution to aluminium - magnesium solutions counteracts the depressive effects of aluminium on magnesium absorption. However, the curve obtained for magnesium in the presence of aluminium after the treatment with oxine is not the same as that obtained from pure magnesium solutions; there is some residual loss in density. It is clear from the results shown in Figs. 1, 2 and 3 that the effect is much more than just the frequently reported "organic enhancement," and it is closely related to the formation of co-ordinate complexes in solution.

Other work carried out in these laboratories indicates that the curve obtained from magnesium in the presence of large excesses of barium (see Fig. 6) is, when the solution is treated with oxine, similar to the aluminium - magnesium curves shown here.

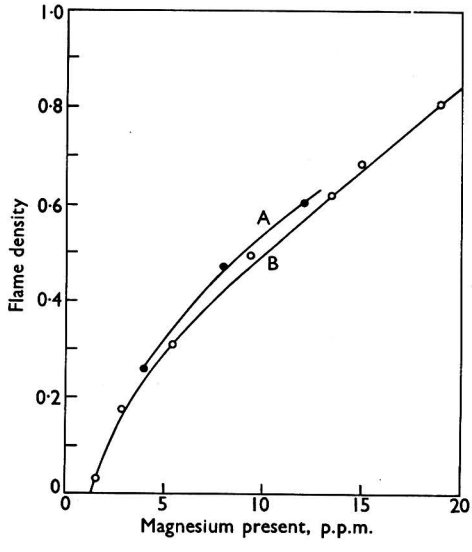


Fig. 6. Comparison of curves for magnesium in presence of: curve A, barium; curve B, aluminium

Elwell and Gidley[1] suggest a stable aluminium - magnesium compound, possibly a mixed oxide, as the cause of interference between magnesium and aluminium. They point out that the production of atoms from organo-metallic compounds is usually exothermic, but the production of metallic atoms from aqueous droplets is almost always endothermic.

Consideration of the facts reported here in the light of the above statements by Elwell and Gidley would seem to lead to the conclusion that, when the droplet enters the flame, it is dried out and disintegrates, possibly explosively, giving an organic vapour in which the magnesium and aluminium compounds are separate from one another. This vapour would then burn, and the magnesium atoms released would not be in close contact with aluminium compounds, so that no opportunity would exist for the formation of a stable mixed-oxide phase.

If this explanation is valid, it would seem reasonable to expect some evidence of the transient existence of free aluminium atoms in the flame. Debras-Guédon and Voinovitch have reported that it is possible to detect significant atomic aluminium emission from a flame into which aluminium oxinate solutions have been sprayed. This would have a definite bearing on the mechanism suggested above, and it was thought that the flame-emission experiment could profitably be repeated. It was found that the emission of the atomic line **3961** Å of aluminium was easily detectable from an oxy-hydrogen flame into which was sprayed a solution containing **200** p.p.m. of aluminium and **25** ml of oxine solution per **100** ml. This would seem to establish that simple atomic species of aluminium exist in the flame under these conditions, although their life is probably extremely short.

Conclusion

It is possible to counteract the interference of aluminium on magnesium atomic absorption by the use of oxine solution. The technique does not compensate for the interference by silicon, but in alloys with high silicon contents this difficulty can be overcome by using calibration standards with similar silicon contents. Alternatively, silicon can be removed by suitable chemical pre-treatment.

The method can be easily fitted into a composite scheme for determining manganese, copper, iron, nickel, titanium, zinc and magnesium in aluminium alloys on one sample weighing.

I thank the Directors of Foseco International Limited for permission to publish this paper, and Messrs. R. F. Boddey and F. E. G. Ravault for helpful discussions during the investigation.

References

1. Elwell, W. T., and Gidley, J. A. F., "Atomic-absorption Spectrophotometry," Pergamon Press, Oxford, London, New York and Paris, 1961.
2. David, D. J., *Analyst*, 1958, **83**, 655.
3. Leithe, W., and Hofer, A., *Mikrochim. Acta*, 1961, 268.
4. Andrew, T. R., and Nichols, P. N. R., *Analyst*, 1962, **87**, 25.
5. Baker, C. A., paper presented at the joint meeting of the Western Section and the Physical Methods Group of the Society for Analytical Chemistry, May, 1960.
6. Baker, C. A., and Garton, F. W. J., U.K.A.E.A. Report AERE R3490, 1961.
7. Debras-Guédon, J., and Voinovitch, I. A., *Chem. Anal., Warsaw*, 1960, **5**, 193.

Received *August 14th*, 1962

The Determination of Constituents of Rocks and Minerals by Gas Chromatography

Part II*. The Determination of Some Gaseous Constituents

BY P. G. JEFFERY AND P. J. KIPPING

(*Department of Scientific and Industrial Research, Warren Spring Laboratory, Stevenage, Herts.*)

When rock and mineral samples are ignited in an inert atmosphere, several gaseous components are evolved. Chromatographic columns containing the appropriate stationary phase and a thermal conductivity cell have been used for separating and detecting these components. Gases that have been determined in this way include hydrogen, helium, oxygen, nitrogen and argon. The presence of carbon monoxide, carbon dioxide and hydrocarbon gases in the mixture of gases evolved on pyrolysis has also been recorded.

THAT rocks and minerals evolve large amounts of gases and vapours when heated is well known; the total volume may be many times that of the solid. Hillebrand,[1] for example, has reported that the volatile products usually comprise hydrogen and carbon dioxide with carbon monoxide, methane, nitrogen, hydrogen sulphide and other gases in smaller amounts. In recent years considerable interest has been shown in the occurrence of gaseous components of rocks and minerals. Stevenson,[2] for example, has described a method for determining nitrogen in rocks and silicate minerals, Iwasaki, Katsura and Yoshida[3] and Cuttitta[4] have described methods for determining oxygen in certain minerals and Shorokhov[5] has described a method for determining hydrogen in sedimentary rocks.

None of these methods is of general application to the determination of gaseous constituents, and none of them is easy to undertake. A somewhat simpler procedure has now been devised, based on gas-chromatographic separation and detection after evolution of the gaseous components in a small reactor attached to the chromatographic apparatus.

A similar technique has been described[6] for the gas-chromatographic determination of carbon dioxide in rocks and minerals. There the problem involved in the design of the reactor was essentially that of dealing with the high pressure developed by the liberated carbon dioxide. In the investigation described here, the problem was to design a small reactor to operate at a temperature high enough to effect complete decomposition of the sample materials in a short time.

DESCRIPTION OF APPARATUS

Initial experiments with gas burners and electric tube furnaces showed that the most satisfactory way of heating a sample was to insert it, in a platinum boat and mixed with a suitable flux, into a silica tube heated by a Meker-type gas burner. This ensured rapid and complete evolution of the gaseous constituents when samples were ground and fused as described below.

Complete expulsion of gaseous constituents is probably not achieved by direct ignition except at temperatures in excess of those permissible in an apparatus fabricated from silica. For this reason it is preferable to decompose the rock and mineral samples by fusion. Several fluxes were tried for this, but the most suitable was found to be the mixture of sodium tungstate and borax glass previously used by Jeffery and Wilson[7] for the decomposition of silicate rocks. When 100-mg portions of rock and minerals were used, decomposition was complete after fusion for a few minutes. This size of sample is sufficient for determining hydrogen and helium, although larger portions are usually required for determining the other constituents.

The reactor is shown in Fig. 1. It consists of a small silica tube, approximately 6 inches long, to which a 9/18 silica ball-joint has been fused. The spring clip supplied did not keep this joint gas-tight, and for this reason two small metal plates, with holes of the appropriate size, were bolted together across the ball-joint. With these in position and the outer part

* For details of Part I of this series, see reference list, p. 271.

of the surface of the ground ball lightly smeared with Apiezon T grease, a gas-tight joint was obtained. The apparatus, as shown, could be improved by using silica ball-joints at both ends of the silica tube used for the ignition. The instructions given above for making the joint gas-tight would also apply to the additional joint. The three-way taps shown in Fig. 1 and the by-pass sample injector[8] were all spring loaded.

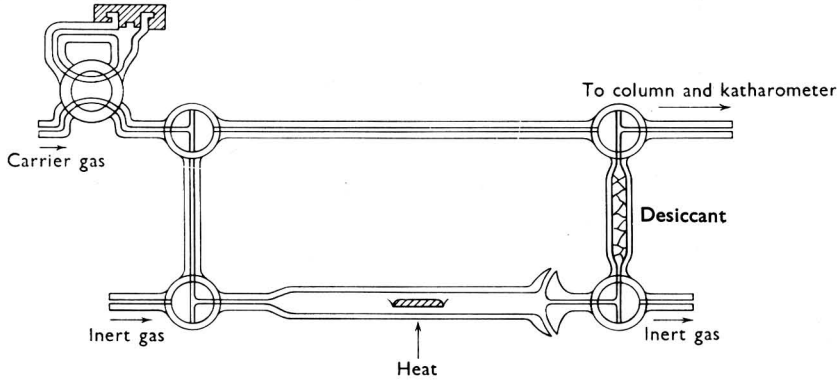


Fig. 1. Apparatus for fusion or ignition of rock and mineral samples

The desiccant is incorporated only to prevent the rapid deterioration of the molecular-sieve column, and is a coarse grade of self-indicating silica gel. Other desiccants could be employed, or the desiccant omitted altogether. If the desiccant were omitted the column material would require regeneration at intervals of about 10 days.

The remainder of the apparatus, not shown in the figure, consists of a thermal conductivity cell with associated Wheatstone-bridge network, potentiometric recorder, gas-flow control and measuring devices. These were all of the conventional type. The chromatographic column used was a 6-foot length of brass tubing (outside diameter $\frac{3}{16}$ inch) packed with molecular sieve No. 5A, ground to −44- to +72-mesh size and activated in the usual way. This column was used to separate hydrogen, helium, oxygen, nitrogen, argon and carbon monoxide. For the separation of carbon dioxide a 2-foot long column of activated charcoal was used. For separating and identifying gaseous hydrocarbons a commercial gas chromatograph was used in conjunction with a modified alumina column as previously described.[9]

The choice of carrier gas, supplied from a cylinder fitted with a two-stage regulator, depends on the constituent being determined. An inert-gas supply was also provided when hydrogen was used as carrier gas.

Method

General procedure—

For the decomposition of a 50-mg portion of ground rock material, weigh approximately 0·15 g of the mixed sodium tetraborate - sodium tungstate flux into a small platinum boat, and fuse thoroughly to ensure complete expulsion of all absorbed gases. Cool rapidly in a desiccator. Accurately weigh approximately 50 mg of the finely ground rock material on to the melt, and transfer the boat and contents to the silica tube. Complete the assembly of the reactor as shown in Fig. 1, and replace the gas in the apparatus by carrier gas. If the ignition is to be conducted in an inert atmosphere, replace the carrier gas in the silica tube by the appropriate inert gas.

With the carrier gas passing directly to the katharometer, heat the tube for sufficient time to ensure fusion of the sample and complete expulsion of the gaseous constituents. Allow the silica tube to cool for about 10 minutes, and then divert the flow of carrier gas to the katharometer via the ignition tube. Once the gas has been swept on to the column, restore the direct flow of carrier gas to the katharometer.

Calibrate the katharometer response by introducing portions of pure gas into the carrier gas stream by means of the by-pass sample injector, as previously described.[8]

DETERMINATION OF HELIUM—

For this determination hydrogen is used as carrier gas, and the ignition is undertaken in an atmosphere of nitrogen. No difficulty was experienced in separating helium from the excess of nitrogen present. To increase the accuracy with which the helium response could be measured, a recorder with a high chart-speed (60 inches per hour) was used. A typical chromatogram is shown in Fig. 2, and some results for a series of uranium-bearing materials are shown in Table I.

TABLE I

HELIUM CONTENT OF SOME URANIUM-BEARING ROCKS

Sample No.	Rock material	Helium found (by weight), %
M182	Pitchblende, Uluguru, Tanganyika	0·05
M229	Autunite, Katanga	0·009
M216	Torbernite, unknown locality	0·010
M230	Davidite, Mozambique	0·062
M151	Monazite, Travancore	0·016

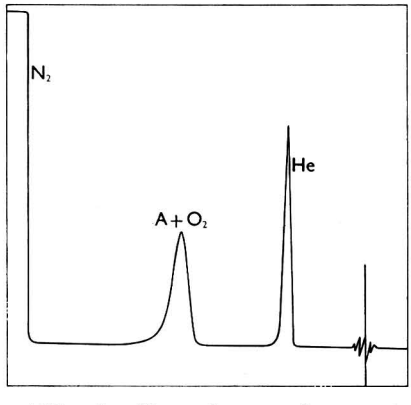


Fig. 2. Chromatogram of gases obtained from pitchblende (sample M182) with hydrogen as carrier gas and the sample fused in an atmosphere of nitrogen

DETERMINATION OF ARGON—

At normal temperatures it is not possible to separate argon from oxygen on a molecular-sieve column, and for this reason oxygen was used as carrier gas in this determination. This also reduced the amount of hydrogen evolved, and hence improved the separation of argon from hydrogen.

The apparatus shown in Fig. 1 was designed for the decomposition by fusion of samples weighing not more than 100 mg. This is insufficient material on which to determine argon. Approximate determinations have, however, been made by igniting samples of approximately 1 g in this apparatus. A typical chromatogram is shown in Fig. 3. When oxygen is used as carrier gas, the response of hydrogen and methane is in the opposite direction to that of argon and nitrogen. The small difference in thermal conductivity between oxygen and nitrogen is the reason for the insensitivity to nitrogen. The ratio of the peak areas of argon to nitrogen is, in the chromatogram shown, greatly in excess of the corresponding ratio for these gases in atmospheric air. The argon response is that of a volume of approximately 0·016 ml, representing 0·003 per cent. by weight of argon in the original material.

DETERMINATION OF HYDROGEN—

For this determination argon is used as carrier gas. The fusion of the sample material is also undertaken in an atmosphere of argon. The great difference in thermal conductivities of hydrogen and argon leads to a large chromatographic response from hydrogen. To measure

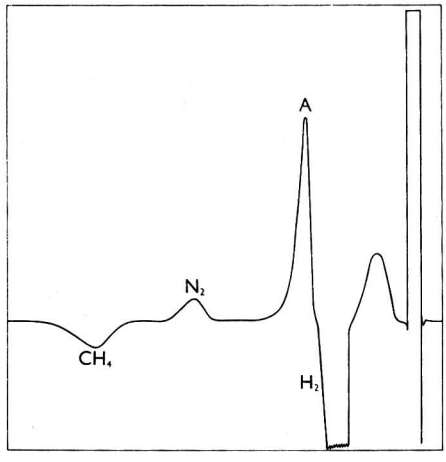


Fig. 3. Chromatogram of gases obtained from a silicate rock (sample R138; granite) with oxygen as carrier gas

this response, the device usually referred to as "backing off"[10] is used. Mixtures of hydrogen and helium would not be resolved on the 6-foot column used, but separate experiments with hydrogen as the carrier gas have shown the absence of measurable amounts of helium in all the silicate materials so far examined. Some results for a few silicate rocks are shown in Table II.

TABLE II

HYDROGEN CONTENT OF SOME ROCK SAMPLES

Sample No.	Rock material	Hydrogen content— found by volume, %	found by weight, %	calculated by weight,* %
R117	Granite, Shetland	55·0	0·0019	0·005
		65·0	0·0022	
R138	Granite, Cornwall	720	0·025	0·030
		700	0·024	
G–1	Granite, Westerly, R.I., U.S.A.	122	0·0042	0·014
		125	0·0043	
		127	0·0045	
W–1	Diabase, Centerville, Va., U.S.A. (Dolerite)	260	0·0087	0·122
		210	0·0068	

* Based on the FeO content, assuming hydrogen to be produced by the reaction—

$$H_2O + 2FeO = Fe_2O_3 + H_2.$$

There is a clear indication that the amount of hydrogen liberated is roughly reproducible for each sample of rock. In all the samples examined, the amounts of hydrogen produced were less than the amounts equivalent to the ferrous iron present, and it is therefore feasible that elemental hydrogen could be produced by reduction of water in this way. Even when sample ignition is undertaken in an atmosphere of pure oxygen, hydrogen is still recorded. The alternative explanation is that elemental hydrogen is an essential component of these materials. The experiments described have given no indication of which of these explanations is correct.

DETERMINATION OF OXYGEN AND NITROGEN—

These gases are separated on a column of molecular sieve, with argon as carrier gas. As for the determination of argon, it is usually necessary to take a large sample (0·5 to 1·0 g) if accurate peak measurements are required. Whatever the size of the sample, prolonged flushing of the reaction vessel with carrier gas is necessary to remove the entrained air. That it is possible to do this has been shown by the failure to detect oxygen in the gas evolved from certain rocks and minerals. The oxygen response appears on the trailing edge of the hydrogen response, but the separation can be improved by the introduction of a second column containing activated charcoal.

DETERMINATION OF CARBON DIOXIDE—

A well known procedure for determining carbon dioxide in rocks and minerals[11] is based on chemical absorption after direct ignition of the sample material. It is clear from the work described here that such an evolution procedure is accompanied by the liberation or production of a wide variety of carbon-containing compounds. For this reason the procedure previously described[6] (for determining carbon dioxide after digestion with phosphoric acid) is preferred to any procedure based on direct ignition of the sample material. It is clearly understood, however, that the preferred procedure will determine only that carbon dioxide readily liberated, *i.e.*, that present in carbonate minerals.

PYROLYSIS OF SILICATE ROCK MATERIAL—

As noted above, a limitation imposed by the use of apparatus made of silica is that complete evolution of gaseous constituents is probably not obtained by direct ignition at the temperatures used. When this technique is used, however, some interesting gaseous products have been detected. These include carbon monoxide, methane, ethane, ethylene and higher hydrocarbons, saturated and unsaturated. These gases are either formed during the pyrolysis or undergo reaction under these conditions, as no successive series of determinations produced any kind of agreement.

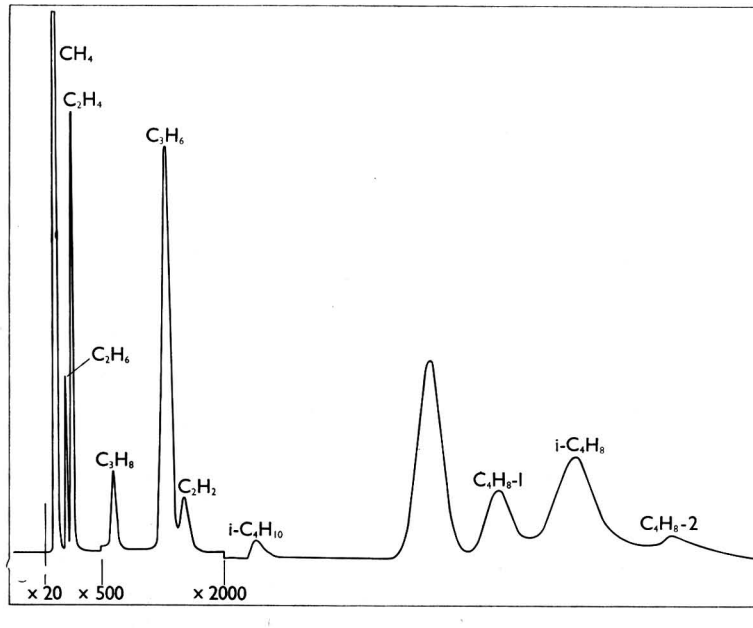


Fig. 4. Chromatogram of hydrocarbon gases obtained on pyrolysis of a silicate rock (sample R 138; granite). Separation on a column of modified alumina, hydrogen - nitrogen mixture as carrier gas and flame ionisation detector

No silicate rock has yet been examined in which carbon dioxide could not be detected, and the presence of ferrous iron in unaltered igneous silicate rocks has long been established. Carbon monoxide could therefore be formed from carbonate minerals by reduction with ferrous iron, and the hydrocarbon gases could be formed from catalytic conversion of the carbon monoxide - hydrogen mixture. The alternative explanation is that these hydrocarbons are essential components of rocks and minerals; we have not, however, been able to extract any hydrocarbons from these materials with a variety of organic solvents.

The hydrocarbons noted on pyrolysis were separated on an alumina column, suitably de-activated by adding liquid paraffin as described previously,[9] and detected by flame ionisation. A chromatogram obtained from one specimen is shown in Fig. 4.

The work described in this paper has been completed as part of a research programme of Warren Spring Laboratory, and is published by permission of the Director.

References

1. Hillebrand, W. F., "The Analysis of Silicate and Carbonate Rocks," U.S. Geological Survey Bulletin No. 700, Washington, D.C., 1919, p. 242.
2. Stevenson, F. J., *Anal. Chem.*, 1960, **32**, 1704.
3. Iwasaki, I., Katsura, T., and Yoshida, M., *Bull. Chem. Soc. Japan*, 1960, **33**, 1340.
4. Cuttitta, F., U.S. Geological Survey, Prof. Paper, No. 400-B, Washington, D.C., 1960, p. 488.
5. Shorokhov, N. R., *Trudy Soyzn. Geologporsk. Kontura Gluagaza pri Sov. Min. U.S.S.R.*, 1960, No. 12, 64.
6. Jeffery, P. G., and Kipping, P. J., *Analyst*, 1962, **87**, 379.
7. Jeffery, P. G., and Wilson, A. D., *Ibid.*, 1960, **85**, 749.
8. Kipping, P. J., and Jeffery, P. G., *Ibid.*, 1961, **86**, 680.
9. "Gas Analysis by Gas Chromatography," D.S.I.R., Warren Spring Laboratory, Stevenage, May, 1962.
10. Kipping, P. J., *Nature*, 1961, **191**, 270.
11. Riley, J. P., *Analyst*, 1958, **83**, 42.

NOTE—Reference 6 is to Part I of this series.

Received *September 13th*, 1962

Rapid Determination of Carbon in Steel by Infrared Gas Analysis

By G. A. TIPLER

(*Richard Thomas & Baldwins Ltd., Midland Section Laboratory, Cookley Works, Brierley Hill, Staffs.*)

A completely automatic apparatus is described for determining carbon in steel. The carbon in the sample is converted to carbon dioxide by combustion in a high-frequency induction heater. Oxygen is used to sweep the combustion products into a dry gasholder, and the gases collected are then passed through an infrared gas analyser.

With the apparatus less than 0·01 per cent. of carbon in a sample can be determined, with an error of ±0·0005 per cent., in 4 minutes.

Several methods are available for determining carbon below the 0·01 per cent. level in steel, but generally the more accurate the determination required the longer it takes to perform. It was with the aim of producing a method capable of determining less than 0·01 per cent. of carbon with an error not greater than ±0·0005 per cent. that could be completed in less than 4 minutes that the technique described in this paper was developed. It was a further aim that the operation should be simple and capable of use under routine control-laboratory conditions.

Principle of the method—

The carbon in the sample is converted to carbon dioxide by combustion in a Radyne 1-kW high-frequency induction heater. The combustion products are collected in a dry gasholder of known volume and then blown through an infrared gas analyser. From the concentration and volume the total carbon dioxide and hence the percentage of carbon in the sample is calculated. The gas analyser is specific for carbon dioxide and there is therefore no need to remove sulphur dioxide or water vapour from the gas stream.

Gas analysers capable of measuring sulphur dioxide are available, and it is possible to determine carbon and sulphur simultaneously by connecting two analysers in series.

A description of the principle of the gas analyser is given on p. 278.

Simple form of the apparatus—

The high-frequency heater is available complete with the combustion chamber and sample loading mechanism. The gasholder is made from Perspex and is sold as a gas sampler. The gasholder is connected to the combustion chamber via a 3-way tap with T-shaped bore, as shown in Fig. 1. After the sample has been placed in the combustion chamber, the 3-way tap is turned so that there is a direct connection to the gas analyser. This allows oxygen to blow out any atmospheric carbon dioxide. The tap is then turned to stop the gas flow from the combustion chamber, and the high-frequency heater is switched on. The sample burns and draws all the oxygen it requires from the purified oxygen supply connected to the inlet of the combustion chamber. Progress of the combustion can be seen through the combustion chamber walls, and, when complete, the tap is turned to blow the combustion products into the gasholder. The dust filter removes any iron or flux oxides that blow out

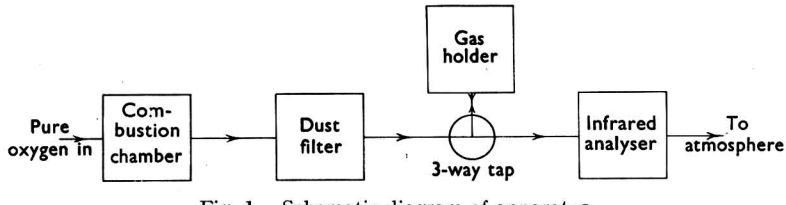


Fig. 1. Schematic diagram of apparatus

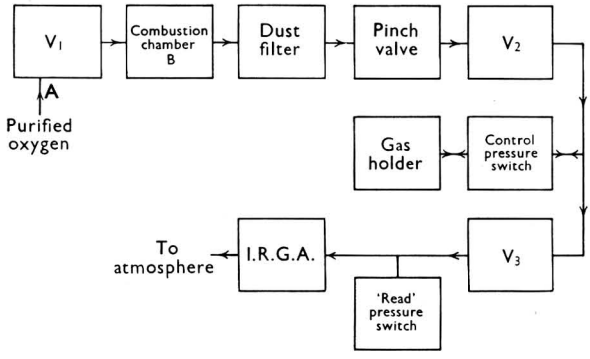


Fig. 2. Schematic diagram of gas flow

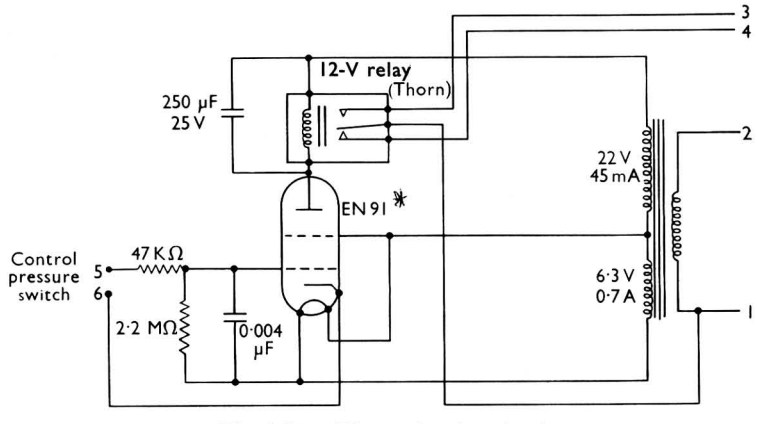


Fig. 3 (*a*). Electronic relay circuit

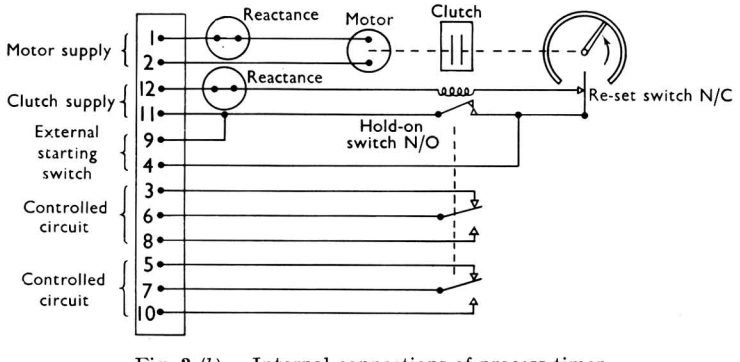


Fig. 3 (*b*). Internal connections of process timer

of the combustion chamber. As the piston in the holder approaches the top, the gas flow is reduced so that there is no pressurisation. Diffusion in the holder is extremely rapid and the mixed gases can be immediately passed into the gas analyser by turning the 3-way tap. The pressure required to raise the piston to the top of the holder can be measured by inserting a gauge between the tap and the gasholder.

This technique is simple, but a more refined apparatus was required that would relieve the operator from all duties after inserting the sample. The automatic apparatus described below was therefore designed.

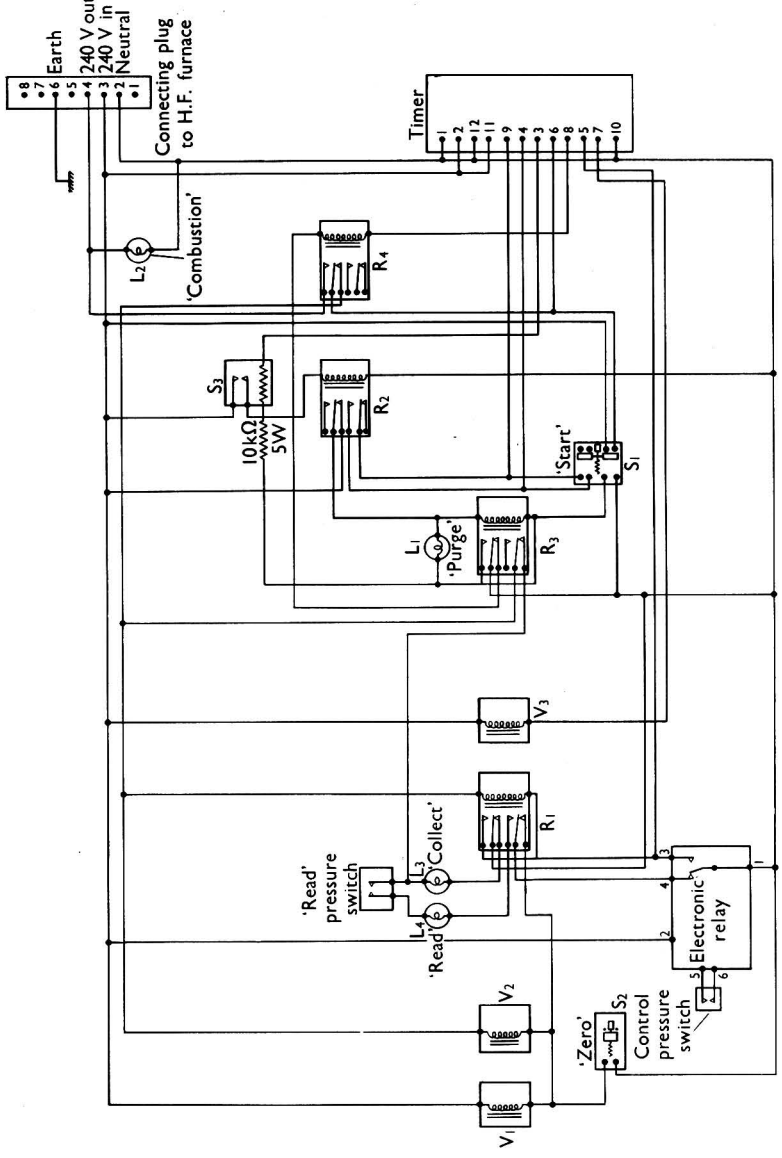


Fig. 3 (*c*). Theoretical circuit of Autocarb unit

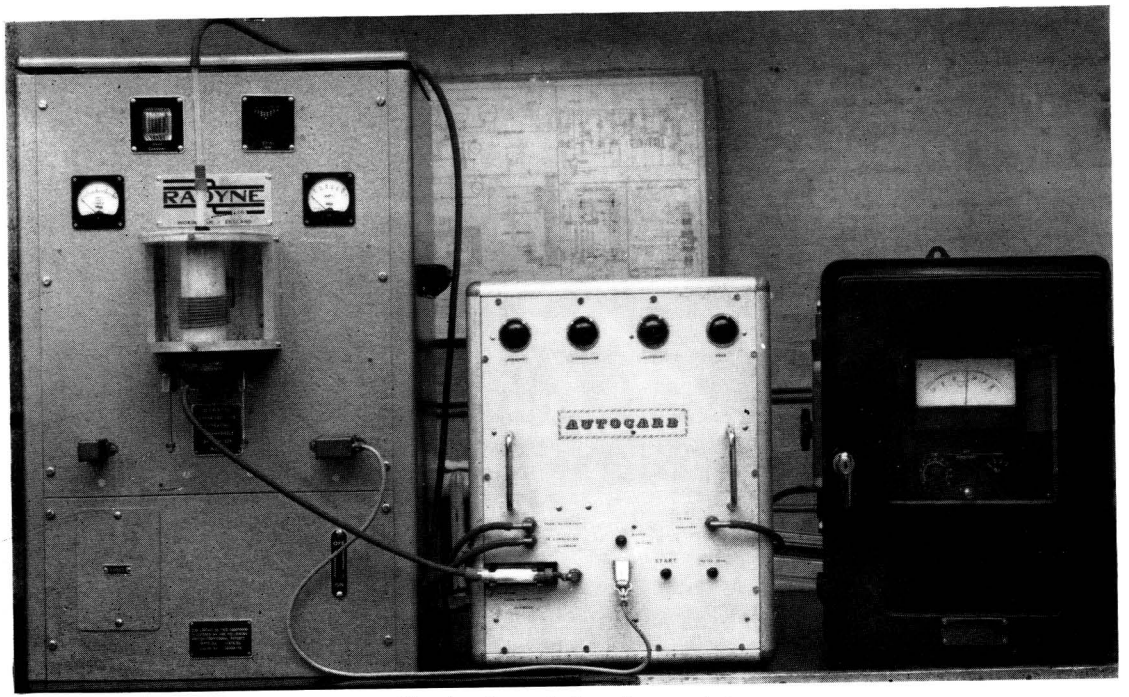


Fig. 4. General view of apparatus

[To face p. 274

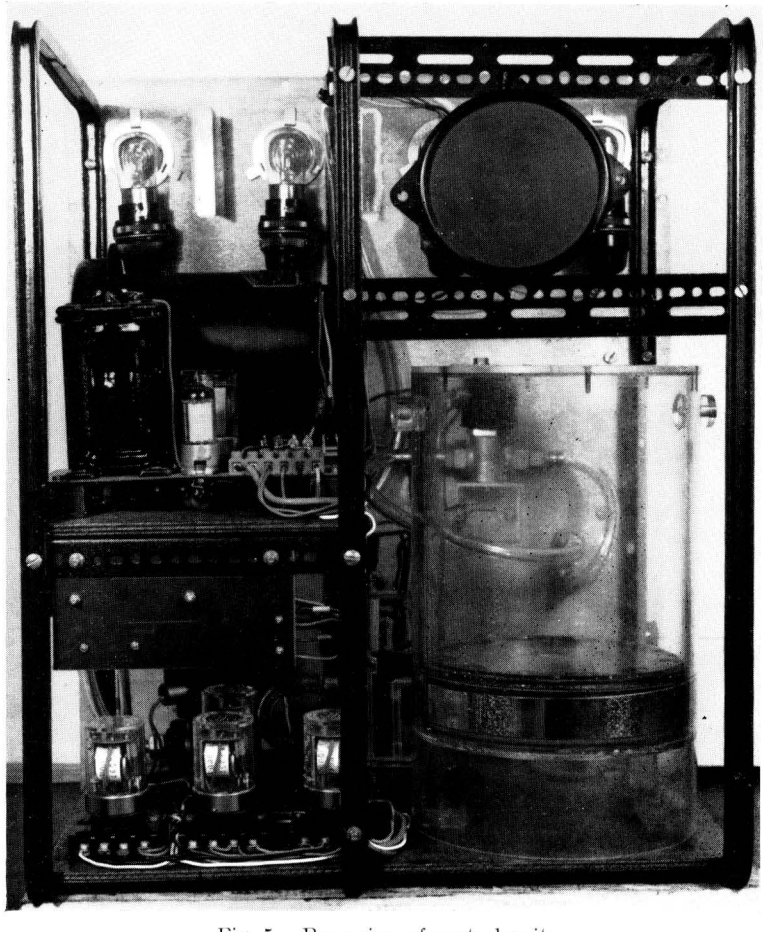


Fig. 5. Rear view of control unit

AUTOMATIC APPARATUS—

Oxygen at 6 lb per sq. inch, purified by conventional means, is fed in at point A (see Fig. 2) and flows out to the combustion chamber at B after passing through valve V_1.

Valve V_1 is normally closed until the sample is placed in the combustion chamber and the start switch is depressed. V_1, V_2 and V_3 then open to allow purified oxygen to sweep out any atmospheric carbon dioxide from the combustion chamber.

After a pre-set time, V_2 and V_3 close and the high-frequency power switches on for a second pre-set time to burn the sample. At the end of the combustion V_2 opens, and the combustion products are collected in the gasholder. When a fixed amount of gas has been collected, valves V_1 and V_2 close and V_3 opens. The gases then flow out to the gas analyser.

The process is completely automatic from when the start switch is depressed. Indicator lights show the progress of the analysis and when to take the reading from the meter, which is calibrated directly in percentage of carbon.

The electrical circuit is shown in Figs. 3 (*a*), (*b*) and (*c*). A general view of the apparatus and the rear view of the control unit are shown in Figs. 4 and 5.

DETAILS OF PRESSURE SWITCH—

To ensure that the same number of moles of gas are collected in the holder under different conditions of temperature and pressure, a special pressure switch was constructed. The design is such that if the temperature remains constant the pressure to which the gas is collected in the holder is always the same and independent of atmospheric pressure. If the temperature alters, however, the pressure to which the gas is collected alters to compensate for the effect of the temperature on the moles of gas in the holder. Perspex block, $\frac{3}{4}$ inch thick, is used for the construction, details of which are shown in Fig. 6. The platinum contacts, C_1 and C_2, are tapped into the blocks and cemented with epoxy resin.

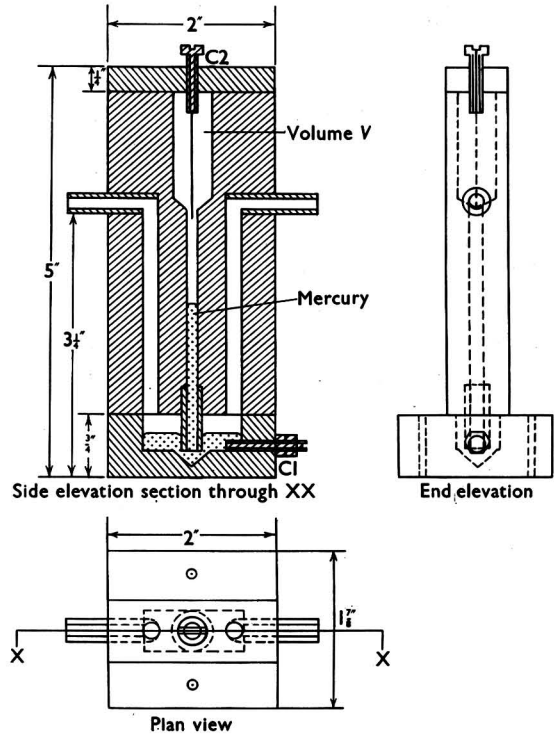


Fig. 6. Detail of pressure switch

As gas flows through the lower chamber of the switch it forces mercury a little way up the central column. When the gasholder piston reaches the top the pressure increases, and the mercury in the central column rises further until it makes contact with C_2 and completes the electrical circuit to C_1. The volume, v, is isolated from the atmosphere so that the pressure required for the mercury to reach C_2 is independent of atmospheric pressure. On the other hand, ambient temperature fluctuations will affect the pressure in volume v in the same sense that they will affect the pressure in the gasholder and will therefore cancel out.

The pressure to which the gas will be collected can be varied by adjustment of the initial height of the mercury in the central column.

SAMPLE WEIGHT AND RANGE OF ANALYSER—

The gas analyser can be obtained from the Infra Red Development Co., and can be calibrated to suit the requirements of the user. The instrument in the equipment described in this paper is provided with two ranges to read from 0 to 0·016 and from 0 to 0·064 per cent. of carbon when 8-g samples are taken. The range can be extended by using either smaller weights of sample or a less sensitive analyser. If it is required to use smaller samples a more sensitive analyser can be used. Sample weights of from 1 to 8 g have been used satisfactorily with this equipment.

GASHOLDER—

The volume of the gasholder is about 2½ litres, and the volume is measured accurately before use by filling it with water. This volume has been found to be more than enough to sweep out the combustion chamber. A simple check of this can be made by allowing the control unit to perform a second cycle without removing the used crucible from the combustion chamber. If any carbon dioxide were left in the chamber, the gas analyser would show a positive reading during the purge period. This does not happen.

CALIBRATION—

Two adjustments are required to calibrate the analyser.

1. Pure oxygen is blown through, and the "zero" control is adjusted to give zero deflection on the scale. A "zero" button is provided on the control unit, which when pressed will allow pure oxygen to flow through the analyser.

2. A standard mixture of carbon dioxide in an inert carrier is blown through, and the "sensitivity" control is adjusted to give the appropriate reading on the scale.

The standard gas mixture is supplied by the instrument manufacturer, but is only accurate to within ±5 per cent. of its stated value. This is a convenient way of setting up and checking the instrument provided that the cylinder of standard gas is recalibrated before use.

The most convenient method of doing this is to "inject" accurately measured volumes of pure carbon dioxide into the gas stream between the combustion chamber and the gasholder. By knowing the amount added, the volume of the gasholder and the temperature and pressure to which the gasholder is filled, it is a simple matter to calculate the percentage of carbon dioxide in the gas delivered to the analyser. The analyser can be adjusted to read this value and then used to standardise the cylinder. Once this is done the cylinder can be used for day-to-day checks of the analyser.

The accuracy of the whole apparatus will depend on the accuracy with which the small volumes of pure carbon dioxide are dispensed. Two methods have been tried and both give satisfactory results. The simpler of the two is to inject the carbon dioxide directly through the rubber tubing with a gas-tight gas-chromatography syringe. The alternative is to construct a simple gas pipette, as shown in Fig. 7, which is connected into the system as indicated. The volume between the taps is calibrated by weighing the amount of mercury

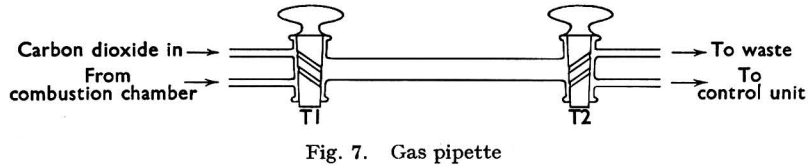


Fig. 7. Gas pipette

required to fill it and should be of such volume that about two-thirds deflection will be given on the meter. To use the pipette pure dried carbon dioxide is blown through it in the direction shown, and taps T_1 and T_2 are turned to the "off" position in that order. T_1 is then turned to connect to the combustion chamber, and the "start" button is depressed. Immediately the "collect" light comes on T_2 is turned to connect to the control unit. The measured volume of carbon dioxide is then collected and fed to the analyser.

With both methods allowance is made for atmospheric temperature and pressure.

BLANK VALUES—

It is more difficult to measure the blank value on a high-frequency combustion furnace than on a normal resistance heating tube because it is not possible to heat a crucible unless there is a sample in it. The blank value is much lower, however, because the crucible does not become as hot as an ordinary combustion boat. This does not prevent the sample itself from attaining an extremely high temperature, but the combustion is completed in a matter of seconds, so that the total heat involved is much less than in a tube furnace. The blank value is not only lower on the crucible, but also lower for the oxygen, because it does not have to pass through a hot tube.

Table I shows mean results for samples analysed by both high-frequency and resistance heating methods. No blank value for crucible or oxygen has been deducted from the high-frequency heating results. There is a favourable comparison between the results indicating that the blank value is negligible. Further, no significant difference has been detected between steel analysed when half- and full-weight samples were taken.

Blank values for tin flux can be measured by analysing standards with different weights of flux and calculating the blank value from the difference in the results obtained. By this procedure an accurate analysis of the standard is not required. The normal blank found on 1 g of tin is about 25 μg of carbon; on an 8-g sample this is equivalent to 0·0003 per cent. of carbon.

TABLE I

COMPARISON OF RESULTS OBTAINED BY DIFFERENT METHODS IN DIFFERENT LABORATORIES

	Carbon content found by—						
	infrared method.	low pressure method. Analyst No.			gravimetric method. Analyst No.		
Sample	Analyst No. 1,* %	1,* %	2,† %	3,† %	1,* %	3,† %	4,† %
A	0·0296	0·0295	0·0306	0·0290	0·0290	0·0290	0·030
B	0·0231	0·0231	0·0244	0·0229	0·0230	0·0230	0·0240
C	0·0043	0·0041	0·0045	0·0036	0·0040	0·0039	0·0042
B.C.S. 264‡	0·0367	0·0361	—	—	0·0360	—	—

* Analyst No. 1 (the author) used high-frequency heating.

† Analysts No. 2, 3 and 4 used resistance heating. Analyst No. 2 was J. Borrowdale, R.T.B. Ltd., Scunthorpe; analyst No. 3 was C. E. A. Shanahan, R.T.B. Ltd., Aylesbury; analyst No. 4 was H. Padget, R.T.B. Ltd., Ebbw Vale.

‡ Carbon content 0·037 per cent.

TABLE II

RANGE OF RESULTS OBTAINED BY THE INFRARED METHOD

Sample	Carbon content, %
A	0·0298, 0·0298, 0·0313, 0·0298, 0·0290, 0·0294, 0·0294
B	0·0229, 0·0229, 0·0232, 0·0230, 0·0232, 0·0234, 0·0230
C	0·0045, 0·0044, 0·0042, 0·0040, 0·0042, 0·0044, 0·0044
B.C.S. 264	0·0366, 0·0366, 0·0372, 0·0374, 0·0354, 0·0371, 0·0371
D	0·0035 to 0·0043*
E	0·0370 to 0·0383†
F	0·0078, 0·0074, 0·0074
G	0·0013, 0·0013

* Range of results of 30 analyses.

† Range of results of 28 analyses.

TABLE III

CARBON DETERMINED IN VARIOUS B.C.S. SAMPLES

Sample	B.C.S. No.	Weight of sample, g	Carbon present, %	Carbon found, %
Rustless steel*	211/1	2	0·24	0·245, 0·243, 0·239
Carbon steel	237	4	0·083	0·082, 0·082, 0·083
18/8 Stainless steel ..	235/1	8	0·042	0·042, 0·039
Carbon steel	265	8	0·047	0·048, 0·048, 0·048
Free-cutting steel† ..	152/2	7	0·06	0·060, 0·061
Carbon steel	238/1	2	0·21	0·204, 0·202, 0·204
Carbon steel	240/1	1	0·45	0·444, 0·443
Pure iron granules ..	149/1	8	0·003	0·0025, 0·0025

* Contains 13 per cent. of chromium.
† Contains 0·26 per cent. of sulphur.

The method was primarily intended for determining carbon in silicon steels with which 1 g of tin is found to be sufficient for 8-g samples. All results quoted in Tables I, II and III were obtained with 1 g of flux, except for B.C.S. 240/1, which, even though only 1-g samples were used, was found to need 2 g of flux to obtain a satisfactory combustion.

PRINCIPLE OF THE GAS ANALYSER—

The principle of operation of the gas analyser is described in a pamphlet entitled "Gas Analysis by Infra Red Spectra," which can be obtained from the Infra Red Development Co. Limited, Welwyn Garden City, Herts. This type of analyser was originally developed by Luft before the second World War, and the general principle is as described below.

The radiation from the two heated filaments, F : F (see Fig. 8), is passed through tubes R and S and falls on to the two halves of the detector, D_1 and D_2. These halves are filled with carbon dioxide and are separated by a thin diaphragm. When equal radiation reaches the two halves of the detector the heating effect is equal and the pressure of the gas in the two halves is the same. If carbon dioxide is present in sample tube S, less heating effect takes place in D_2 and the diaphragm is deflected. To convert this deflection into an electrical signal a shutter is rotated between the sources and the tubes. This causes the beam to be pulsed so that the diaphragm oscillates. By making the diaphragm one plate of a condenser an a.c. signal can be produced, which, when amplified and rectified, can be fed to a meter. By using this sytem there is no need to monochromate the infrared radiation, because the detector can only respond to radiation in the carbon dioxide absorption band.

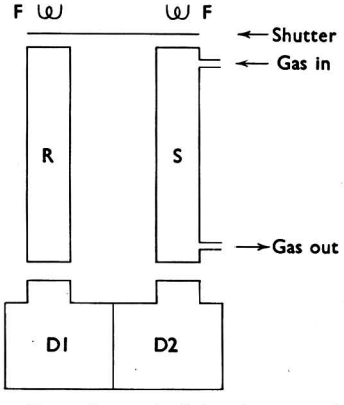


Fig. 8. Operation principle of gas analyser

RESULTS—

Table I compares results from different laboratories and by different methods. Table II shows the individual results and indicates the precision obtained. Other results on B.C.S. samples are shown in Table III.

It has been found that the method more than satisfies the objective accuracy of $\pm 0{\cdot}0005$ per cent. below 0·01 per cent. of carbon. Operation of the apparatus is extremely simple, the only training required being in the preparation of sample and crucibles and the weighing of the sample.

Excluding weighing and sample preparation, the time required for drilled samples is approximately $2\frac{1}{2}$ minutes before the result is obtained and a further minute before the apparatus is ready for the next sample. During this latter time, however, the next sample can be placed in the combustion chamber so that repeat analyses can be obtained in 3 minutes. Up to a minute longer may be required with chipped samples because of their slower heating in a high-frequency field.

I thank Richard Thomas & Baldwins Limited for permission to publish this paper, the Infra Red Development Co. Limited for their assistance and the other chemists in the Company who have provided the results quoted.

Received *October 2nd*, 1962

The Determination of Hydrogen in Magnesium Alloys

By R. BERRY and J. A. J. WALKER

(*United Kingdom Atomic Energy Authority, Reactor Materials Laboratory, Culcheth, Warrington, Lancs.*)

A method is described for determining hydrogen in magnesium alloys involving the simultaneous sublimation of magnesium from a graphite crucible and the removal of hydrogen from the furnace system. The amount of hydrogen extracted from a known weight of sample is determined by collecting the gas in a calibrated volume and measuring the pressure with a McLeod gauge before and after diffusion through a palladium tube. The results for the reproducibility tests show the coefficient of variation to be not greater than 8 per cent. for samples weighing between 0·05 and 2 g and having hydrogen contents between 6 and 140 ml per 100 g. Possible sources of error and the precautions necessary to minimise the errors are discussed.

Magnesium alloys are used as a canning material for uranium fuel elements in nuclear reactors of the Calder Hall pattern. The alloys of particular interest contain 0·8 per cent. of aluminium (Magnox AL.80), 0·5 per cent. of zirconium (Magnox ZR.55) or 0·7 per cent. of manganese (Magnox MN.70). Since the presence of gases in many metals is known to affect their physical properties, a method of determining hydrogen was investigated to facilitate the assessment of its effect on these magnesium alloys. The normal level of hydrogen is 10 ml per 100 g (approximately 10 p.p.m.), rising to 150 ml per 100 g for hydrided Magnox ZR.55.

Preliminary work[1] with (*a*) a vacuum extraction procedure and (*b*) a vacuum fusion in a tin bath suffered from the long extraction times required by both methods. In order to deal with many samples, a more rapid analytical technique was needed. The vacuum sublimation method was therefore developed. In this method the sample is inductively heated at 1000° C in a graphite crucible. The magnesium sublimes on to the cooler parts of the furnace tube, and the hydrogen evolved is pumped away and measured. At this operating temperature interstitial hydrogen is released, and any hydride contained in the sample is decomposed.

Method

Apparatus—

The furnace assembly (see Fig. 1) consists of a vertical silica tube, housing a graphite crucible, and a horizontal sample-loading arm; the assembly is connected to the analytical part of the apparatus via a mercury diffusion pump. The sample is moved into position by a mild-steel pusher and magnet. The graphite crucible is heated by a 1-kW 5-Mc/s high-frequency generator, and the temperature is measured with a disappearing-filament pyrometer.

The analytical system (see Fig. 2) consists of an Edwards GM2 mercury diffusion pump backing into a calibrated volume. Supplementary volumes in the form of three 1-litre bulbs may be included when large volumes of gas are being handled. Two McLeod gauges, ranges 0 to 0·1 mm and 0 to 2 mm of mercury, and a Pirani gauge are included in the system to measure the pressure before and after the hydrogen has been diffused out of the analytical system via a palladium thimble. Two cold traps are incorporated in the system, one to protect the palladium thimble, the other to prevent the mercury vapour from the GM2 diffusion pump reaching the furnace.

The whole apparatus is evacuated by an Edwards 1M2 mercury diffusion pump backed by an Edwards 1S50 rotary pump. The apparatus can measure volumes down to 0·01 μl (at N.T.P.).

Procedure—

Prepare the samples (maximum of 20) by cutting with a clean grease-free hack-saw to give a sample weight in the range 0·1 to 1 g. Cut 0·5 g of magnesium as a "getter." Thoroughly abrade the surfaces with a clean grease-free file or by dry turning to a depth of several thousandths of an inch. Wash samples with carbon tetrachloride, and dry in air. Weigh, and immediately load samples and getter into the side-arm of the furnace assembly

with clean forceps. Position the graphite crucible in the furnace tube, and evacuate the system. De-gas the graphite crucible at 1300° C overnight or until an acceptable blank rate has been achieved (less than 5 μl per hour of total gas at N.T.P.).

Drop the getter into the graphite crucible after its temperature has been lowered to 1000° C. De-gas the apparatus until the original blank rate is reached. Drop a sample into the graphite crucible, and de-gas as before. Measure the total hydrogen evolved by plotting the pressure of hydrogen against time and extrapolating the straight-line portion of the graph (equivalent to the blank rate) to zero time. Calculate the volume of hydrogen per 100 g of sample.

Results

Samples of the different alloys (including hydrided material) in the form of strip or extruded bar were analysed. The weights of specimen in these tests varied from 0·05 to 2 g. The standard deviation and other relevant information are shown in Table I. The weight of sample taken has no significant effect on the reproducibility. All variations within a run are random; there is no evidence of a drop in hydrogen values from beginning to end of a run. Similarly, the variation in blank values determined in between analysing individual specimens was random throughout.

Table I

Reproducibility of results for hydrogen content of magnesium alloys

Material		Range of sample weights, g	Hydrogen content (at N.T.P.), ml per 100 g	No. of samples	Standard deviation, ml per 100 g	Extraction time, minutes
Magnox AL. 80	(*a*)	0·4 to 0·6	7·1	10	0·4	12
	(*b*)	0·9 to 1·1	6·7	11	0·4	12
	(*c*)	1·4 to 2·1	6·8	12	0·4	20
Magnox MN. 70		0·2 to 1·1	17	19	0·9	13
Pure magnesium		0·1 to 1·1	15	20	0·8	12
Magnox ZR. 55	(*a*)	0·1 to 1·1	7·2	21	0·6	12
	(*b*)	0·1 to 0·4	8·6	16	0·6	12
		Apparatus blank rate	0·002*	11	0·001*	—
	(*c*)	0·1 to 0·2	140	16	6	10
	(*d*)	0·05 to 0·2	136	22	3	10
		Apparatus blank rate	0·004*	15	0·002*	—

* Figures for apparatus blank rate are in ml per hour.

Errors caused by desorption of gases

Sorbed moisture of apparatus—

Preliminary work on this technique indicated some gettering, since the first sample in a run always gave a much higher result. This is caused by the reaction of magnesium vapour with adsorbed moisture on the silica furnace walls.

It is essential to remove sorbed moisture from the walls of the apparatus by heating in a flame and collecting the vapour in a cold tap or by using the first specimen as a getter. A sample of Magnox AL.80 analysed by this procedure was shown to contain 38 ml of hydrogen per 100 g of alloy, but, if none of these precautions was taken, values as high as 44 ml per 100 g were obtained.

Surface effects on magnesium—

Any method for determining hydrogen in magnesium is prone to errors owing to the surface contamination of the sample. Moisture reacts with magnesium at ambient temperature to produce a film of magnesium hydroxide, $Mg(OH)_2$, or hydrated oxide, $MgO.xH_2O$. When heated, these compounds release hydrogen by the reactions—

$$Mg(OH)_2 \longrightarrow MgO + H_2O \text{ (slow)}$$
$$H_2O + Mg \longrightarrow MgO + H_2 \text{ (fast)}.$$

Bobalek and Shrader[2] reported that water was released when an uncleaned specimen of magnesium was heated in an inert atmosphere at temperatures up to 420° C. Above 440° C only hydrogen was evolved, owing to the reaction of water with the magnesium. During the investigation described here, specimens of surface-contaminated Magnox AL.80 were heated

in argon at different temperatures up to 600° C; release of gas began at approximately 400° C, and only hydrogen was detected.

Table II shows the errors inherent in the results when uncleaned samples are analysed.

TABLE II

SURFACE EFFECTS OF MAGNESIUM

Specimen	Hydrogen found (at N.T.P.), ml per 100 g	Sample preparation
A	63	No abrading of surface
B	35	Few thousandths of an inch removed from surface by filing

CONTAMINATION OF PREPARED SAMPLES BY HYDROGEN—

If abraded specimens are exposed to the atmosphere, fresh contamination of the surface occurs owing to its reaction with moisture and results in an increased hydrogen content. Six samples from the same sheet of Magnox AL.80 were abraded and left exposed in the laboratory for 3 days. Three specimens were analysed without further treatment, the remainder were re-abraded before analysis; the results (N.T.P.) were—

Hydrogen in once abraded specimens, ml per 100 g ..	17	19	20
Hydrogen in twice abraded specimens, ml per 100 g ..	13	13	13

This contamination can occur on storage, even in an atmosphere that might be presumed to be extremely dry. For example, several specimens cut from the same extruded bar of Magnox AL.80 previously machined to remove surface contamination were de-gassed by heating *in vacuo* at 400° C for 7 days. They were then stored in a desiccator containing fresh phosphorus pentoxide for various periods, and analysed without further treatment. The results are shown in Table III.

TABLE III

HYDROGEN CONTAMINATION BY DRIED AIR

Sample No.	Hydrogen found (at N.T.P.), ml per 100 g	Storage period
1	0·3	Immediate analysis
2	1	10 days
3	3·3	25 days

The equilibrium pressure of water vapour over phosphorus pentoxide is extremely low, yielding an atmosphere containing less than 1 p.p.m. by volume of water. Under such conditions no reaction with magnesium would be expected. However, in practice it is difficult to attain such dry conditions unless elaborate precautions are taken. The method of storage used in this instance, a standard desiccator containing phosphorus pentoxide, would certainly result in the moisture content of the air around the specimen being an order of magnitude greater than the equilibrium value. Moisture is continually being desorbed from the glass surface and is only slowly removed from the gas phase, the rate being determined by its diffusion in the air at normal pressure and the condition of the surface of the desiccant. This is a possible explanation for contamination of the samples during storage.

GETTERING OF HYDROGEN BY MAGNESIUM FILMS

Tests have been carried out to determine the gettering properties of magnesium for hydrogen. From other published work[3] it seemed unlikely that the sorption of hydrogen by a magnesium film at or slightly above room temperature would present a problem. The apparatus used for these experiments consisted of that shown in Figs. 1 and 2, with the exception of the furnace tube.

A modified furnace tube and standard-volume gas-addition device (see Fig. 3) was connected via a B29 cone and socket to the sample-loading arm. The furnace tube contained a graphite crucible heated by induction. The graphite crucible was de-gassed at 1300° C, and a sample of magnesium was dropped into the crucible after the temperature had been

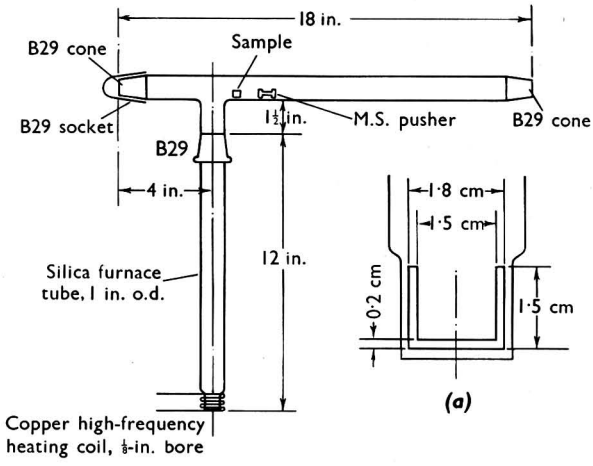
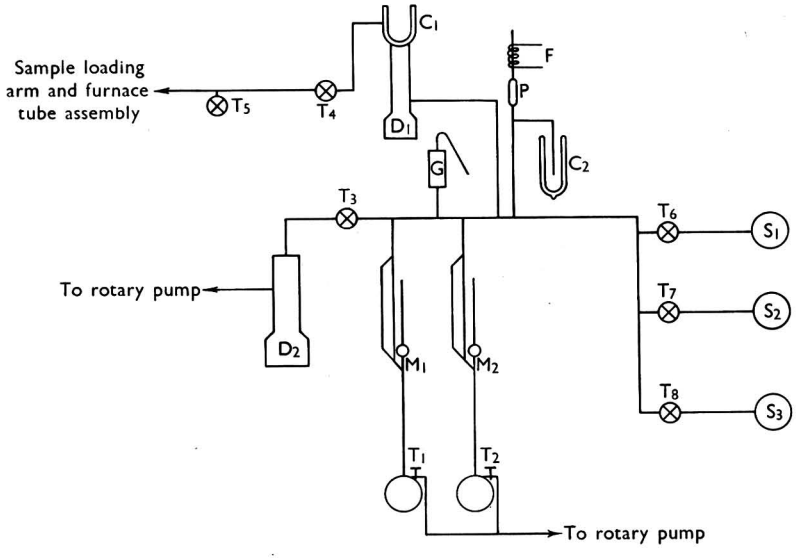


Fig. 1. Sample loading arm and furnace tube assembly: (*a*), enlarged view of furnace tube and graphite crucible.



C_1, C_2 = Cold traps (—196° C).
D_1, D_2 = Mercury diffusion pumps.
F = Nichrome furnace for palladium thimble.
G = Pirani gauge.
M_1, M_2 = McLeod gauges.
P = Palladium thimble.
S_1 to S_3 = Supplementary volumes.
T_1 to T_9 = Taps.

Fig. 2. Schematic diagram of apparatus for extracting hydrogen in metals.

adjusted to 1000° C. Operating temperatures above 1000° C were tried, but it was observed that mechanical agitation of the sample occurred owing to rapid evolution of magnesium vapour. With Magnox ZR.55 it was feared that too rapid a sublimation might cause loss of zirconium hydride by entrainment in the vapour. This would probably lead to low results for hydrogen since (*a*) gettering of hydrogen by particles of zirconium in the cooler part of the furnace might occur and (*b*) incomplete decomposition of ejected hydride particles would be likely.

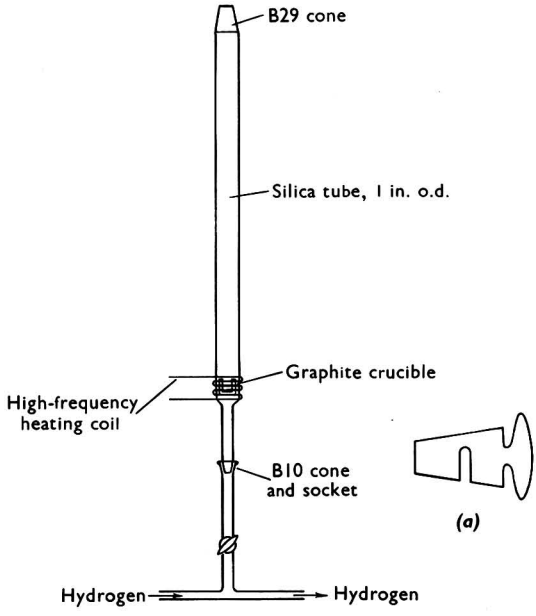


Fig. 3. Furnace tube for gettering experiments: (*a*), enlarged view of sampling stopcock.

The magnesium was sublimed from the graphite crucible on to the walls of the silica furnace tube, and the system was de-gassed to the original blank rate. A known volume (0·344 ml at N.T.P.) of hydrogen was introduced into the apparatus via the sampling stopcock at the operating temperature of 1000° C. The hydrogen flowed over the surfaces of the graphite crucible and magnesium film, and was pumped into the analytical system where it was measured. This procedure was then repeated, so as to simulate conditions of analysis. In both experiments all the hydrogen was recovered within 1 minute.

In the second test the same volume of hydrogen was introduced into the furnace after it had been isolated from the analytical system. After the hydrogen had been in contact with the magnesium surfaces for 5 minutes, the gas was pumped away, collected and measured in the normal manner; this experiment was repeated. All the hydrogen was recovered within 1 minute.

Gettering of hydrogen by magnesium vapour—

Although the experiments described above indicated that hydrogen was not removed by a pre-formed film of magnesium, the possibility of its removal by condensing magnesium vapour could not be disregarded. This was examined with the same apparatus and procedure as were used in the previous gettering tests, except that (*a*) the sample was introduced into the cold de-gassed crucible and (*b*) a known amount of hydrogen was added to the furnace system before the magnesium was sublimed. The magnesium was then volatilised in the presence of hydrogen. After a period of 5 minutes the gas was pumped away and measured. In none of the three experiments was less than 97 per cent. of the hydrogen recovered.

De-gassing of zirconium residues

Interstitial hydrogen is released by subliming magnesium from a graphite crucible. With magnesium - zirconium alloys a residue of zirconium hydride remains in the crucible. An experiment was therefore devised to determine the optimum temperature for the rapid extraction of hydrogen from zirconium. A known weight of zirconium purified by the iodide process was de-gassed at 1050° C for 5 hours, after which time the hydrogen content had been reduced to negligible proportions. A measured volume of hydrogen was then introduced into the extraction apparatus and was allowed to react with the zirconium at a

temperature of 800° C. When the reaction was complete the specimen of zirconium was cooled to room temperature, and the residual hydrogen was measured. The zirconium was then heated again to 1050° C under high vacuum; recovery of the added hydrogen was 100 per cent. The results of several tests show that a temperature higher than 1050° C would be advisable for rapid extraction of hydrogen from the massive zirconium. However, when magnesium is present as in Magnox ZR.55 rapid evolution of vapour at temperatures in the region of 1200° C can cause loss of zirconium by entrainment; it was therefore decided to carry out the operation at 1000° C.

Mode of sublimation

Two different procedures have been used for subliming magnesium. In the original experiments[1] the crucible and furnace tube were heated at 750° C (initial de-gassing at 1000 °C) by use of an external Nichrome-wire resistance element. A high de-gassing rate and severe attack of the silica furnace tube by magnesium resulted from this method of heating. The second method, high-frequency heating, permits operating temperatures of 1000° C (initial de-gassing at 1300° C) to be attained. Under such conditions, only a small proportion of the furnace tube is heated by radiation from the crucible; the blank rate is much lower and the service life of the silica tube is increased twenty-fold.

Reproducibility tests on a sample of Magnox AL.80 show the superiority of the high-frequency heating procedure (see Table IV).

Table IV

Results with different methods of heating

Mode of heating	Nominal hydrogen content, ml per 100 g	Standard deviation, ml per 100 g	No. of determinations
Resistance (750° C)	10	2	12
High frequency (1000° C) ..	10	0·4	11

Preparation of hydrogen standards

A 5-g sample of de-gassed Magnox AL.80 cut from $\frac{5}{8}$-inch diameter extruded bar was heated for 1 hour at 350° C and a further hour at 500° C in an atmosphere of hydrogen at a pressure of $3\frac{1}{2}$ mm of mercury. No detectable decrease in the hydrogen pressure was observed; a repeat experiment carried out at a pressure of 7 mm of mercury at 400° C confirmed this.

A sample of Magnox AL.80 similar to that used in low-pressure hydrogenation was heated in the temperature range 350° to 550° C at a pressure of 2 atmospheres of hydrogen. Some absorption of hydrogen by the Magnox AL.80 occurred, and this was detected by a differential oil manometer. Results are shown in Table V.

Table V

Sorption of hydrogen by Magnox AL.80

Temperature, °C	Time, hours	Pressure drop, mm of oil	Original pressure, atmospheres of hydrogen
350	$\frac{1}{2}$	Nil	2
400	1	1	2
450	$\frac{1}{2}$	5	2
550*	1	19	2

* Some volatilisation occurred.

The volume of the system containing the hydrogen was 270 ml, and the specific gravity of the oil was 0·89. Approximately 0·4 ml of hydrogen (at N.T.P.) was absorbed by the 5-g sample of Magnox AL.80 at 550° C, which is equivalent to 8 ml per 100 g. This pick-up of hydrogen was not high or rapid enough for preparing reliable standards, especially as some volatilisation of the sample occurred.

Unsuccessful attempts were made to introduce hydrogen at low pressure (3 mm of mercury) into Magnox AL.80 by activating the hydrogen with an electrodeless discharge produced by a 20-Mc/s high-frequency generator.

The tests described above were repeated with Magnox ZR.55, and the results were similarly disappointing.

No satisfactory method of preparing standards has been found.

Conclusion

The rapid extraction of hydrogen from magnesium alloys by the sublimation of magnesium at 1000° C is a convenient routine method. Results obtained with high-frequency heating of the crucible at 1000° C are superior to those obtained with external resistance heating at 750° C. Under the conditions described, reproducibility is good, although the surface of the samples and apparatus must be carefully prepared otherwise they can cause significant errors. The weight of sample does not affect the reproducibility of the method. Small specimens permit an increased number to be loaded into the apparatus, and therefore are preferable.

References

1. Berry, R., Walker, J. A. J., and Clarke, A. E., "The Determination of Hydrogen in Magnox A.12," U.K.A.E.A. DEG. Report 107 (C), 1961.
2. Bopalek, E. G., and Schrader, S. A., *Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 343.
3. Reimann, A. L., *Phil. Mag.*, 1933, **16**, 673.

Received *May 28th*, 1962

A Dithizone Method for determining Zinc in Organic Material

By GUNNEL WESTÖÖ

(*Department of Nutrition and Food Hygiene, The National Institute of Public Health, Stockholm, Sweden*)

A rapid and selective one-colour dithizone method is described for determining zinc in food products or other organic material.

After wet oxidation of the sample, an aliquot is treated with cyanide in alkaline solution to mask cobalt and is then extracted with a solution of dithizone in carbon tetrachloride at pH 5·5 in the presence of thiosulphate, cyanide and citrate. All the zinc and some cadmium are dissolved as dithizonates in the carbon tetrachloride, whereas other metals that can form dithizonates soluble in carbon tetrachloride are masked by the thiosulphate or the cyanide and remain in the aqueous phase. The dithizonate solution is shaken with a solution of sodium sulphide buffered with borate in order to remove cadmium dithizonate, excess of dithizone and dithizone oxidation products. The optical density of the zinc dithizonate solution is measured at 535 mμ.

In 1960 Margerum and Santacana[1] published an excellent critical examination of 8 different methods for determining microgram amounts of zinc. They stated that Butts, Gahler and Mellon's[2] method with bis(2-hydroxyethyl)dithiocarbamate, cyanide and citrate as masking agents was superior to the other procedures compared. The method described here, which is a development and simplification of the procedures proposed by Fischer and Leopoldi[3] and Baggot and Willcocks,[4] is more rapid for routine analyses and gives yields of zinc dithizonate as good as those obtained by Butts, Gahler and Mellon's method.

The proposed method differs from Fischer and Leopoldi's and Baggot and Willcocks's methods in, among other things, the use of one combined buffer and masking agent solution containing sodium citrate and citric acid as buffer, cyanide to mask nickel and cobalt and a large amount of sodium thiosulphate to mask other metals. The pH of the solution is 5·5, and it is the only reagent solution used that has to be purified. The amounts of masking agents have been chosen to be sufficiently large to allow considerable amounts of foreign metals to be present (see Table I), but not so large that the formation of zinc dithizonate is affected (see Table III). Only cobalt has to be masked at a pH above 5·5 and necessitates extra additions of cyanide and citric acid solutions.

Baggot and Willcocks remove excess of dithizone, dithizone oxidation products and remaining traces of cadmium dithizonate with a sulphide solution buffered with phosphate at pH 11; the phosphate buffer is purified by tedious extractions with dithizone solution. In the proposed method a sulphide solution buffered with borate (pH 11·5) is used, which gives smaller blank values than does the phosphate–buffered solution, despite the fact that it is not purified.

Method

The laboratory should be free from dust and, if possible, be used only for determinations of trace metals. As zinc is an extremely common metal, it is necessary to work with scrupulous care to ensure low and constant blank values and good results.

Glassware—

All dishes and storage bottles must be of zinc-free borosilicate glass. Before use they should be washed with diluted hydrochloric acid (1 + 1) and metal-free water. The Kjeldahl flasks used for digestion of the organic material, if they are new or have previously been used for other analyses, should be boiled with concentrated sulphuric and nitric acids for half an hour, and then washed with metal-free water. Immediately before use all glass articles should be shaken with a mixture of 10 ml of a 0·01 per cent. solution of dithizone in carbon tetrachloride and 25 ml of 0·02 N ammonium hydroxide. This procedure must be repeated until the glass is free from adsorbed metal ions, *i.e.*, until the carbon tetrachloride solution does not show any trace of red colour. The residues of the solutions should then be

removed by rinsing with carbon tetrachloride and metal-free water. It is recommended that the same glassware should be kept for all determinations of zinc and not be used for other determinations. After use the glassware should be cleaned with metal-free water, and Kjeldahl flasks and separating funnels should be stored stoppered.

REAGENTS—

All reagents should be of recognised analytical grade, and only metal-free water (see below) should be used.

Ammonium hydroxide, concentrated—Distil 1 litre of ammonium hydroxide, density 0·91 g per ml, into 500 ml of water at $<10°$ C until the density of the distillate is 0·90 to 0·91 g per ml. (Ammonia gas from a cylinder, if available, can also be used. It should be purified by passage through a gas washing bottle containing concentrated ammonium hydroxide and then passed into cooled water until a solution of the right density is obtained.)

Ammonium hydroxide, N—Dilute 75 ml of the concentrated ammonium hydroxide to 1 litre with water.

Ammonium hydroxide, 0·02 N—Dilute 10 ml of the N ammonium hydroxide to 500 ml with water.

Buffered masking agent solution—This solution should be prepared under an efficient extraction hood. Dissolve 715 g of sodium thiosulphate pentahydrate, 60 g of trisodium citrate dihydrate and 10·0 g of potassium cyanide in 1 litre of water. Add, with stirring, a solution of 15·0 g of citric acid in 100 ml of water. Divide the solution into two portions, and shake each portion thoroughly for several minutes in a separating funnel twice with 50-ml portions of 0·04 per cent. dithizone solution and twice with 50-ml portions of 0·01 per cent. dithizone solution. Each time the layers should separate cleanly. Shake the last carbon tetrachloride layers with 15 ml of buffered sodium sulphide solution (see below). If the carbon tetrachloride layers are red or pink, repeat the extractions with dithizone and sulphide solutions until the carbon tetrachloride turns colourless (or pale green or yellow). Then wash the remaining dithizone from the two portions of masking agent solution with two or three portions of carbon tetrachloride. Combine the masking agent solutions, filter, and make up to 2 litres. The pH of the solution should be 5·5. Do not use the solution when it is more than 1 month old.

Citric acid solution—Dissolve 33 g of citric acid monohydrate in water, and make up to 500 ml.

Cotton-wool—Wash cotton-wool with dilute dithizone solution and carbon tetrachloride.

Dithizone stock solution, 0·04 *per cent. w/v*—Dissolve 0·200 g of dithizone in 500 ml of carbon tetrachloride, and filter into a dark flask. Store the solution in a refrigerator.

Dithizone extraction solution, 0·01 *per cent. w/v*—Dilute 100 ml of dithizone stock solution with 300 ml of carbon tetrachloride. Store this solution in a dark flask in a refrigerator for not more than 2 days. Allow the solution to reach room temperature before use.

Methyl red solution—Dissolve 0·04 g of methyl red (sodium salt) in 100 ml of water.

Potassium cyanide solution—Dissolve 5·0 g of potassium cyanide in water, and make up to 100 ml. The solution should not be stored for more than 1 month.

Buffered sodium sulphide solution—Pass hydrogen sulphide into 500 ml of 0·25 N sodium hydroxide until the pH is just below 8, as measured with thymol blue indicator. Do not use if it is more than 1 month old.

Dissolve 61·8 g of boric acid and 40·5 g of sodium hydroxide in water, and make up to 1 litre. The pH of the solution should be 11·5.

Before use, mix 10 ml of the sodium sulphide solution with 90 ml of the borate buffer solution.

Thymol blue solution—Grind 100 mg of thymol blue with 4·3 ml of 0·05 N sodium hydroxide in a mortar until the solid has dissolved, and make up to 100 ml with water.

Water—Pass distilled water through a column of cation-exchange resin and then do not allow it to come into contact with objects from which it can pick up metal ions, *e.g.*, certain plastic or rubber tubes. The water can be examined for metal ions by shaking 25 ml with 0·5 ml of N ammonium hydroxide and 10 ml of dithizone extraction solution; the carbon tetrachloride layer should not be red or pink.

Standard zinc solution—Dissolve 0·1000 g of zinc metal in 10 ml of diluted hydrochloric acid (1 + 1), and make up to 1 litre with water. Prepare a solution containing 2 μg of zinc per ml by diluting 20·00 ml of the stock solution to 1 litre.

PROCEDURE—

Digestion—Digest the sample, containing 5 to 100 μg of zinc, by any suitable wet-oxidation method.[5] Concentrate the acidic solution to 2 to 3 ml, and make up to 50·0 ml with water.

Separation and colorimetric determination of zinc—The analyses should be carried out under an efficient extraction hood. Transfer 10·00 ml of the sample solution to a 125-ml separating funnel, and adjust the pH to 5·5 (at room temperature) by adding ammonium hydroxide solution (concentrated and N) with methyl red as indicator. Add 1·0 ml of potassium cyanide solution, mix, and dilute with water to 25 ml. Add 25 ml of buffered masking agent solution and 1·0 ml of citric acid solution. (If the samples do not contain cobalt, the addition of potassium cyanide solution and citric acid solution can be omitted.) Ensure that the colour of the indicator corresponds to pH 5·5. Add 15·00 ml of dithizone extraction solution, and shake the funnel vigorously for 2 minutes. The funnels should be ventilated through the neck, not through the stopcock.

When the layers have separated, transfer the carbon tetrachloride layer to a 125-ml separating funnel containing 15 ml of buffered sodium sulphide solution, and shake the funnel for about 30 seconds. After the layers have been separated, filter the carbon tetrachloride layer through cotton-wool into a test-tube, and keep the tube stoppered until the optical density of the solution has been measured at 535 mμ (1-cm cell) against carbon tetrachloride. This zinc dithizonate solution should be protected from direct sunlight, and, if its optical density is not measured within 2 hours, the solution should be stored in a dark place.

Blank test—For each determination of zinc carry out at the same time a blank digestion on the same amounts of oxidising agents as are needed for digesting the sample. Take the solution from the blank digestion through the entire procedure, and measure the optical density of the final solution; subtract this value from that obtained for the sample. The optical density of the blank solution (1-cm cell) does not usually exceed 0·120.

Calibration curve—In each of six 125-ml separating funnels place 0, 2·00, 4·00, 6·00, 8·00 or 10·00 ml of standard zinc solution. Carry out the proposed procedure on these standard samples from the addition of 1·0 ml of potassium cyanide solution. Subtract the optical density for the sample without zinc from the values for the other standard samples, and plot the corrected optical densities as a function of the zinc content. A straight line passing through origin and relating 1·0 μg of zinc to an optical density of 0·096 (1-cm cell) is obtained. If the reagents are good, the optical density measured in a 1-cm cell for the sample without zinc should not exceed 0·070.

INTERFERENCES

Foreign metals—Appreciable amounts of foreign metals can be present without seriously affecting the determination of zinc (see Table I). The effects of amounts of foreign metals larger than 5 mg have not been tested.

Only small amounts of ferrous salts may be present, but this is unimportant as iron is oxidised to the tervalent state during digestion.

Effect of light—Zinc dithizonate is decomposed when its solution in carbon tetrachloride is exposed to direct sunlight. In diffuse light the solution does not change noticeably in 1 to 2 hours; no effect was observed after 24 hours in the dark (see Table II).

TABLE I

EFFECT OF FOREIGN METALS ON THE DETERMINATION OF ZINC

Foreign metal	Amount present, μg	Zinc found: No zinc added, μg	Zinc found: 8·0 μg of zinc added, μg	Foreign metal	Amount present, μg	Zinc found: No zinc added, μg	Zinc found: 8·0 μg of zinc added, μg
Fe^{3+}	5000	0·15	8·0	Cr^{3+}	5000	0·1	8·0
Fe^{2+}	250	0·0	8·0	Co^{2+}	5000	0·1	7·9
Ni^{2+}	5000	0·1	8·0	Sn^{2+}	1000	0·05	7·6
Cu^{2+}	5000	0·2	8·0	Sn^{4+}	1000	0·0	7·75
Mn^{2+}	5000	0·1	8·05	Bi^{3+}	1000	0·1	7·75
Ag^{+}	5000	0·3	8·0	Cd^{2+}	1000	0·05	7·75
Pb^{2+}	5000	0·1	7·9	Hg^{2+}	1000	0·15	7·8

TABLE II

EFFECT OF LIGHT ON OPTICAL DENSITY OF ZINC DITHIZONATE SOLUTIONS

Optical density of zinc dithizonate solution (535 mμ; 1-cm cell)				
Immediately	After 24 hours in the dark	After 1½ hours in diffuse light	After 4 hours in diffuse light	After 1½ hours in sunlight
0·836	0·837	0·837	0·784	0·383
1·030	1·030	1·026	0·970	0·528

TABLE III

EFFECT OF MASKING AGENTS ON RECOVERY OF ZINC

Zinc added, μg	Zinc found— at pH 5·5 with masking agent, μg	at pH 5·5 without masking agent, μg
6·0	6·05	6·1
8·0	8·0	8·05
10·0	10·0	10·0

CONTROL OF THE METHOD

Masking agents—No loss of zinc was caused by the masking agents used (see Table III).

Stability of zinc dithizonate solution in contact with borate-buffered sulphide solution—Margerum and Santacana[1] have shown with zinc-65 that no loss of zinc is caused by shaking a dithizone - zinc dithizonate solution with a solution of sodium sulphide. The results in Table IV show that this is also true when a borate-buffered sulphide solution is used.

TABLE IV

RECOVERY OF ZINC IN PRESENCE OF BORATE-BUFFERED SULPHIDE SOLUTION

Zinc added, μg	Time of shaking, minutes	Borate-buffered sulphide solution present, ml	Zinc found, μg
8·0	½	10	7·95
8·0	½	20	8·05
8·0	½	30	8·0
8·0	½	15	8·05
8·0	1	15	8·10
8·0	2	15	8·05
8·0	3	15	8·15

Effect of pH on the formation of zinc dithizonate—Irving, Bell and Williams[6] have shown that, at pH 3·88, shaking for 30 minutes is necessary to achieve equilibrium in the reaction of aqueous solutions of zinc salts with dithizone in carbon tetrachloride. With increasing pH the rate of reaction increases and, at pH 4·9, equilibrium is reached within a few minutes.[6] Thus the rate of reaction at pH 5·5 should be satisfactory. Table V indicates that the extraction of zinc is complete by the proposed method (excess of dithizone in carbon tetrachloride; pH 5·5; time of shaking 2 minutes), as, even at pH 7·5 without masking agents (time of shaking 2 minutes) the amount of zinc extracted is not appreciably greater.

TABLE V

EFFECT OF pH ON RECOVERY OF ZINC

Zinc added, μg	Zinc found— at pH 5·5 with masking agent, μg	at pH 7·0 without masking agent, μg	at pH 7·5 without masking agent, μg
8·00	8·0	8·05	8·05
10·00	10·0	10·05	10·0

Variation of the method

If the sample to be analysed contains so much zinc that a portion containing 100 μg of zinc is too small to be representative, a larger portion must be taken. A smaller aliquot can then be used or the sensitivity of the method can be reduced by increasing the volume of dithizone solution used for extraction. In the latter instance only part of the dithizone - dithizonate solution is shaken with an equal volume of buffered sulphide solution.

I thank Miss Linnéa Eriksson and Mrs. Judith Antonsson for valuable technical assistance.

References

1. Margerum, D. W., and Santacana, F., *Anal. Chem.*, 1960, **32**, 1157.
2. Butts, P. G., Gahler, H., and Mellon, M. G., *Sewage and Ind. Wastes*, 1950, **22**, 1560.
3. Fischer, H., and Leopoldi, G., *Z. anal. Chem.*, 1937, **107**, 241.
4. Baggot, E. R., and Willcocks, R. G. W., *Analyst*, 1955, **80**, 53.
5. Analytical Methods Committee, *Ibid.*, 1960, **85**, 643.
6. Irving, H., Bell, C. F., and Williams, R. J. P., *J. Chem. Soc.*, 1952, 356.

Received *August 7th*, 1962

Hydroxocobalamin: Its Examination and Determination in Parenteral Injection Solutions by Paper Chromatography

By J. L. MARTIN and W. H. C. SHAW

(*Glaxo Laboratories Ltd., Greenford, Middlesex*)

The separation of hydroxocobalamin (aquocobalamin salt) from its analogues and decomposition products by conversion to the corresponding cyano-compounds, with subsequent paper chromatography, is described; coloured impurities are eluted and determined spectrophotometrically. The method can be applied to the solid material or solutions for parenteral injection after extraction to eliminate electrolytes and other interfering substances. Decomposition during storage produces additional coloured impurities, and paper chromatography in conjunction with spectrophotometry can be used as an alternative to microbiological assay during stability trials on parenteral injection solutions.

In an earlier paper[1] it was shown that any "red acids" and neutral cobalamins that may occur as impurities in commercial hydroxocobalamin could be separated by chromatography on basic and neutral celluloses and then determined spectroscopically. Other possible impurities that cannot be separated in this way include analogues containing nucleotide bases other than 5,6-dimethylbenzimidazole and some yellow degradation products. It was also noted that any analogues present could be separated (after removal of red acids on a column of basic cellulose) by paper chromatography with a wet s-butanol - acetic acid system in the presence of hydrogen cyanide vapour,[2] but that direct paper chromatography of hydroxocobalamin as such gave slow-running diffuse zones. Further work has shown that the preliminary removal of red acids is not necessary, provided that hydroxocobalamin is first converted to the cyano-form, and that paper chromatography is suitable for determining impurities in hydroxocobalamin and for the assay of hydroxocobalamin injection products. The proposed method is more precise and yields more information than microbiological assay, whether this is carried out on the hydroxo- or the cyano-form.

There is, however, a resulting disadvantage from converting to the cyano-form for paper chromatography, in that it is not then possible to detect the presence of any cyanocobalamin in the sample. For solid samples of hydroxocobalamin this is of no consequence, as cyanocobalamin is measured, together with any other neutral cobalamin, by column chromatography on basic and acidic celluloses.[1]

However, for solutions, particularly those of unknown origin when no corresponding solid can be tested and samples are small, column chromatography is not particularly suitable and preliminary extraction with phenol[2] must first be carried out to remove electrolytes. For either solid or solutions the ratio of the optical density at 361 mμ to that at 351 mμ, determined on a suitable dilution, provides a simple means of ensuring that the sample is substantially free from cyanocobalamin. This ratio for mixtures of hydroxo- and cyanocobalamin in different proportions is shown below—

Hydroxocobalamin, % ..	100	95	90	85	80	0
Ratio, E_{361}/E_{351}	0·65	0·69	0·72	0·76	0·79	1·60

Although some variations in the ratio for authentic samples of hydroxocobalamin are to be expected, it would appear that limits of 0·63 to 0·69 would exclude more than about 5 per cent. of cyanocobalamin.

Method

Determination of coloured impurities in hydroxocobalamin—

Shake 1 litre of a mixture of equal parts of water and s-butanol, allow it to separate overnight at a constant temperature between 25° and 30° C, and transfer the lower layer to the bottom of a paper-chromatography tank with the addition of 1 per cent. of acetic acid and 0·5 g of potassium cyanide.

Dissolve about 2 mg of sample, accurately weighed, in 0·5 to 1·0 ml of water, and add 1 drop only of freshly prepared 0·3 per cent. aqueous hydrocyanic acid. After it has been mixed, set the solution aside for 15 minutes, and then place on a filter-paper (Whatman 3 MM paper, 20 inches × 9 inches) in a narrow line 4 inches from one end, leaving 1 inch clear at both sides. Dry each superimposed streak in a stream of nitrogen and without applying heat. Serrate the lower edge, suspend from a trough in the paper-chromatography tank, and allow the paper to equilibrate overnight at the same temperature as before.

Add 5 per cent. of s-butanol and 1 per cent. of acetic acid to the upper layer, and fill the trough with this liquid. Allow the chromatogram to run until the cyanocobalamin has travelled about two thirds of the length of the paper, transfer to a fume cupboard, remove the paper, and dry in a current of air. Cut out and discard the main cyanocobalamin band, and from both remaining areas of paper trim off and discard any uncoloured top and bottom zones. Form both strips of paper into cylinders by fastening the vertical edges of each together, and stand them on wet filter-paper so that the coloured material is eluted to the top edges. Unfasten and trim off the uncoloured paper, and elute the coloured impurities by descending-solvent chromatography with the minimum amount of water. Combine the eluates, and dilute to a convenient volume with water (preferably not more than 25 ml). Filter through sintered glass, and measure the optical density at the maximum near 361 mμ in a 2-cm cell. Repeat the procedure on paper similar in size and position, cut from a blank chromatogram, and deduct the optical density observed at the same wavelength.

Calculate the coloured impurities ($E^{1\%}_{1cm} = 207$) as a percentage of the dry solid.

In the experimental work on which this method is based, it became clear that certain precautions are necessary if accurate results are to be obtained. The addition of the initial cyanide is critical; if the normal procedure via the dicyanide is carried out (addition of potassium cyanide in alkaline solution and then acidification with acetic acid) extra bands appear in the chromatogram—in particular, a strongly adsorbed red band remains at the origin. Hydrocyanic acid solution is the most convenient alternative. The amount and concentration must be carefully controlled, as even moderate excess can cause formation of red impurities. Under these conditions, conversion of the hydroxocobalamin to the cyano-form is only about 90 per cent. complete, the remainder being converted by the cyanide in the atmosphere of the chromatography tank.

A sample of hydroxocobalamin (approximately 5 mg) was purified by paper chromatography in a cyanide-free atmosphere. The main hydroxocobalamin band was then eluted and re-run in an acidic cyanide tank without adding hydrocyanic acid to the solution before streaking on to paper. Only the main cyanocobalamin band was detected.

Although complete conversion to cyanocobalamin can be effected during equilibration in the tank, it is desirable to minimise, by adding cyanide to the sample solution, the main losses that may occur with hydroxocobalamin during the loading of the paper. This preliminary conversion is essential before applying the phenol extraction if the sample contains electrolytes (see below).

The most likely source of cyanocobalamin loss is irreversible adsorption on the paper. The recoveries shown below are calculated as a percentage of the initial optical density at 361 mμ. For each test 30 to 60 mg were dissolved in 10 ml of water, and a portion (0·4 to 0·6 ml) was streaked on to paper. A similar portion was diluted to 100 ml, and the optical density at 361 mμ was measured; the results were—

Dry sample taken for chromatogram, mg	3·37	1·55	2·33	2·35	2·35	2·37
Recovery, %	100	101	98	98	99	96

These figures, obtained under favourable conditions with considerable attention to detail, show that the recovery is not reliably 100 per cent. (this is hardly to be expected with paper chromatography), and under routine conditions a recovery of less than 95 per cent. may occasionally be recorded.

The losses of impurities would not, however, be important if they were proportional to those of cyanocobalamin. Two tests were therefore made to show that, with impurities of the amount and type normally found, the losses were, in fact, proportional.

A chromatogram was produced from a small amount of monobasic red acids (equivalent to a few per cent. in a 5-mg sample of cyanocobalamin) and another from a vitamin B_{12} analogue containing benzimidazole as the nucleotide base.

The results in Table I were calculated by comparing the optical densities at about 361 mμ of solution before and after chromatography. During these tests it was found that any paper of the developed chromatogram showing no pink colour when examined wet against the light could safely be discarded. Blank values determined on the paper were found to be about 1 per cent., provided that the paper was not old or discoloured.

TABLE I

THE RECOVERY OF RED ACIDS AND BENZIMIDAZOLE FROM CHROMATOGRAMS

Sample	Weight of sample chromatographed, mg	Recovery, %
Monobasic "red acids"	0·096	103
"Benzimidazole" analogue of cyanocobalamin	0·19	99

The method of expressing results for coloured impurities requires comment. The cyano-compounds separated have maxima at about 360 mμ, but although an $E^{1\%}_{1cm}$ of 204 has been reported[3] for pseudo-vitamin B_{12} and for factor A, and a sample of red acids examined in this laboratory had an $E^{1\%}_{1cm}$ of approximately 195 at 361 mμ, the mean $E^{1\%}_{1cm}$ at 361 mμ cannot be known precisely.

It is necessary for the purpose of the test, therefore, to define the coloured impurities as the ones separable in their cyano-forms by the method described, and then to specify an $E^{1\%}_{1cm}$ value for the calculation. The adoption of 207 has been shown to be unlikely to introduce any substantial error. Results are then conveniently expressed as a percentage of the dry solid taken.

Examination of commercial hydroxocobalamin from three different sources by this method indicated the presence of 0·5 to 6·4 per cent. of coloured impurities, the majority lying between 2 and 4 per cent.

DETERMINATION OF HYDROXOCOBALAMIN IN INJECTION PRODUCTS—

It is normally necessary to extract hydroxocobalamin from preparations before it can be chromatographed, as any electrolyte present will interfere with the separation, and other ingredients also may be eluted from the paper and interfere spectrophotometrically. Preliminary experiments had shown that hydroxocobalamin was sensitive to heat and, to some extent, to light and that degradation was less likely to occur if, before extraction, it were converted to the cyano-form. Moreover, it is much easier to obtain a clean precipitation with ether in the presence of cyanide.

Extraction procedure—Place in a 50-ml separating funnel an accurately measured amount of the sample containing not less than 2 mg and not more than 5 mg of hydroxocobalamin. Dilute, if necessary, to 5 ml with water. For each 2 ml of solution add 1 drop of freshly prepared 0·3 per cent. w/v aqueous hydrocyanic acid, mix, and set aside for 15 minutes. Extract with 2 ml of a 50 per cent. solution of phenol in chloroform. Repeat the extraction with 1-ml portions of phenol - chloroform mixture until no more colour is extracted. Collect all the extracts in a 100-ml centrifuge tube, and add 15 ml of acetone and 80 ml of anaesthetic ether. Mix, and spin in a centrifuge at 1500 **g** for 10 minutes. Decant the colourless ether layer, mix the residue with a further 50 ml of ether, spin in a centrifuge, and again decant. Repeat the washing of the residue with ether, until no odour of phenol remains after the ether has been allowed to evaporate from the residue. Dissolve the residue in the minimum amount of water, and transfer the solution quantitatively to paper for chromatography and subsequent determination of coloured impurities as described in the procedure for hydroxocobalamin. Measure the optical density at the maximum near 351 mμ on a suitable dilution of the sample, and calculate the apparent hydroxocobalamin in the sample; use $E^{1\%}_{1cm} = 190$. Deduct from the value obtained the proportion of coloured impurities found, and report the results as micrograms of hydroxocobalamin per millilitre. The ratio of the optical density at 361 mμ to that at 351 mμ should also be determined on the original solution, to permit calculation of the amount of any cyanocobalamin in the sample.

The amount of phenol - chloroform mixture used for extraction should be as small as possible, so as to minimise the volume of ether required for precipitation. This is preferable to re-extraction into water, as the cyanocobalamin is obtained solid and no further concentration step is needed.

As a check on possible losses during the extraction, the hydroxocobalamin in 50 ml of a solution containing 1000 μg per ml was partially degraded by steaming for 4 hours. One 5-ml portion to which two drops of 0·3 per cent. w/v hydrocyanic acid had been added was set aside for 15 minutes and was then loaded on to paper for determining coloured impurities. Another 5-ml portion was treated with hydrocyanic acid in the same way, but was then extracted with phenol - chloroform mixture before chromatography. Duplicate results for coloured impurities were 17 and 16 per cent. without and 17 and 18 per cent. with the extraction step. Before it was steamed the hydroxocobalamin contained 4 per cent. of coloured impurities.

Consideration of possible impurities in injection solutions suggests that the coloured impurities determined by the above method may provide a good indication of any deterioration. Although the small amounts of analogues and other cobalamins present (even if less stable than the hydroxocobalamin) are unlikely to affect substantially the total amount of impurities occurring after storage, the amount of red acids increases, and the total coloured impurities increase by the same amount. Provided that analogues and interfering substances are present in negligible amounts, determination of the coloured impurities permits correction of the optical density at 351 mμ and an accurate determination of hydroxocobalamin.

To check this, 100-ml portions of an aqueous solution of hydroxocobalamin (1000 μg per ml) were steamed for different times to produce a substantial degree of breakdown. These solutions were assayed microbiologically (three consecutive daily tests, in duplicate, at two levels with *Escherichia coli* M200); the optical density at 351 mμ was measured, and the coloured impurities were determined. The results are shown in Fig. 1. It will be noted that for heat-induced breakdown the drop in optical density is approximately half the drop in microbiological potency, and the differences between the two sets of values are well correlated with the amounts of coloured impurities. The figures for the solution that has not been treated in an autoclave are anomalous in that the microbiological assay results (fiducial limits for a single day's assay, 2 operators at 2 dose-levels, are $\pm$11 per cent., P = 0·95) are higher than the corresponding ultraviolet figure, although absorbing impurities are present. The hydroxocobalamin sample chosen, however, was not of high purity and *E. coli* M200 responds to a wide range of cobalamin-like substances of different stabilities and with different effects on zone size. Factor B, for example, produces a large rather diffuse zone in comparison with the same amount of hydroxocobalamin. The higher microbiological results obtained may be

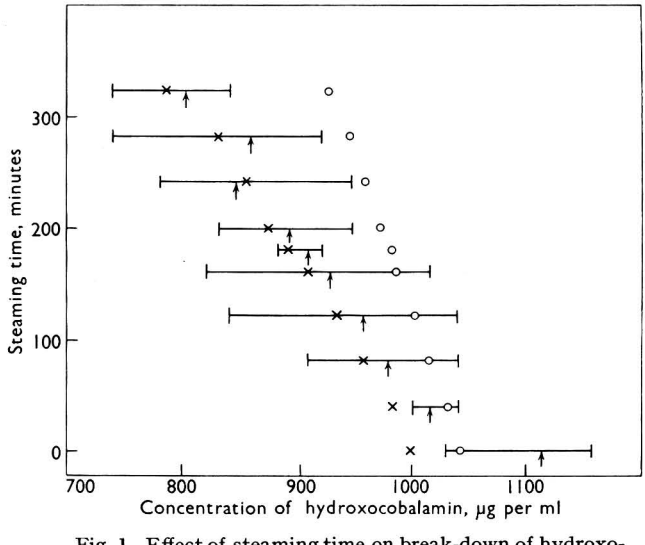


Fig. 1. Effect of steaming time on break-down of hydroxocobalamin: ○, ultraviolet spectrophotometric results; ×, ultraviolet spectrophotometric results *minus* red impurities; ——, microbiological results (range over 3 days); ↑, mean of biological results

attributed to the presence of small amounts of such compounds, having comparatively low stability.

It has not so far been possible to prove that solutions of hydroxocobalamin for injection (100 to 1000 μg per ml) stored at ambient temperatures undergo the same type of breakdown, but we have found that the increase in coloured impurities in typical preparations is less than 1 per cent. at room temperature and less than 2 per cent. at 31° C over a period of 7 months.

It is suggested that a limit of not more than 5 per cent. for total coloured impurities determined by the proposed method should be attainable for hydroxocobalamin; a further 10 per cent. may be considered permissible for their development during manufacture and later storage, provided that this is offset by an adequate overage of hydroxocobalamin itself.

We thank Mr. C. J. Sparkes and Miss M. Armitage for practical assistance and Mr. S. Varsanyi for the microbiological assays.

References

1. Smith, E. Lester, Martin, J. L., Gregory, R. J., and Shaw, W. H. C., *Analyst*, 1962, **87**, 183.
2. Armitage, J. B., Cannon, J. R., Johnson, A. W., Parker, L. F. J., Smith, E. Lester, Stafford, W. H., and Todd, A. R., *J. Chem. Soc.*, 1953, 3849.
3. Dion, H. W., Calkins, D. G., and Pfiffner, J. J., *J. Amer. Chem. Soc.*, 1954, **76**, 948.

Received *September 10th*, 1962

The Determination of 4-Methyl-2,6-di-t-butyl Phenol in Liquid Paraffin B.P.

By B. BRAITHWAITE and G. E. PENKETH

(Imperial Chemical Industries Limited, Heavy Organic Chemicals Division, Research Department, Billingham, Co. Durham)

A colorimetric procedure is described for determining the antioxidant 4-methyl-2,6-di-t-butyl phenol at the 10 p.p.m. level in liquid paraffin B.P. The antioxidant is converted to the intensely coloured 3,5,3′,5′-tetra-t-butyl stilbene-4,4′-quinone by oxidation with lead dioxide in isopropanol azeotrope. The quinone is extracted into cyclohexane, and the optical density is measured at 420 mμ. A sample of uninhibited liquid paraffin is necessary, but, for the instances when this is not available, a method is described for extracting the antioxidant and recovering the liquid paraffin.

Liquid paraffin B.P. is a mixture of hydrocarbons derived from petroleum, which, under the present requirements of the British Pharmacopoeia, may be stabilised by the addition of not more than 10 p.p.m. of α-tocopherol. From 1963 onwards, however, it is proposed to permit the additional use of up to 10 p.p.m. of 4-methyl-2,6-di-t-butyl phenol (4M26B), and as a result of this a request was received from the B.P. commission for a suitable method of determining 4M26B in the presence of α-tocopherol.

The general problem of determining low concentrations of antioxidants in the wide range of materials to which they are now added poses two difficult analytical problems—separation of the additive and development of sensitive and preferably specific methods for its determination.

Among the procedures for separating antioxidants reported in the literature in recent years are extraction,[1,2] steam-distillation,[3,4,5,6] column chromatography[7] and gas - liquid chromatography.[8,9] In our hands each of these procedures has given satisfactory results for particular types of inhibited materials, but none proved satisfactory for liquid paraffin, and a novel extraction procedure was eventually adopted. Methods for determining 4M26B have been based mainly on measurement of ultraviolet absorption[10,11] or colorimetry,[3,6,12] but the variable background absorption of liquid paraffin ruled out ultraviolet measurement, and the colorimetric methods lacked sensitivity or specificity or both.

For the work described here it was decided to investigate the oxidation of 4M26B with lead dioxide as the basis of a colorimetric method. An effective separation procedure for removing 4M26B from liquid paraffin was simultaneously developed, but was ultimately not required owing to the high sensitivity of the final colorimetric method.

Experimental

Extraction of 4-methyl-2,6-di-t-butyl phenol—

Although continuous liquid extraction has been used with success for separating 4M26B from several solid materials, little has been reported about the analogous use of continuous liquid - liquid extraction. It was therefore decided to explore this possibility by using the apparatus shown in Fig. 1. A survey of common organic solvents, in which 4M26B is soluble and liquid paraffin virtually insoluble, showed that lower alcohols should be the most suitable extractants. Preliminary experiments with ethanol and methanol on a sample of liquid paraffin containing 20 mg of 4M26B per 100 ml gave recoveries of only 70 to 80 per cent., and observations of the operation of the liquid - liquid extractor suggested that poor mixing, owing to the high viscosity of the liquid paraffin, might well be the cause of these low recoveries. The same sample of liquid paraffin was therefore diluted with cyclohexane and again extracted with methanol for 6 hours. Recoveries of 96·7 and 98·0 per cent. were then achieved. When applied to a sample of liquid paraffin containing 10 p.p.m. of 4M26B, complete extraction was again achieved.

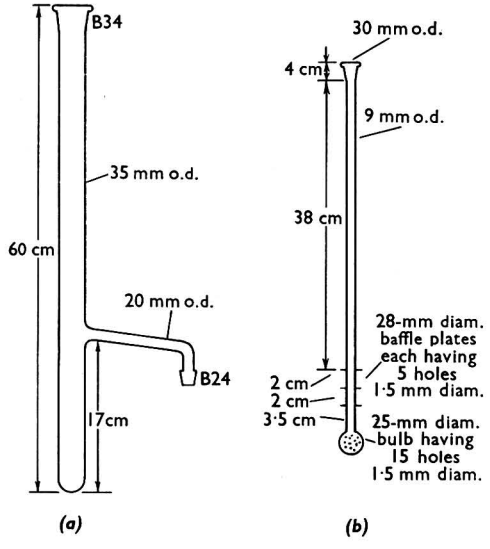


Fig. 1. Continuous liquid extraction apparatus: (*a*) extractor; (*b*) solvent diffuser

The measurement of ultraviolet absorption, although satisfactory for the extraction experiments, could not, however, be adopted for the final method. Liquid paraffin exhibits variations in absorption from batch to batch, thus making it necessary to use in the blank test the identical base oil present in material under test. Since this is impracticable in a routine method, attention was turned to the development of a more generally applicable colorimetric procedure. The method ultimately developed proved so sensitive that removal of 4M26B by continuous extraction was unnecessary, and, although a blank test was still required, any liquid paraffin free from 4M26B was suitable. However, when uninhibited liquid paraffin is not available or when any doubt exists, the extraction procedure provides a convenient way of preparing liquid paraffin suitable for the blank test, and for this reason full experimental details are given below.

EXTRACTION PROCEDURE—

Measure 90 ml of liquid paraffin B.P. into a 250-ml beaker, and add 30 ml of cyclohexane; mix well, and then transfer to the liquid - liquid extractor. Introduce the solvent diffuser, and fit a double-surface water condenser to the top of the extractor. The liquid level in the extractor should be about 2 cm below the bottom of the side-arm. Connect the side-arm to a round-bottomed 250-ml flask containing 100 ml of analytical-reagent grade methanol, and place in a suitably sized heating mantle (alternatively, a 250-ml conical flask and a hot-plate may be used). Bring the contents of the flask to the boil, and extract the liquid paraffin - cyclohexane mixture for 6 hours. Cool to room temperature, and then transfer the contents of the extractor to a 250-ml separating funnel. Allow the layers to separate, and then run off the liquid paraffin - cyclohexane layer into a round-bottomed 250-ml flask. Connect the flask to the vacuum-distillation apparatus, and distil off all the cyclohexane at a pressure not exceeding 10 mm of mercury.

Cool the contents of the boiler, and store in a clean bottle until required.

DEVELOPMENT OF COLORIMETRIC METHOD—

When an alcoholic solution of 4M26B is boiled under reflux with lead dioxide, a yellow colour is formed that has an absorption maximum at 420 mμ. Attempts to carry out the reaction directly on a solution of 4M26B in liquid paraffin, or on liquid paraffin diluted with cyclohexane, did not, however, produce a colour. Addition of the latter alone to an ethanolic solution of the antioxidant also resulted in considerable diminution of the colour, thereby

demonstrating that the hydrocarbons have this common property. All experiments on the colour reaction were carried out in 250-ml conical flasks fitted with double-surface water condensers. The flasks and contents were heated on a hot-plate for the appropriate period, and then cooled to room temperature. After filtration through a No. 4 sintered Gooch crucible, the solutions were made up to a suitable volume, and the optical densities were measured at 420 mμ.

EFFECT OF AMOUNT OF LEAD DIOXIDE USED—

Different weights of lead dioxide in the range 0·1 to 2·0 g were boiled under reflux for 3 hours with 75-ml portions of a methanol solution containing 2 mg of 4M26B per 100 ml. Within the limits of experimental error, optical densities were identical. On the grounds of economy of reagents, 0·1 g of lead dioxide was adopted as standard for all subsequent work in which methanol was used. Subsequently, however, when isopropanol azeotrope was found to be a more suitable solvent, it was necessary to increase this to 1 g in order to obtain reproducible colours.

EFFECT OF TIME—

Portions (75 ml) of the same methanol solution as above were boiled under reflux for different periods up to 4 hours, and then diluted to 100 ml with methanol. The results were—

Time, hours	½	1	1½	2	3	4
Optical density (1-cm cell)	0·492	0·588	0·555	0·555	0·545	0·505

Blank experiments carried out at the same time did not produce any perceptible colours.

Later it was realised that addition of water resulted in a considerable enhancement in the intensity of the colour, and the work was repeated with 78 per cent. v/v aqueous methanol containing 1 mg of 4M26B per 100 ml. Similar experiments were carried out with isopropanol azeotrope as solvent. In each test the solutions were diluted to 100 ml and the optical densities measured in ½-cm cells. The results were—

Time, hours		½	1	1½	2	2½	3	4
Optical density (½-cm cell)	Aqueous methanol	0·282	0·348	0·435	0·460	0·475	0·473	0·475
	Isopropanol azeotrope	0·013	0·156	0·284	0·322	0·358	0·355	0·365

As a result of these experiments a standard reaction time of 3 hours was adopted.

EFFECT OF WATER—

Experiments were carried out to determine the effect of adding water to the reaction mixture. In each test 1 mg of 4M26B was boiled under reflux with 90 ml of aqueous methanol or isopropanol containing various proportions of water. The solutions were diluted to 100 ml before measurement. The results were—

Ratio of alcohol to water		85/5	80/10	70/20	60/30	45/45	30/60	20/70
Optical density (½-cm cell)	Methanol	0·263	0·350	0·448	0·225	0·258	—	—
	Isopropanol	0·228	0·315	0·475	0·552	0·594	0·495	0·025

These results show that water considerably enhances the intensity of the developed colours. At the optimum ratio of 70 parts of methanol to 20 parts of water (78 per cent. v/v of ethanol) the optical density is almost twice that obtained when no water is present. With isopropanol the optimum ratio is 45/45, but, because of solubility difficulties, isopropanol azeotrope was adopted for subsequent work.

EFFECT OF α-TOCOPHEROL—

α-Tocopherol does not produce a colour when oxidised with lead dioxide in methanol or isopropanol solution.

EXTRACTION OF COLOUR INTO CYCLOHEXANE—

During attempts to apply the colour reaction to methanol extracts of 4M26B, it was observed that the developed colour partitioned between the alcohol and the small amount of liquid paraffin that separated out on cooling. This suggested that considerable concentration of the intensity of the colour would be achieved by extraction into a hydrocarbon solvent after development of the colour.

Portions of liquid paraffin (5 ml) containing 0·5, 1·0 and 1·5 mg of 4M26B were boiled under reflux for 3 hours with 100 ml of isopropanol azeotrope and 1 g of lead dioxide. After the solutions had been cooled they were diluted with 100 ml of water, and then extracted 3 times with 10-ml portions of cyclohexane, which at this stage does not affect the colour reaction. The bulked extracts were filtered into 50-ml calibrated flasks, made up to volume with cyclohexane, and the optical densities measured at 420 mμ. Results of these determinations are shown in Fig. 2 and indicate that a considerable increase in sensitivity had in fact been achieved.

Although these results show good repeatability, it will be seen that the best straight line fitting these points does not pass through the origin. This effect was noticed in all subsequent work, and it was concluded that there is a certain minimum concentration of 4M26B (about 0·2 mg) below which the Beer - Lambert law is not obeyed. This difficulty was obviated in the final form of the method by the addition of known amounts of 4M26B to samples, to increase the minimum concentration above the 0·2-mg level.

DIRECT COLORIMETRIC DETERMINATION—

From the initial experiments it was known that liquid paraffin had a deleterious effect on the colour reaction. It was considered, however, that this could be more than counterbalanced by the increase in sensitivity gained by extracting the colour into cyclohexane. In experiments to prove this point, isopropanol azeotrope was chosen as solvent, since liquid paraffin is more soluble in this solvent than in methanol and it provides a convenient way of adding water necessary for the reaction. Various amounts of liquid paraffin were added to 100-ml portions of isopropanol azeotrope containing 1 mg of 4M26B, 1 g of lead dioxide was added, and the mixture was boiled under reflux for 3 hours. After dilution with water, the colour was extracted into 50 ml of cyclohexane, and the optical density was measured. The results shown below indicate that the reduction in optical density produced by liquid paraffin is directly proportional to the amount present.

Volume of liquid paraffin, ml	0	5	10	20
Optical density (½-cm cell)	0·805	0·695	0·595	0·419

By comparison with the results showing the effect of water, p. 299, it can be seen that, even in the presence of 10 ml of liquid paraffin, the optical density for 1 mg of 4M26B shows an increase of about 30 per cent. over the maximum value obtained with aqueous methanol. It was decided, therefore, to adopt a sample size of 10 ml as standard for a liquid paraffin containing 10 p.p.m. of 4M26B.

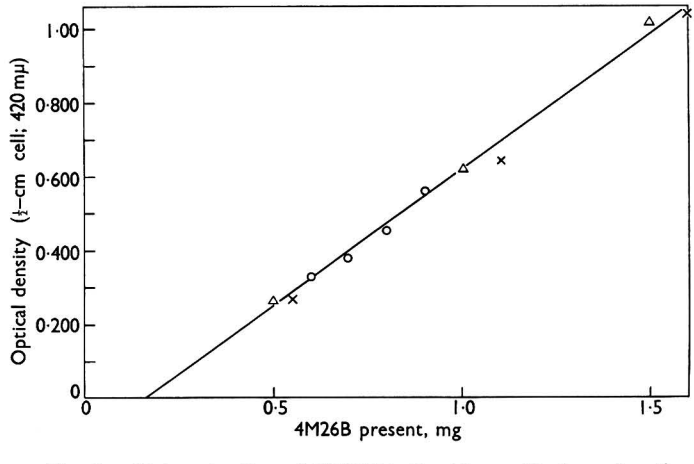


Fig. 2. Determination of 4M26B in liquid paraffin by extraction of the colour into cyclohexane

METHOD

APPARATUS—

Spectrophotometer—A Unicam SP500, or any suitable instrument capable of measuring optical density at 420 mμ, and 1-cm glass or silica cells.

REAGENTS—

Lead dioxide.

Isopropanol.

Isopropanol azeotrope—Dilute 85 parts of isopropanol with 15 parts of distilled water, and mix well.

Cyclohexane.

Standard solution of 4-methyl-2,6-di-t-butyl phenol—Dissolve 0·1000 ± 0·0005 g of pure 4M26B in 50 ml of analytical-reagent grade methanol contained in a 100-ml calibrated flask. Make up to the mark with analytical-reagent grade methanol, and mix well. Dilute 10 ml of this solution to 100 ml with analytical-reagent grade methanol, and mix well.

1 ml ≡ 0·1 mg of 4M26B.

PROCEDURE—

In each of four 500-ml conical flasks place exactly 10 ml of the liquid paraffin under test and then 0, 2, 3 or 4 ml of the standard solution of 4M26B (equivalent to 0, 0·2, 0·3 and 0·4 mg of 4M26B). In a further four identical flasks place 10-ml portions of "blank" liquid paraffin (*i.e.*, one containing α-tocopherol, but no 4M26B—see Note 1) and then 0, 2, 3 or 4 ml of the standard solution of 4M26B. Add to the contents of all the flasks 2 g of lead dioxide, 200 ml of isopropanol azeotrope and a few boiling-beads. Connect the flasks to double-surface water condensers, and boil under reflux for 3 hours on a hot-plate. At the end of this period cool to room temperature, and then quantitatively transfer the contents of each flask in turn to 750-ml separating funnels. Add 300 ml of distilled water to the contents of each funnel, and then extract the colours with three 10-ml portions of cyclohexane. Filter the extracts through Whatman No. 42 filter-papers into 50-ml calibrated flasks. Add exactly 10 ml of isopropanol to the contents of each, and then make up to the mark with cyclohexane, and mix well. Measure the optical density in 1-cm cells of each solution at 420 mμ against cyclohexane. Plot graphs relating optical density to milligrams of 4M26B added for both sample and blank liquid paraffins (see Fig. 3).

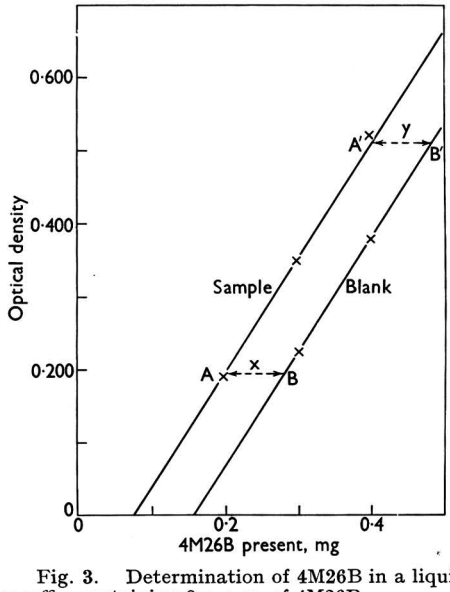


Fig. 3. Determination of 4M26B in a liquid paraffin containing 8 p.p.m. of 4M26B

Concentration of 4M26B, p.p.m. = 100 x
or = 100 y

CALCULATION—

Let the point on the sample graph corresponding to 0·2 mg of added 4M26B be A and that corresponding to 0·4 mg of added 4M26B be A′.

Draw lines parallel to the X axis from A and A′ to intersect the "blank" graph at B and B′.

Let the number of milligrams of 4M26B corresponding to AB and A′B′ be x and y, respectively (x and y will normally be identical; if they differ by more than 0·01 mg, repeat the determination).

Then—

Concentration of 4M26B in sample, p.p.m. = 100 x

or = 100 y

NOTE—

1. If a blank liquid paraffin (*i.e.*, one containing no 4M26B) is not available, a suitable blank can be prepared by the extraction procedure described on p. 298.

RESULTS AND DISCUSSION

Some results on factitious mixtures of 4M26B in liquid paraffin were—

4M26B added, p.p.m. . .	6	10	12	15	20
4M26B found, p.p.m. . .	6,6	8, 9, 9	12, 11, 12	13, 14, 15	18, 18

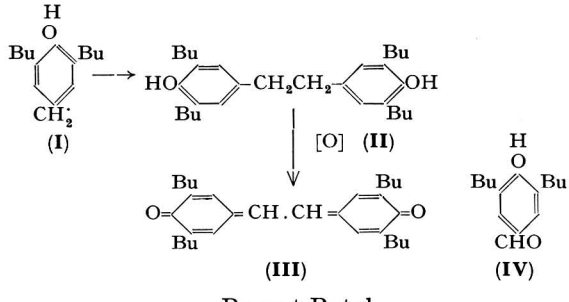
These results demonstrate that the method is sufficiently precise for determining 4-methyl-2,6-di-t-butyl phenol in liquid paraffin.

DISCUSSION—

Although the mechanism of the colour reaction is not fully understood, it seems certain that the main product of the oxidation is 2,5,3′,5′-tetra-t-butylstilbene-4,4′-quinone (**III**), which is a brilliant red and responsible for the colour produced.

Cosgrove and Waters[13] have reported the formation of **III** by the action of benzoyl peroxide on 4M26B, and suggest that this product is formed via a 3,5-di-t-butyl-4-hydroxybenzyl radical (**I**). This view is supported by the fact that, in our work, the oxidation of 4M26B in methanol with lead dioxide nearly always gave an initial blue colour before development of the yellow. Cook[14] also confirms this view, and suggests that an expected intermediate oxidation product would be **II**.

The probable reaction is therefore—



Bu = t-Butyl.

Cook found that the oxidation of 4M26B with limited amounts of oxygen, lead dioxide, alkaline ferricyanide or hydrogen peroxide always gave mixtures of **II** and **III**. He isolated **II** from the lead dioxide oxidation of 4M26B in diethyl ether as a light yellow powder (m.p. 171° to 175° C), which, on recrystallisation from alcohol, gave white needles (m.p. 174° to 175° C). **III** was obtained, by concentration of the mother liquor, as bright red crystals (m.p. 296° to 301° C; 314° to 315° C after recrystallisation).

Wasson and Smith[15] have reported that oxidation of 4M26B with chromic anhydride - acetic acid gives a white aldehyde (m.p. 185° C) believed to be 3,5-di-t-butyl-4-hydroxybenzaldehyde (**IV**). Cook does not report finding any trace of this. We have shown, however, that if the oxidation of 4M26B with lead dioxide is carried out in methanol, and more especially if water is also present, the aldehyde **IV** is indeed formed.

One gram of 4M26B was oxidised with 3 g of lead dioxide in 120 ml of analytical-reagent grade methanol. A copious white precipitate was formed, which proved to be lead hydroxide (lead found, 85·9 per cent.; theory, 86·0 per cent.). Dilution of the red methanol filtrate with water gave a yellow powder (m.p. 172° to 176° C) after one recrystallisation from ethanol. This material had a C=O value of 6·3 per cent. (theory for **IV**, 11·9 per cent.) and was probably a mixture of **II** and **IV**. Cook quotes a mixed melting-point for this mixture of 174° C. Concentration of the mother liquor gave dark red crystals (m.p. 300° to 304° C); this was the quinone **III**.

In a second similar experiment the oxidation was carried out in the presence of 25 ml of water. Dilution of the filtrate in the manner described above yielded a pale yellow precipitate (m.p. 182° to 184° C). After sublimation, this material was isolated as an almost white powder (m.p. 185° to 188° C, C=78·0 per cent., H=9·7 per cent., m.p. of 24DNP derivative 239° to 241° C; theory, m.p. 189° C, C=77·2 per cent., H=9·4 per cent., m.p. of 24DNP 236° C). This material was 3,5-di-t-butyl-4-hydroxybenzaldehyde (**IV**). Concentration of the mother liquor again gave the red quinone **III** (m.p. 304° to 308° C, C=82·9 per cent., H=9·6 per cent.; theory, C=83·1 per cent., H=9·7 per cent.).

Cook's preparation of **II** and **III** by oxidation of 4M26B in diethyl ether with lead dioxide was next duplicated. **II** was isolated as a dark yellow powder (m.p. 169° to 172°C, C=81·9 per cent., H=10·7 per cent.) and **III** was obtained as scarlet red crystals (m.p. 315° to 319° C, C=82·3 per cent., H=9·6 per cent.). No trace of aldehyde was found. It is evident, therefore, that although the final product of oxidation is the stilbene quinone (**III**) in each instance, the intermediate oxidation products are dependent on the solvent in which the reaction is carried out.

We thank Mrs. L. F. C. Underwood for her valuable assistance with the experimental work.

References

1. Mahon, J. H., and Chapman, R. A., *Anal. Chem.*, 1951, **23**, 1116.
2. ——, ——, *Ibid.*, 1951, **23**, 1120.
3. Anglin, C., Mahon, J. H., and Chapman, R. A., *J. Agric. Food Chem.*, 1956, **4**, 1018.
4. Shell Development Co., Emeryville, California, Emeryville Method Series, 1956, EMS 3H, 31/56R.
5. Filipic, V., and Ogg, C., *J. Ass. Off. Agric. Chem.*, 1960, **43**, 795.
6. Sloman, K. G., Romagnoli, R. J., and Cavagnol, J. C., *Ibid.*, 1962, **45**, 76.
7. Phillips, M. A., and Hinkel, T. D., *J. Agric. Food Chem.*, 1957, **5**, 379.
8. Jennings, E. C., Curran, T. D., and Edwards, D. G., *Anal. Chem.*, 1958, **30**, 1946.
9. Buttery, R. G., and Stuckey, B. N., *J. Agric. Food Chem.*, 1961, **9**, 283.
10. Wadelin, C. W., *Anal. Chem.*, 1956, **28**, 1530.
11. Hanson, P. V., Kaufman, F. L., and Widerman, L. H., *J. Amer. Oil Chem. Soc.*, 1959, **36**, 1933.
12. American Society for Testing Materials, A.S.T.M. Committee D.2, Volume 2, 1960, Method D, 1473.
13. Cosgrove, S. L., and Waters, W. A., *J. Chem. Soc.*, 1951, 388.
14. Cook, C. D., *J. Org. Chem.*, 1953, **18**, 261.
15. Wasson, J. I., and Smith, W. M., *Ind. Eng. Chem.*, 1953, **45**, 197.

Received *October 11th*, 1962

The Determination of the Total Sulphur Dioxide Content of Ciders

By L. F. BURROUGHS and A. H. SPARKS

(*Research Station, Long Ashton, Bristol*)

The factors involved in determining total sulphur dioxide in alcoholic beverages are discussed, and an iodimetric method is described in which reflux distillation from dilute orthophosphoric acid and a carrier-stream of nitrogen are used. The reflux condenser effectively retains other iodine-reducing substances, and the iodimetric titration eliminates interference due to the presence of acetaldehyde.

In principle, the determination of sulphur dioxide in a foodstuff requires the distillation of the sample with acid and collection of the sulphur dioxide in some suitable solution in which it can be determined. The fact that so many methods and modifications of conditions of distillation and determination have been proposed[1,2,3] indicates that no entirely satisfactory general procedure has yet been devised. The extensive literature on the determination of sulphur dioxide is confused and sometimes contradictory, possibly owing to lack of appreciation of the errors involved in a particular method, especially if it is applied to a product for which it is not entirely suitable. In most methods there are several possible sources of error, some causing high results and some low, so that apparently correct results can be obtained fortuitously by combination of these effects. Unless, therefore, the separate errors are recognised and each minimised or evaluated, even the apparent complete recovery of added sulphur dioxide is no guarantee of the general reliability of a method. Further, different foodstuffs present different problems owing to the presence of interfering substances.

Any method for the accurate determination of sulphur dioxide in alcoholic beverages should take account of (*a*) the presence of volatile substances other than sulphur dioxide that will give appreciable blank values and (*b*) the presence of acetaldehyde, which can react with sulphur dioxide before it is oxidised (with hydrogen peroxide or iodine) in the receiver. This latter requirement is frequently overlooked; the error involved is usually small (1 to 2 per cent.), but cannot be neglected in an accurate determination.

The various methods for determining sulphur dioxide in wines have been fully reviewed by Deibner.[3] Apart from Ripper's method[4,5] of direct titration with iodine, which is well known to be subject to errors, the most commonly used methods all involve distillation and can be divided into two main groups.

Alkalimetric methods—The acidified sample is boiled under reflux and the sulphur dioxide carried over in a stream of air or inert gas into neutralised hydrogen peroxide, where it is oxidised to sulphuric acid and, finally, titrated with sodium hydroxide. The result can be checked gravimetrically by precipitation as barium sulphate. This is essentially Monier Williams' method.[6] The presence of volatile acids in the sample may lead to high results by titration (depending on the efficiency of the reflux condenser), but does not affect results determined gravimetrically.

Iodimetric methods—The sulphur dioxide distilled from the acidified sample is collected in a suitable receiving solution and subsequently titrated with iodine. Various receiving solutions have been proposed, *e.g.*, sodium hydroxide,[3] sulphuric acid,[7] and iodine.[8] The advantage of this method is the convenience and sensitivity of the iodimetric titration.

Two methods for determining total sulphur dioxide in wines have been described recently, one alkalimetric and the other iodimetric. In describing the first, Kielhöfer and Aumann[9] discussed possible sources of error in the iodimetric titration of sulphur dioxide and concluded that Paul's alkalimetric method is preferable; Paul later described his method in more detail.[10] The wine (10 or 20 ml) is boiled under reflux with 5 ml of 25 per cent. phosphoric acid for 15 minutes in a Lieb - Zacherl apparatus[11]; the liberated sulphur dioxide is carried over in an air stream into 3 ml of 0·3 per cent. hydrogen peroxide (previously neutralised to Tashiro

indicator) and titrated with 0·01 N sodium hydroxide. The essential feature of this apparatus is a highly efficient reflux condenser. Blank determinations of any volatile acids not retained by the condenser can be made by adding a trace of hydrogen peroxide to the sample before distillation. Paul, however, claims that blank determinations for normal wines are negligible, and this was confirmed by Kielhöfer and Aumann. Although this method is satisfactory from the point of view of the blank value, it cannot allow for the error due to acetaldehyde.

In the second method, described by Diemair, Koch and Hess,[12] the sample is distilled from dilute phosphoric acid, and the distillate is collected in buffer at pH 3·4 to prevent loss of sulphur dioxide by volatilisation. The distillate is titrated with 0·01 N iodine, first at pH 3·4 to oxidise the free sulphur dioxide and then after solid sodium hydrogen carbonate has been added to dissociate any acetaldehyde bisulphite. Although this method caters for the presence of acetaldehyde, it makes no allowance for the blank value of volatile iodine-reducing substances other than sulphur dioxide. With ciders this blank value has been found to represent about 5 p.p.m. of sulphur dioxide.

The proposed method combines the advantages of both the procedures described above, since it gives zero blank corrections with ciders and complete recovery of sulphur dioxide in the presence of acetaldehyde.

Method

General principles—

The development of this method was based on the considerations listed below:—

1. In order to be able to titrate acetaldehyde bisulphite it is essential to use an iodimetric method, since the adjustment of pH necessary to dissociate the bound sulphur dioxide is not permissible in the alkalimetric procedure.

2. In principle, Diemair, Koch and Hess's method[12] appeared to offer a rapid and convenient means of determining total sulphur dioxide, particularly if it could be done by steam-distillation in a Markham apparatus.[13] Recovery of sulphur dioxide (10 ml at 300 p.p.m.) from acetaldehyde bisulphite was 99·5 per cent. by this procedure. When, however, ciders that were shown to be completely free from sulphur dioxide (by acid distillation and colorimetric determination[14] by pararosaniline and formaldehyde) were analysed by this method, the cider blank value, due to volatile iodine-reducing substances, corresponded to 3 to 8 p.p.m. of sulphur dioxide. No means could be found of reducing the magnitude of this blank value. The alternative, of determining the blank value by adding a trace of hydrogen peroxide to the cider to oxidise the sulphur dioxide before distillation, was tested on a sulphur dioxide free cider. This was unsuccessful since the blank value was much greater (18 p.p.m. as sulphur dioxide) after treatment with hydrogen peroxide than without (3 p.p.m.); further, traces of hydrogen peroxide in the distillate reacted slowly with potassium iodide to liberate iodine. It was therefore impossible to differentiate chemically between sulphur dioxide and the other volatile iodine-reducing substances present in the distillate obtained by steam-distillation.

3. A physical separation of the volatile iodine-reducing substances, which produce the cider blank value, from sulphur dioxide was achieved by distillation under reflux, as in the alkalimetric procedures. The combination of iodimetric titration with distillation under reflux in a stream of nitrogen is made possible by the fact that the vapour pressure of iodine in potassium iodide solution is greatly reduced by increasing the concentration of potassium iodide. In the proposed method the small amount of iodine volatilised by passing nitrogen through 0·01 N iodine in 5 per cent. potassium iodide solution is completely absorbed in a second tube containing 5 per cent. potassium iodide. This second tube also acts as a second absorber for sulphur dioxide, for which purpose a trace of iodine is initially added to the contents. Since it is essential that no iodine should escape from the second tube, a third absorption tube is included, containing 5 per cent. potassium iodide solution and a drop of starch indicator. This tube normally remains colourless during several determinations.

4. During the distillation, a small amount of the sulphur dioxide re-combines (presumably in the vapour phase) with acetaldehyde, which also distils over. In order to determine this bound sulphur dioxide, the combined contents of tubes 1 and 2 are made weakly alkaline (pH 8·3) to allow the acetaldehyde bisulphite to dissociate. After 30 seconds the solution is acidified and the residual iodine titrated with 0·01 N sodium thiosulphate. Control experiments showed that acetaldehyde itself is not oxidised by iodine under these conditions.

5. In the alkalimetric methods described by Paul[10] and by Kielhöfer and Aumann[9] the sulphur dioxide is carried over in a stream of air. The good recoveries quoted by them are surprising (as recognised by Kielhöfer and Aumann), since most other workers have found it necessary to carry out the distillation in the presence of carbon dioxide or nitrogen. The necessity of avoiding oxidation in the method described below was established by comparing recoveries of sulphur dioxide from 10 ml of a solution containing 245 p.p.m. When the sulphur dioxide was carried over in a stream of nitrogen, the recovery was 245·5 p.p.m.; 223·5 p.p.m. were recovered when a stream of air was used.

APPARATUS—

The apparatus (see Fig. 1) is based on the Monier Williams principle of distillation under reflux in a stream of a carrier gas. It is similar to the Lieb - Zacherl apparatus used by Paul, except that the latter has only one absorption tube. The flask (round-bottomed, 250 ml, with side inlet), double-walled condenser (B24 joints) and tubes (200 mm × 25 mm) are standard pieces of glassware, obtainable from Messrs. Quickfit and Quartz, Ltd. The absorption heads are made from B24 cone-joints with the internal delivery tubes drawn out to a jet. The separate absorption units are joined together by small lengths of polyvinyl chloride tubing. Blood urea distillation tubes, obtainable from Messrs. Quickfit and Quartz, Ltd., can be used but are more difficult to rinse.

The flask is heated by a micro-bunsen burner, with a shield of asbestos with a hole ($1\frac{1}{2}$ inches in diameter) being used to prevent charring of the sample. The absorption tubes are shielded from the heat of the bunsen burner by a vertical sheet of asbestos.

The nitrogen supply is taken from a cylinder of oxygen-free nitrogen, reduced to a pressure of about 15 cm of water.

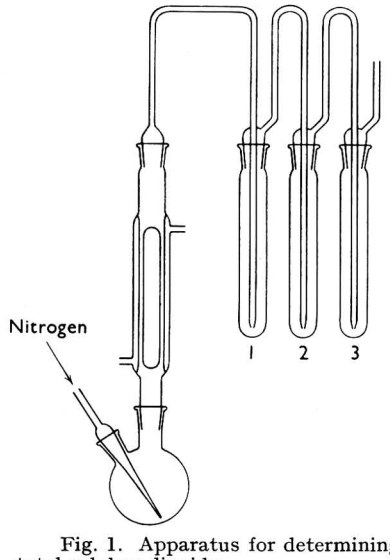


Fig. 1. Apparatus for determining total sulphur dioxide

REAGENTS—

All materials should be of analytical-reagent grade.

Iodine, 0·01 N *in 5 per cent. w/v potassium iodide solution*—This need not be freshly prepared and can be kept as a stock solution.

Potassium iodide, 5 *per cent. w/v*—Freshly prepare free from iodine.

Sodium thiosulphate, 0·1 N—Standardise accurately against potassium iodate. Dilute to 0·01 N as required for use.

Sodium phosphate, 0·2 M—A solution containing 72 g of disodium hydrogen orthophosphate, $Na_2HPO_4.12H_2O$, per litre.

Orthophosphoric acid, 25 per cent. v/v—A solution containing 250 ml of 85 per cent. orthophosphoric acid per litre.

Sulphuric acid, N.

Starch indicator—Prepare a fresh 1 per cent. w/v solution of soluble starch.

PROCEDURE—

By pipette transfer 5-ml portions of 5 per cent. potassium iodide solution to tubes 2 and 3, and add one drop of starch indicator to the contents of tube 3; transfer 10 ml of 0·01 N iodine to tube 1.

By pipette place the sample (10 or 20 ml, containing up to 3 mg of sulphur dioxide) in the 250-ml flask, and add 5 ml of 25 per cent. phosphoric acid. Attach the flask to the condenser. Adjust the flow of nitrogen to a moderate rate (2 to 3 bubbles per second). Heat the flask for 15 minutes with a small flame, and gently boil the contents.

Remove the flame, but keep the nitrogen flowing. Disconnect tube 2, and rinse the delivery tube. Similarly disconnect and rinse tube 1.

Pour and rinse the contents of tube 2 into tube 1, and add 5 ml of 0·2 M sodium phosphate solution. After 30 seconds add 2 ml of N sulphuric acid, and titrate the excess of iodine with 0·01 N sodium thiosulphate solution, adding 5 drops of starch indicator near the end-point. During the titration it is convenient to stir the solution in the tube with a magnetic stirrer having a small polythene-coated iron rod (1 cm long). Subtract the volume of titre from the volume of sodium thiosulphate equivalent to 10 ml of 0·01 N iodine *plus* 5 ml of 5 per cent. potassium iodide found by direct titration. One millilitre of 0·01 N sodium thiosulphate is equivalent to 0·32 mg of sulphur dioxide.

NOTES—

1. A blank titration, carried out by distilling 20 ml of distilled water instead of the sample, should agree with the direct titration of 10 ml of 0·01 N iodine to within ±0·02 ml of sodium thiosulphate solution. This shows that any iodine volatilised from tube 1 is completely retained in tube 2. This is confirmed by the fact that the contents of tube 3 normally remain colourless, and therefore do not need to be replaced during several determinations.

2. Duplicate determinations usually agree within ±0·02 ml of 0·01 N sodium thiosulphate, equivalent to 0·6 p.p.m. of sulphur dioxide in a 10-ml sample.

3. For samples containing more than 3 mg of sulphur dioxide, the volume of 0·01 N iodine in tube 1 can be suitably increased. Similarly, for extremely small amounts of sulphur dioxide the volume of iodine can be reduced, but 5 per cent. potassium iodide solution should then be added to keep a total volume of 10 ml.

4. Samples in which most of the sulphur dioxide is in the free state (*i.e.*, not combined as bisulphite compounds) rapidly evolve their sulphur dioxide in the early stages of boiling, and traces of sulphur dioxide may fail to be absorbed in tube 1. With such samples it is advisable to prolong the flow of nitrogen before starting to heat the solution, so that part of the free sulphur dioxide is carried over in the cold and sufficient iodine is volatilised into tube 2 to retain any sulphur dioxide escaping past tube 1. Alternatively, 2 or 3 drops of 0·01 N iodine may be placed in tube 2 from the pipette before the remainder is placed in tube 1.

TESTS OF RECOVERY OF SULPHUR DIOXIDE

The accuracy of the method was established by investigating (*a*) recovery of free sulphur dioxide from sodium bisulphite solution, (*b*) recovery of bound sulphur dioxide from acetaldehyde sodium bisulphite added to cider and (*c*) the magnitude of the cider blank value due to volatile iodine-reducing substances other than sulphur dioxide.

A solution of sodium bisulphite, calculated to contain approximately 250 p.p.m. of sulphur dioxide, was assayed by the distillation method and by direct iodine titration. In the latter procedure, 10 ml of solution was added by pipette, with stirring, to 10 ml of 0·01 N iodine and 5 ml of 5 per cent. potassium iodide solution; the excess of iodine was then titrated

as in the distillation method. Blank determinations on 10 ml of distilled water were made, both by distillation and by direct titration. The results expressed as millilitres of 0·01 N sodium thiosulphate solution were—

	Distillation	Direct titration
10 ml of distilled water	9·76, 9·74	9·75, 9·74
10 ml of sodium bisulphite	2·08, 2·10	2·09, 2·10

It can be seen that free sulphur dioxide is completely recovered by the distillation procedure, but, in order to ensure this, it is important to have the initial trace of iodine in tube 2 (see Note 4 above). The close agreement between the blank values obtained by distillation and by direct titration supports the observation in Note 1 above.

Recovery of bound sulphur dioxide from acetaldehyde bisulphite added to cider was tested on three ciders that were shown to be completely free from sulphur dioxide (by distillation from acid solution and determination of sulphur dioxide by the colorimetric para-rosaniline - formaldehyde method). Results are shown in Table I.

TABLE I

RECOVERY OF BOUND SULPHUR DIOXIDE FROM ACETALDEHYDE BISULPHITE ADDED TO CIDER

Recovery expressed as a percentage of the results obtained by direct titration

	Cider A			Cider B			Cider C*		
Procedure (see text)	Thio-sulphate titre, ml	Sulphur dioxide found, mg	Recovery, %	Thio-sulphate titre, ml	Sulphur dioxide found, mg	Recovery, %	Thio-sulphate titre, ml	Sulphur dioxide found, mg	Recovery, %
I	7·42	2·375	100·0	7·43	2·378	100·0	8·14	2·605	100·0
	7·44	2·380		7·42	2·375		8·12	2·599	
II	7·43	2·378	100·0	7·40	2·370	99·7	8·11	2·595	99·8
	7·42	2·375	99·9	7·41	2·372	99·8	8·12	2·599	99·9
III	7·34	2·350	98·8	7·30	2·336	98·3	8·02	2·565	98·7
	7·35	2·352	98·9	7·28	2·330	98·0	8·00	2·560	98·4
IV	0·01	0·003	—	0·02	0·006	—	0·02	0·006	—
	0·01	0·003	—	0·02	0·006	—	0·02	0·006	—
V	0·01	0·003	—	0·00	0·000	—	0·00	0·000	—
	0·01	0·003	—	0·00	0·000	—	0·00	0·000	—
VI	0·01	0·003	—	0·01	0·003	—	0·01	0·003	—
	0·01	0·003	—	0·02	0·006	—	0·01	0·003	—

* The acetaldehyde bisulphite solution used with cider C was different from that used with ciders A and B.

Portions (10 ml) of acetaldehyde bisulphite solution, prepared from sodium metabisulphite and redistilled acetaldehyde, were added by pipette to 10 ml of 0·01 N iodine and 5 ml of 5 per cent. potassium iodide solution and titrated as described in the distillation method (Procedure I). Further 10-ml portions were distilled in the presence of 10 ml of cider and 5 ml of 25 per cent. phosphoric acid, and the sulphur dioxide was determined (*a*) by the full procedure described above and (*b*) by omitting the treatment with 0·2 M sodium phosphate. The former (Procedure II) gave complete recovery of sulphur dioxide, but when sodium phosphate was omitted (Procedure III) recovery was slightly low, owing to recombination of sulphur dioxide with acetaldehyde. Blank distillations (Procedure V) were also made on 20-ml portions of the ciders to ensure that none of the sulphur dioxide recorded in the previous distillations was due to other iodine-reducing substances.

Although these three ciders showed zero blank values, it cannot be assumed that all ciders would behave similarly, and some means of measuring the blank value of a cider containing sulphur dioxide is needed. Paul[10] added a trace of hydrogen peroxide to the contents of the flask in order to oxidise the sulphur dioxide before distillation. In Procedure IV, therefore, 10 ml of acetaldehyde bisulphite solution, 10 ml of cider and 5 ml of 25 per cent. phosphoric acid (*i.e.*, as in Procedure II) were treated with 10 drops of 1 per cent. w/v hydrogen peroxide before distillation; the sulphur dioxide was completely oxidised, and the blank values were negligible. In Procedure VI, 20 ml of cider, without added acetaldehyde bisulphite, were oxidised with hydrogen peroxide before distillation; the blank

values were again negligible. Thus, although hydrogen peroxide was found to increase the amount of steam-voltaile iodine-reducing compounds (see Note 2 under "General Principles," p. 305), these substances were effectively retained by the reflux condenser in the apparatus described.

For ordinary purposes and with normal ciders, there seems to be no need to carry out routine blank determinations. The above treatment with hydrogen peroxide would only be needed if other evidence suggested the presence of volatile iodine-reducing substances that might interfere with the determination of sulphur dioxide.

REPRODUCIBILITY—

The method gives highly reproducible results; seven replicate determinations on 20-ml portions of a cider, containing 130 p.p.m. of sulphur dioxide, gave results between 8·06 and 8·08 ml of 0·01 N sodium thiosulphate, with a coefficient of variation of only 0·10 per cent.

REFERENCES

1. Joslyn, M. A., and Bravermann, J. B. S., *Adv. Food Res.*, 1954, **5**, 97.
2. Amerine, M. A., *Ibid.*, 1958, **8**, 133.
3. Deibner, L., *Rev. Ferment.*, 1959, **14**, 179.
4. Ripper, M., *J. prakt. Chem.*, 1892, **46**, 428.
5. "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists," Third Edition, Washington, D.C., 1930, p. 344.
6. Monier Williams, G. W., *Reports of Public Health and Medical Subjects*, No. 43, Ministry of Health London, 1927, p. 1.
7. Deibner, L., and Bénard, P., *Ind. Agric. Aliment.*, 1955, **72**, 565 and 673.
8. Nichols, P. F., and Reed, H. M., *Ind. Eng. Chem., Anal. Ed.*, 1932, **4**, 79.
9. Kielhöfer, E., and Aumann, H., *Mitt. Wein u.-Obstbau, Wien, A*, 1957, **7**, 287.
10. Paul, F., *Ibid.*, 1958, **8**, 21.
11. Leib, H., and Zacherl, M. K., *Hoppe-Seyl. Z.*, 1932, **211**, 211.
12. Diemair, W., Koch, J., and Hess, D., *Z. anal. Chem.*, 1961, **178**, 321.
13. Markham, R., *Biochem. J.*, 1942, **36**, 790.
14. Stone, I., and Laschiver, C., *Wallerstein Lab. Commun.*, 1957, **20**, 361.

Received *August 23rd*, 1962

The Spectrophotometric Determination of Caffeine in Coffee and Tea Products, with Special Reference to Coffee and Chicory Mixtures

BY R. F. SMITH AND D. I. REES

(*The Laboratories, J. Lyons & Co. Ltd., Kensington, London, W.*14)

A spectrophotometric method that can be applied to coffee and chicory mixtures is described for determining caffeine. The sample is first extracted with boiling water and then filtered on a magnesium oxide - filter-aid column. The caffeine content of the sample is calculated from the difference between the optical densities at 273 mμ of the clarified extract before and after extraction with chloroform. High results were obtained when caffeine was determined in mixtures of coffee and chicory owing to the presence in roasted chicory of 5-hydroxymethylfurfural, which was removed from the clarified water extracts by reduction with aluminium amalgam. This method gave results that agreed closely with those obtained by a semi-micro method, and there was a considerable saving in time.

THE use of a spectrophotometer provides a simple and rapid method for determining caffeine in tea and coffee and their products because of the characteristic absorption peak of caffeine at 272–3 mμ. Most methods used for determining caffeine involve the preparation of a suitably clarified water extract from which the caffeine is extracted with chloroform. After purification, the chloroform extract is weighed or its caffeine content is obtained from a Kjeldahl determination of nitrogen. This determination can, however, be avoided by spectrophotometric measurement of the chloroform extract, but, as chloroform is an unsuitable solvent for spectrophotometry, we found it advisable to confine all spectrophotometric measurements to aqueous solutions. First an examination was made of suitable methods for preparing clarified aqueous extracts of the samples. Ishler, Finucane and Borker's method[1] for removing interfering substances, by using heavy magnesium oxide and zinc ferrocyanide, was found by us to remove chlorogenic acid, but did not remove trigonelline, which has an absorption peak at 266 mμ, close to the 273-mμ peak of caffeine. We finally based our clarification technique on that used by Bower, Anderson and Titus,[2] and used a column of heavy magnesium oxide and diatomaceous filter-aid. Portions of the aqueous extracts were used for spectrophotometric measurements made on suitably diluted solutions both before and after extraction of caffeine with chloroform. The caffeine contents of the solutions were then obtained from the changes in optical density after extraction with chloroform. We found this more convenient and less liable to errors than removal of the chloroform by distillation and solution of the residue in water. Trigonelline, being insoluble in chloroform, remained in the aqueous solutions and by this procedure did not cause interference. To check these results caffeine was also determined in portions of the same clarified aqueous extracts by extraction with chloroform and then by micro-Kjeldahl determination of nitrogen in the chloroform extracts by the original method.[2]

Satisfactory agreement was obtained between the results by the two methods on samples of coffee and tea, but the spectrophotometric method gave results that were too high and variable with samples containing both coffee and chicory. Lee Kum-Tatt[3] has also reported the presence of substances interfering with the spectrophotometric determination of caffeine in coffee mixtures containing chicory, which made it necessary for him to determine the nitrogen contents of the chloroform extracts of such samples.

We have found that a sample of pure chicory gave an apparent caffeine content of 1·5 per cent. determined by the spectrophotometric method, owing to the presence in chicory of substances soluble in chloroform and having an absorption maximum at 282 mμ. We have now established that the interference is due mainly to 5-hydroxymethylfurfural, and to a lesser extent to furfural. Both these compounds have been identified among the aromatic

principles of roasted chicory by Reichstein and Beitter.[4] We found that two-dimensional paper partition chromatography of a chloroform extract of roasted chicory, first with n-butanol - acetic acid - water (63 : 10 : 27) and then with 2 per cent. acetic acid, gave one spot having a similar R_F value to those of 5-hydroxymethylfurfural and which contributed almost entirely to the absorption at 282 mμ. It appeared as a dark spot in ultraviolet light of wavelength 253·7 mμ and as a yellow spot in daylight after exposure to ammonia vapour, and gave other colour reactions characteristic of 5-hydroxymethylfurfural. Thus the apparently high caffeine contents of samples of coffee and chicory mixtures were accounted for, being due to the presence of 5-hydroxymethylfurfural in the clarified extracts and to its removal by extraction with chloroform affecting the optical density at 273 mμ.

A search was then made for a simple method of removing the interference due to 5-hydroxymethylfurfural from the clarified extracts. Complete elimination was not readily obtained by steam-distillation or ether extraction, but almost complete elimination of the absorption maximum of 5-hydroxymethylfurfural at 282 mμ was obtained by reduction with aluminium amalgam, as reported by Mizuguchi *et al.*[5] It was found that treatment of pure aqueous solutions with aluminium amalgam for 30 minutes at 100° C (on a boiling-water bath) almost completely eliminated the absorption due to 5-hydroxymethylfurfural, and only slightly reduced the absorption due to caffeine, *viz.*, by about 5 per cent. The spectrophotometric method used for determining caffeine was therefore modified for products containing chicory, the extracts being reduced with aluminium amalgam before extraction with chloroform. As a result, the apparent "caffeine" content of chicory was reduced from as much as 1·5 per cent. to as little as 0·07 per cent. In the same way, the interference was removed from extracts of coffee and chicory mixtures. Aluminium amalgam reduction also made small but significant differences to the optical densities of extracts of pure coffee, confirming

TABLE I

CAFFEINE CONTENT OF SAMPLES OF COFFEE, CHICORY AND TEA

Sample	Sample number	Caffeine content found by— micro-Kjeldahl method, %	proposed spectrophotometric method: without reduction, %	proposed spectrophotometric method: with reduction, %
Roasted coffee	1	1·25	1·43	1·30
	2	1·99	—	1·94
	3	2·01	—	2·07
	4	2·19	—	2·06
	5	1·94	—	2·03
	6	1·92	—	2·03
	7	1·56	—	1·50
Instant coffee	1	3·26	—	3·51
	2	3·62	—	3·79
"Caffeine-free" instant coffee		0·60	0·52	0·48
Roasted chicory		0·00	1·54	0·07
Coffee and chicory mixture (1 + 1)		0·68	—	0·74
Dried extracts of coffee and chicory with carbohydrate	1	0·35	—	0·37
	2	0·43	1·36	0·47
	3	1·02	—	0·98
	4	1·10	—	1·03
Liquid coffee and chicory essences	1	0·26	0·64	0·25
	2	0·20	0·55	0·25
	3	0·35	0·38	0·35
	4	0·30	—	0·30
Ceylon tea BOPF		2·75	2·88	—
Assam tea PF		3·62	3·64	—
Ceylon tea BOP		2·75	2·80	—
Darjeeling tea		4·17	4·16	—
Instant tea		7·92	8·05	—
Instant tea with carbohydrate		3·08	3·10	—

the presence of small amounts of furfural compounds in coffee (which was also confirmed by paper chromatography); the results of the caffeine determinations then agreed more closely with those obtained by the micro-Kjeldahl method. When this method was used for determining caffeine in samples of tea, which contains no 5-hydroxymethylfurfural, the reduction stage was omitted as being unnecessary.

In making the spectrophotometric measurements, corrections were made for background absorption by use of the "base line" procedure of Morton and Stubbs,[6] deducting the mean of the readings obtained at 250 and 296 mμ (*viz.*, at wavelengths on the ascending and descending portions of the extinction curve at equal intervals on either side of the maximum at 273 mμ) from the reading at 273 mμ. The results of the determinations of caffeine in roasted coffee, and in products containing coffee and chicory, obtained by the proposed spectrophotometric method were in close agreement with those obtained by micro-Kjeldahl determinations. Similarly, with the omission of the aluminium amalgam reduction stage, there was close agreement in the caffeine contents of samples of tea determined by the two methods (see Table I).

Method

Preparation of the extracts—

Weigh accurately in a beaker a suitable amount of the sample, according to its expected caffeine content (*e.g.*, 1 g of coffee, 0·5 g of tea or 0·25 g of dried coffee extract). Add 3 g of heavy magnesium oxide and 15 ml of boiling water, and stand the beaker on the boiling-water bath for at least 10 minutes, stirring at intervals with a glass rod.

Prepare a packed column in a Soxhlet filter tube (180 mm × 22 mm) with a sintered-glass plate (porosity 2) with a suspension in water of 5 g of a mixture of equal weights of heavy magnesium oxide and a diatomaceous filter-aid (*e.g.*, Dicalite or Celite) by suction under reduced pressure; ensure that the column is not sucked dry in the process.

Transfer the contents of the beaker to the column with boiling water, and filter under reduced pressure. Continue washing the column with boiling water until at least 150 ml of filtrate have been collected (ensure that the column is not sucked dry). Transfer the extract with water to a 500-ml conical flask, add 5 ml of 10 per cent. v/v sulphuric acid, and boil for 5 minutes or until the volume of liquid has been reduced to about 150 ml. Cool, transfer the extract to a 200-ml calibrated flask with water, and dilute to the mark.

Reduction with aluminium amalgam—

Prepare the aluminium amalgam as described below. Cut sheet aluminium (0·02 inches thick) into pieces about 1 cm square, and clean the surface by heating in 10 per cent. sodium hydroxide solution on a water bath until there is an evolution of hydrogen. Wash with distilled water and then with 95 per cent. ethanol. Cover with 2 per cent. mercuric chloride solution for 3 minutes to allow a film of amalgam to form on the surface. Decant off the solution, wash the aluminium amalgam with distilled water and then with 95 per cent. ethanol, and store in ethanol until required for use.

By pipette, transfer 20 ml of the prepared extract of the sample to a beaker, add one piece of aluminium amalgam, and stand the beaker on a boiling-water bath for 30 minutes. Cool the beaker, and filter the contents through a Whatman No. 41 filter-paper into a 50-ml calibrated flask, and dilute to the mark (solution *a*).

Preparation of the solutions for spectrophotometric measurement—

By pipette transfer 10 ml of solution *a* to a 50-ml calibrated flask, and dilute to the mark (solution *b*). Measure 20 ml of solution *a* into a separating funnel, extract 5 times, each time with 15 ml of chloroform, and discard the chloroform extracts. Transfer the contents of the separating funnel to a beaker with water, boil to expel any residual chloroform, and reduce the volume to less than 50 ml. Cool, transfer the contents of the beaker to a 50-ml calibrated flask with water, and dilute to the mark (solution *c*).

SPECTROPHOTOMETRIC READINGS AND CALCULATIONS—

Measure the optical densities (E) of solutions *b* and *c* in 1-cm silica cells against distilled water with a spectrophotometer, such as a Unicam SP500, at 250, 273 and 296 mμ*; calculate the caffeine content, C, of the sample from the equation—

$$C = \frac{(2E_b - E_c)\ 1250}{410 \times w}$$

where w = weight of sample taken and E_b and E_c are the values of—

$$E_{273} - \frac{(E_{250} + E_{296})}{2}$$

for solutions *b* and *c*, respectively. The value of this expression for a 0·001 per cent. solution of caffeine is 0·410.

RESULTS

The results shown in Table I have been obtained on samples of coffee, coffee and chicory mixtures, dry and liquid extracts of coffee and chicory, tea and dried extracts of tea. Caffeine was determined by Bower, Anderson and Titus's (micro-Kjeldahl) method,[2] and by the proposed spectrophotometric method.

REFERENCES

1. Ishler, N. H., Finucane, T. P., and Borker, E., *Anal. Chem.*, 1948, **20**, 1162.
2. Bower, R. S., Anderson, A. D., and Titus, R. W., *Ibid.*, 1950, **22**, 1056.
3. Lee Kum-Tatt, *Analyst*, 1961, **86**, 825.
4. Reichstein, T., and Beitter, W., *Ber.*, 1930, **63**, 816.
5. Mizuguchi, J., Suzuki, S., Motoi, S., and Horike, K., *Kôgyô Kagaku Zasshi*, 1957, **60**, 445; *Chem. Abstr.*, 1959, **53**, 7637.
6. Morton, R. A., and Stubbs, A. L., *Analyst*, 1946, **71**, 348.

Received *September 27th*, 1962

* The wavelength of maximum absorption and the optical density of a 0·001 per cent. solution of pure caffeine should be checked for each instrument used for determining caffeine by this method.

2,2′-Thiodiethanethiol as a Colorimetric Reagent for the Determination of Nickel

BY J. SEGALL, M. ARIEL AND L. M. SHORR

(*Israel Mining Industries Laboratories, P.O. Box* 313, *Haifa, Israel, and Department of Chemistry, Technion, Israel Institute of Technology, Haifa, Israel*)

A quantitative spectrophotometric method has been developed for determining nickel with 2,2′-thiodiethanethiol. In ammoniacal medium the reagent forms a red complex with nickel, insoluble in water and soluble in chloroform, which is suitable for determining the metal in the concentration range 2 to 25 p.p.m. with an error of 1 to 3 per cent. Copper, cobalt and palladium interfere with the determination; however, these ions can be removed by standard techniques before the analysis. No other ions were found to interfere.

THIS paper describes an investigation of a new colorimetric reagent, 2,2′-thiodiethanethiol, for determining nickel. In procedures involving use of dimethylglyoxime,[1] α-furildioxime[2] or cyclohexane-1,2-dione dioxime,[3] iron interferes and has to be complexed or removed before the colorimetric determination. Quinoxaline-2,3-dithiol[4] appears to be the only reagent reported for nickel with which this interference is not encountered. However, the limited stability of this reagent in solution is disadvantageous.

The preparation and the synthesis of 2,2′-thiodiethanethiol (TDT) have been described by Mathias[5] and Harley-Mason.[6] The latter used ammoniacal nickel salt solutions and thiourea for determining mustard gas in air; a coloured complex formed between nickel and TDT, which was generated *in situ*.

TDT is not specific for nickel alone, but the reagent permits the determination of this metal in the presence of many other ions. The complex is extracted from the aqueous phase with a water immiscible solvent, such as chloroform.

METHOD

REAGENTS—

2,2′-Thiodiethanethiol, $HSCH_2CH_2SCH_2CH_2SH$, is a colourless liquid, boiling at 138° C (13 mm), having a specific gravity of 1·191 at 20° C and a refractive index of 1·5958 at 20° C. It is immiscible with water, but soluble in halogenated organic solvents. The compound is not available commercially, but can be synthesised readily by the procedure described by Mathias.[5]

2,2′-Thiodiethanethiol solution, 0·2 M—Prepared by diluting 12·7 ml of TDT to 500 ml with chloroform.

Standard nickel solutions—A stock solution of nickel sulphate (approximately 0·05 M) was prepared by dissolving 7·2709 g of analytical-reagent grade nickel sulphate heptahydrate in water, and diluting to 500 ml with water. One millilitre of this solution was found to contain (by gravimetric analysis with dimethylglyoxime) 3·23 mg of nickel.

Ammonium hydroxide solution, 25 *per cent. w/w, aqueous*—Analytical-reagent grade.

Chloroform—Analytical-reagent grade.

APPARATUS—

Spectrophotometric measurements were made in 1-cm glass cells with a Zeiss model PMQ II spectrophotometer at a slit width of 0·01 cm. The measurements were made at 25° C.

PROCEDURE—

Dilute a 20·0-ml portion of the standard nickel solution to 1000 ml in a calibrated flask; transfer 2·0-, 5·0-, 10·0-, 20·0- and 25·0-ml portions to 50- to 100-ml separating funnels. Add ammonium hydroxide solution dropwise until the pH is 10 to 11, and then add 5 to 10 ml of the 0·2 M TDT solution. Shake for a few minutes, then run off the lower organic phase. Extract the aqueous phase three times, with a different portion of the reagent each time, and then wash the residual aqueous phase with 5- to 10-ml portions of chloroform,

and separate. Collect the organic phases in 50-ml calibrated flasks, and dilute to the mark with chloroform. Measure the optical densities of these pink to red solutions at 520 mμ against pure chloroform. Plot a graph relating the optical density to the amount of nickel present; the graph should be linear and pass through the origin.

Samples containing approximately 0·1 to 1 mg of nickel should be analysed in the same way as described for the preparation of the calibration graph, the concentration of nickel in the sample being read from the calibration curve.

Results and discussion

The nickel complex of 2,2′-thiodiethanethiol in chloroform is red, whereas the solution of the reagent itself is colourless. The optical-density spectrum for the coloured complex shows a maximum at 520 mμ.

The formation of the complex is dependent on the pH of the aqueous solution. Fig. 1 indicates that a pH above 10 is needed for quantitative formation of the complex between nickel and TDT.

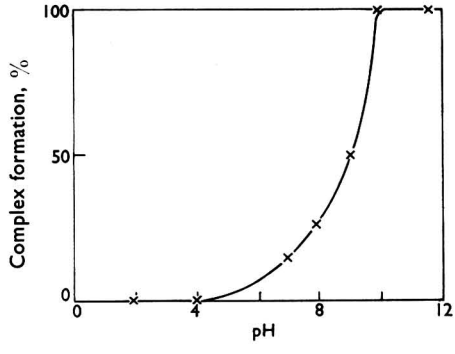


Fig. 1. Complex formation as a function of pH

The optical density of the solution of the complex in chloroform is stable for a long period of time. Solutions measured immediately after their preparation and again after 1 or 2 weeks did not show any appreciable change in optical density (see Table I).

Table I

Colour stability as a function of time

Solution A contained 3·8 p.p.m. of Ni^{2+}; solution B contained 12·9 p.p.m. of Ni^{2+}

Time after preparation of solution, weeks	Optical density of Solution A	Optical density of Solution B
0	0·075	0·234
1	0·072	not determined
2	not determined	0·231

Samples of the reagent as well as samples of solutions of the reagent in chloroform were kept at room temperature in glass-stoppered bottles for 12 months with no change in concentration or any deterioration.

Job's method of continuous variations[7] indicated that every atom of nickel in the complex is bound to two molecules of TDT and that a single complex species is formed in solution.

Effect of other ions—

Cobalt and palladium interfere in the determination of nickel with TDT by forming coloured complexes equally soluble in chloroform. Copper also interferes, possibly by oxidising the TDT to its disulphide and thus repressing the reaction with the nickel. However, many of the common ions have no effect on the determination (see Table II).

TABLE II

EFFECT OF VARIOUS IONS ON THE DETERMINATION OF NICKEL

Ion	Ratio of foreign ion to nickel by weight	Error in nickel determination, %
Na^+	5	−0·3
K^+	5	−0·3
Ca^{2+}	0·3	−0·3
Mg^{2+}	5	−0·3
Pb^{2+}	3·5	+1·0
Zn^{2+}	2	0
Sn^{2+}	4	+1·5
Mn^{2+}	25	0
Sb^{3+}	4	0
Fe^{3+}	5	−0·3
Cr^{3+}	9	0
Al^{3+}	1	−0·3
Cl^-	25	−0·3
NO_3^-	1000	+0·6
CH_3COO^-	6	0
SO_4^{2-}	9	0
PO_4^{3-}	30	0

The interference of cobalt, palladium and copper can be overcome by using one of the known methods for their removal. Thus the complexes of cobalt and palladium with ammonium thiocyanate[3] are extracted with ethyl acetate and pentanol, respectively, and copper is removed by precipitation with thioglycollic acid.[8] The residual solutions can then be used for determining nickel. Table III shows the results obtained for the determination of nickel, in the presence of interfering ions, by these modifications of the procedure.

TABLE III

DETERMINATION OF NICKEL IN THE PRESENCE OF INTERFERING IONS

Interfering ion	Ratio of foreign ion to nickel by weight	Error in nickel determination, %
Co^{2+}	3	+0·6
Pd^{2+}	4	0
Cu^{2+}	40	+1·7

ACCURACY OF DETERMINATION—

The versatility and applicability of this analytical procedure were investigated by determining nickel in standard samples of the National Bureau of Standards (U.S.A.). After the sample had been dissolved in the usual way, the nickel steel, No. **33d**, and the chromium - molybdenum steel, No. **133**, were analysed without prior removal of any ion. The copper - nickel - zinc alloy, No. **157**, and the manganese bronze, No. **62c**, were analysed after precipitation of the copper with thioglycollic acid. Tables IV to VII summarise the results of these analyses.

TABLE IV

DETERMINATION OF NICKEL IN NICKEL STEEL STANDARD SAMPLE **33d***

In each test the portion taken for the measurement of optical density was 2 ml from 50 ml

Weight of sample, g	Optical density at 520 mμ	Nickel found, %	Error, %
0·2608	0·142	3·7	+2·8
	0·142	3·7	+2·8
0·2678	0·140	3·6	0
0·3023	0·160	3·6	0
	0·161	3·6	0
		Average ..	+1·1

* Nickel steel 33d contains: 95 per cent. of Fe, 0·5 per cent. of Mn, 0·25 per cent. of Mo, 0·25 per cent. of Si, 0·17 per cent. of C, 0·14 per cent. of Cr, 0·12 per cent. of Cu and 3·6 per cent. of Ni.

TABLE V

DETERMINATION OF NICKEL IN CHROMIUM - MOLYBDENUM STEEL 133*

In each test the portion taken for the measurement of optical density was 10 ml from 50 ml

Weight of sample, g	Optical density at 520 mμ	Nickel found, %	Error, %
0·4787	0·093	0·27	− 6·9
	0·103	0·29	0
0·4945	0·110	0·30	+ 3·5
	0·113	0·31	+ 6·9
0·4994	0·115	0·32	+10·0
	0·116	0·32	+10·0
		Average ..	+ 3·9

* Chromium - molybdenum steel 133 contains: 83·8 per cent. of Fe, 13·6 per cent. of Cr, 0·8 per cent. of Mn, 0·6 per cent. of Mo, 0·4 per cent. of Si, 0·4 per cent. of S, 0·1 per cent. of C and 0·29 per cent. of Ni.

TABLE VI

DETERMINATION OF NICKEL IN COPPER - NICKEL - ZINC ALLOY STANDARD SAMPLE 157*

In each test the portion taken for the measurement of optical density was 1 ml from 50 ml

Weight of sample, g	Optical density at 520 mμ	Nickel found, %	Error, %
0·1539	0·200	17·9	0
	0·203	18·3	+2·2
0·1549	0·210	18·7	+4·5
	0·214	18·8	+5·0
0·1584	0·208	18·0	+0·6
		Average ..	+2·5

* Copper - nickel - zinc alloy 157 contains: 72·1 per cent. of Cu, 9·7 per cent. of Zn, 0·14 per cent. of Co, 0·05 per cent. of Fe and 17·9 per cent. of Ni.

TABLE VII

DETERMINATION OF NICKEL IN MANGANESE - BRONZE STANDARD SAMPLE 62c*

Weight of sample, g	Portion taken, ml	Optical density at 520 mμ	Nickel found, %	Error, %
0·4264	5 from 25	0·090	0·29	0
	10 from 25	0·200	0·28	−3·5
0·4938	10 from 25	0·210	0·29	0
	5 from 25	0·106	0·29	0
0·4983	5 from 25	0·107	0·30	+3·5
0·5029	5 from 25	0·115	0·31	+6·9
0·5120	10 from 25	0·220	0·29	0
			Average ..	+1·0

* Manganese bronze 62c contains: 59·2 per cent. of Cu, 37·2 per cent. of Zn, 1·2 per cent. of Al, 0·7 per cent. of Fe, 0·7 per cent. of Mn, 0·4 per cent. of Sn, 0·2 per cent. of Pb, 0·07 per cent. of Si and 0·29 per cent. of Ni.

We thank the Israel Mining Industries for permission to publish this work, which is based on a thesis submitted by one of us (J.S.) to the Technion, Israel Institute of Technology, Haifa, as partial fulfilment of the requirements of the M.Sc. degree in the Science Faculty.

REFERENCES

1. Feigl, F., *Ber.* **1924**, **57B**, 759.
2. Taylor, C. G., *Analyst*, 1956, **81**, 369.
3. Monnier, D., and Haerdi, W., *Anal. Chim. Acta*, 1959, **20**, 444.
4. Skoog, D. A., Lai, M., and Furst, A., *Anal. Chem.*, 1958, **30**, 365.
5. Mathias, S., *Bol. Fac. Filos. Ciênc. S. Paulo*, 1942, **14**, Chim. 1, 75.
6. Harley-Mason, J., *J. Chem. Soc.*, 1952, 146.
7. Job, P., *Ann. Chim.*, 1928, **10**, 113.
8. Blundy, P. D., and Simpson, M. P., *Analyst*, 1958, **83**, 558.

Received *September 3rd*, 1962

SHORT PAPERS

Colorimetric Determination of Low Concentrations of 2,2-Dichlorovinyl Dimethyl Phosphate in the Atmosphere

By J. T. HUGHES

(*Glasshouse Crops Research Institute, Worthing Road, Rustington, Littlehampton, Sussex*)

2,2-Dichlorovinyl dimethyl phosphate (DDVP) is an anticholinesterase insecticide of relatively high volatility (vapour pressure = 0·1 mm of mercury at 20° C) and moderately low mammalian toxicity.[1] In addition to its use in conventional spraying or aerosol formulations, or in baits, the compound may also be effectively applied purely as a fumigant by incorporation in a suitable base, such as polyvinyl chloride, from which it is continuously released as a vapour into the atmosphere.[2] To investigate the use of DDVP as a fumigant against mushroom pests, a method was required for determining atmospheric concentrations of 0·1 μg per litre or less i cropping houses and other enclosed spaces. The method reported by Geiger and Fürer,[3] in which the test solution is treated with resorcinol and sodium carbonate, fails to distinguish between DDVP and any free dichloroacetaldehyde present, and apparently determines both together. Although the method is sensitive it also suffers from the further disadvantage of producing an unstable coloured product and a somewhat inconsistent and high reagent blank value. A method is reported here for determining low concentrations of both unhydrolysed DDVP and free dichloroacetaldehyde present in the same aqueous solution. A stable coloured product is formed, and the reagent blank value is low.

The method is based on that already proposed by Hodgson and Casida[4] for dichloroacetaldehyde, in which use is made of its reaction with 2,4-dinitrophenylhydrazine, the product forming a blue solution on treatment with an excess of alkali and a mutual solvent. The reaction conditions have been modified to increase the over-all sensitivity of the method, and, by introducing a controlled hydrolysis stage, it has been found possible to determine microgram amounts of both dichloroacetaldehyde and DDVP present in the same solution. Hodgson and Casida[4] state that hydrolysis of the vinyl - phosphate bond of DDVP is complete in 0·2 N sodium hydroxide in 15 minutes at 27° C. However, determinations of the dichloroacetaldehyde produced from known amounts of pure DDVP, hydrolysed under these conditions, showed recoveries of only 50 per cent. or less, losses evidently being due to secondary alkaline decomposition of the dichloroacetaldehyde. Geiger and Fürer[3] found, by measuring the amount of alkali consumed, that hydrolysis of DDVP is quantitatively complete if carried out at 0° to 1° C for 20 minutes in N/6 sodium hydroxide. Measurements by the proposed colorimetric procedure of the hydrolysis of pure DDVP under closely similar conditions (15 minutes in 0·2 N sodium hydroxide at 0° C) confirmed that the recovery—even with microgram amounts—was substantially complete. Tests with known amounts of dichloroacetaldehyde ranging from 3·4 to 27·5 μg submitted to the latter hydrolysis conditions indicated a constant recovery of at least 90 per cent.

As shown by Hodgson and Casida,[4] DDVP does not readily hydrolyse and react with 2,4-dinitrophenylhydrazine, under mild treatment, so that if conditions are correctly chosen the free dichloroacetaldehyde can be determined in the presence of the ester. A further determination on some of the same solution after quantitative hydrolysis gives a measure of the total aldehyde present. By difference, the amount of unchanged DDVP originally present can be calculated.

To test the usefulness of the method a series of mixtures of DDVP and dichloroacetaldehyde was prepared and analysed. Dichloroacetaldehyde was prepared from dichloroacetal by the method proposed by De Bièvre and co-workers.[5] Although the aldehyde polymerised readily when stored at room temperature, and slowly at −20° C, de-polymerisation could be effected by distillation and the freshly distilled product (b.p. 89·9° C) was immediately dissolved in water. Pure samples of DDVP were kindly provided by the Shell Chemical Company Ltd. and Ciba Laboratories Ltd.

Results obtained on a series of mixtures, each equivalent to about 60 μg of DDVP, are shown in Table I.

Experiments to check recoveries of low concentrations in the atmosphere have been carried out by evaporating 0·01- and 0·1-ml portions of dilute aqueous solutions of DDVP in 1- and 10-litre

TABLE I

AMOUNTS OF DDVP AND DICHLOROACETALDEHYDE FOUND IN PREPARED MIXTURES

Mixture	DDVP— taken, μg	DDVP— found, μg	Dichloroacetaldehyde— taken, μg	Dichloroacetaldehyde— found, μg
1	60·0	58·2	0	0·4
2	56·0	52·3	3·0	3·4
3	45·0	44·0	7·5	8·0
4	30·0	29·3	15·0	15·3
5	15·0	13·9	22·5	22·9
6	6·0	5·2	27·0	27·8
7	0	0	30·0	30·5

glass vessels. Metered volumes of air were drawn through the vessels, which were fitted with taps, into three bubblers containing distilled water. The mean recovery obtained, in 5 experiments, with simulated mean air concentrations ranging from 0·16 to 4·6 μg per litre, was 93 per cent. At the lowest concentration 70 per cent. of the total was recovered as DDVP, the mean for the series being 83 per cent. Most of the recovered material was always found in the first absorber. Under the least favourable conditions of sampling used (350 litres at a rate of 0·5 to 1 litre per minute) the proportions found in the first, second and third absorbers were 0·84, 0·14 and 0·02, respectively.

To obtain a convenient optical-density reading in the test, a volume of at least 0·25 cu. metre would be required for an air sample containing 0·1 μg of DDVP per litre. To take sample volumes in excess of 1 cu. metre (which can be taken overnight) would in general require inconveniently long times, so that for practical purposes the lowest measurable concentration can be regarded as about 0·02 μg per litre.

METHOD

SAMPLING—

Aspirate a sufficient volume of the air through two or three bubblers in series, each containing 10 ml of distilled water, by means of a suitable pump. The rate should not exceed 1 litre per minute, and the volume of the air may be measured with a gas meter or other device.

DETERMINATION OF TOTAL DICHLOROACETALDEHYDE—

Adjust the sample volume to 10 ml. Place a 5-ml portion of each sample solution in a 10-ml graduated tube, and immerse this in crushed ice until the temperature is reduced to 0° C. Add 1·0 ml of 1·2 N sodium hydroxide (the temperature of which has previously been adjusted to 0° C), mix, and maintain at 0° C for exactly 15 minutes. Add 0·6 ml of 0·1 per cent. 2,4-dinitrophenylhydrazine solution in 4 N hydrochloric acid, mix, and place in a water bath at 37° C for 60 minutes. Add 0·6 ml of 4 N sodium hydroxide solution, shake, add ethanol to give a volume of approximately 10 ml, and mix thoroughly. Cool to 20° C, and adjust the volume to exactly 10 ml with ethanol. Measure the optical density at 580 mμ against a reagent blank solution. Determine the amount of dichloroacetaldehyde present by reference to a calibration graph obtained from known amounts of dichloroacetaldehyde submitted to the same procedure, including the hydrolysis stage.

DETERMINATION OF FREE DICHLOROACETALDEHYDE—

To the remaining 5 ml of sample in each bubbler add 1·0 ml of water and 0·6 ml of 0·1 per cent. 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid, and maintain at 37° C for 60 minutes. Complete the procedure as before, and determine the dichloroacetaldehyde present by reference to a calibration graph obtained with known amounts of dichloroacetaldehyde.

Calculate the amount of combined dichloroacetaldehyde present by difference, and express this as DDVP by applying the theoretical conversion factor (1·956).

REFERENCES

1. Klotzsche, C., *Z. angew. Zool.*, 1956, **43**, 87.
2. Miles, J. W., Pearce, G. W., and Woehst, J. E., *J. Agric. Food Chem.*, 1962, **10**, 240.
3. Geiger, M., and Fürer, R., *Z. anal. Chem.*, 1960, **174**, 401.
4. Hodgson, E., and Casida, J. E., *J. Agric. Food Chem.*, 1962, **10**, 208.
5. De Bièvre, P. J., van der Kelen, G. P., Cornille, G., and Eeckhaut, Z., *Bull. Soc. Chim. Belg.*, 1959, **68**, 550.

Received *November 14th*, 1962

A Method for the Rapid Determination of Alcohol in Body Fluids

By R. M. MABON

(*The Department of Physiology, The Hannah Dairy Research Institute, Ayr, Scotland*)

Various methods of determining alcohol in body fluids have been described in recent years. Kent-Jones and Taylor[1] reported the results of an investigation into the merits of two methods—the micro Cavett and that of Kozelka and Hine. The micro Cavett method was found to be the more accurate, but it suffered from serious inconsistencies in reproducibility; Kozelka and Hine's method, although less accurate, gave good reproducibility but was arduous and time-consuming.

Nickolls[2] reported a modification of the micro Cavett method that appears to give results as accurate as those obtained by the unmodified method and even better reproducibility than that given by Kozelka and Hine's method. The simplicity of the procedure recommended its use for routine work in the laboratory.

In this laboratory work is in progress to assess the acclimatisation of cattle to tropical environments, and one of the important variables being considered is the change in total body water content at various environmental temperatures. Budtz-Olsen, Cleeve and Oelrichs[3] investigated the total body water of sheep, and recommended the use of alcohol, on the dilution principle, as offering the simplest means of determining this parameter. In preliminary investigations Nickolls's method has been simplified (*a*) by reducing the time for the determination and (*b*) by assessing the reduction of dichromate photometrically.

Method

Apparatus—

Jars—Kilner jars (1-lb size) fitted with two rubber gaskets.
Glass triangles with 1-inch legs.
Petri dishes—5 cm in diameter.
Incubator—Temperature controlled at approximately 45° C.

Reagent—

Potassium dichromate solution—Dissolve 4·9 g of potassium dichromate in 1 litre of 50 per cent. v/v sulphuric acid.

Procedure—

By pipette put 10 ml of potassium dichromate solution into a Kilner jar, place a glass triangle in the jar, and on top of the triangle place a Petri dish. From a pipette place 2 ml of blood or other body fluid containing the alcohol in the Petri dish, and then add 2 ml of water directly on top of the blood to ensure a good mixture. Place the two rubber washers and the glass top of the jar in position, and screw on the metal cap tightly. (It is of the utmost importance that the jars be completely airtight.) Place the jar in an oven controlled at 45° C for 2 hours, then remove it, and set it aside to cool. When it has cooled, open the jar and remove the Petri dish. Wash the legs of the glass triangle with distilled water from a wash bottle, and then remove the triangle from the jar. Transfer the contents of the jar, with several washings, to a 100-ml calibrated flask, and make up to the mark with distilled water.

Measure the optical density of the solution in a 1-cm cuvette with a spectrophotometer at 450 mμ; use distilled water in the reference cuvette. Construct a calibration graph by measuring the reducing effect of known weights of alcohol on the dichromate solution. A straight line is produced for amounts of alcohol between 0 and 3·5 mg. A calibration graph must be constructed with each new batch of alcohol or dichromate solution.

The results of tests to determine the accuracy of the method in which three lots of blood containing different amounts of alcohol were analysed are shown in Table I. A sample of blood without added alcohol was included with each series, and in no instance was there any reduction of dichromate.

TABLE I

RESULTS

Alcohol content of blood, mg per 2 ml	No. of determinations	Mean alcohol recovered, mg	Standard deviation
0·600	20	0·613	0·030
1·708	20	1·694	0·034
2·996	20	3·006	0·016

REFERENCES

1. Kent-Jones, D. W., and Taylor, G., *Analyst*, 1954, **79**, 121.
2. Nickolls, L. C., *Ibid.*, 1960, **85**, 840.
3. Budtz-Olsen, O. E., Cleeve, J. D., and Oelrichs, B. A., *Aust. J. Agric. Res.*, 1961, **12**, 681.

Received *November 6th*, 1962

A Rapid Method for determining Sulphate in Water Extracts of Soils

BY A. MASSOUMI AND A. H. CORNFIELD

(*Chemistry Department, Imperial College of Science and Technology, London, S.W.7*)

THIS paper describes modifications of Butters and Chenery's method[1] (for determining total sulphur in soils and plants) that increase its sensitivity and make it suitable for determining sulphate in water extracts of soils.

The main modification involves the use of an extremely dilute seed suspension of barium sulphate added to the test solution. This was done since it was found that, in the original method when the portion taken contained less than 10 μg of sulphate-sulphur, no absorption occurred. The use of the seed suspension overcame this and gave good recovery of as little as 2 μg of sulphate-sulphur. Further, the sensitivity of the method was also increased, and as little as 2 p.p.m. of soil sulphate-sulphur could be determined with a fair degree of accuracy.

It was also found that the use of barium chloride crystals of less than 1-mm sieve size without removal of particles less than 52 mesh, as described in the original method, gave more consistent results. The final main modification involves the incorporation of the gum acacia reagent with the acetic acid reagent. This mixed reagent was stable indefinitely, whereas the aqueous gum acacia solution described in the original method tended to become turbid after a few days.

Since several other minor modifications were also made, the method finally adopted for determining sulphate in water extracts of soils is described in full below.

METHOD

REAGENTS—

Nitric acid, 25 per cent. v/v—Prepare from analytical-reagent grade nitric acid.

Acetic - phosphoric acid—Mix 900 ml of analytical-reagent grade acetic acid with 300 ml of analytical-reagent grade orthophosphoric acid.

Gum acacia - acetic acid solution—Dissolve 5 g of gum acacia in 500 ml of hot water. Filter the hot solution through a Whatman No. 42 filter-paper, cool the filtrate, and then dilute to 1 litre with analytical-reagent grade acetic acid.

Barium sulphate seed suspension—Dissolve 18 g of analytical-reagent grade barium chloride in 44 ml of hot water, and add 0·5 ml of the concentrated standard sulphur solution (see below). Bring this to the boil, and then cool quickly. Then add 4 ml of the gum acacia - acetic acid solution. Prepare this seed suspension freshly each day before use.

Barium chloride—Grind analytical-reagent grade barium chloride to pass a 1-mm sieve.

Concentrated standard sulphur solution, 2 mg per ml—Dissolve 1·088 g of oven-dried analytical-reagent grade potassium sulphate in water, and dilute the solution to 100 ml.

Working standard sulphur solution, 10 μg per ml—Each day, dilute 5 ml of the concentrated standard to 1 litre with water.

Purified animal charcoal—Boil 20 g of Norit NK charcoal with about 200 ml of concentrated hydrochloric acid for about 10 minutes, filter under suction, and wash until free of chloride. Dry in an oven at about 100° C.

PROCEDURE—

Place 10 g of soil (air-dried and ground to pass a 2-mm sieve) and 20 ml of water in a 6-inch × 1-inch boiling tube. Stopper with a rubber bung, and shake in a mechanical reciprocating shaker for 15 minutes. Add 0·04 g of purified animal charcoal, and shake for another 15 minutes. Filter through a Whatman No. 42 filter-paper; use dry apparatus and filter-paper. Place a suitable portion of the filtrate (not more than 15 ml and containing not more than 120 μg of sulphate-sulphur) in a 6-inch × ¾-inch test-tube calibrated at 25 ml, add 2·5 ml of 25 per cent. nitric acid and 2 ml of acetic - phosphoric acid, and dilute to about 22 ml. Insert the bung, shake the tube, and then add, successively, 0·5 ml of the barium sulphate seed suspension (which must be shaken just before use) and 1 g of barium chloride crystals. Insert the bung, and invert the tube 3 times. After 10 minutes invert 10 times, and after another 5 minutes invert 5 times. After another 5 minutes add 1 ml of the gum acacia - acetic acid solution. Dilute to volume, invert 3 times, and set aside for 1½ hours. Then invert the tube 10 times, pour the contents into a 4-cm cell, and measure the optical density with a Hilger Biochem absorptiometer; use the dark blue filter (optimum transmission at approximately 440 mμ).

PREPARATION OF STANDARDS—

Place 0, 1, 3, 5, 8, 10 and 12 ml of the working standard sulphur solution in separate 25-ml calibrated test-tubes, and continue as described above. Prepare standards each time a batch of solutions is analysed, and also run a reagent blank test.

The addition of charcoal during extraction usually produces a colourless extract. When this does not occur, treat a portion of the solution with all the reagents except barium chloride and the barium sulphate seed suspension. Subtract the optical-density value obtained with this from that obtained with another portion given the normal full reagent treatment. This will allow for any absorption owing to colour in the extract.

Preliminary tests showed that the charcoal does not absorb any sulphate from the extract.

RESULTS AND DISCUSSION

The method was tested by determining the recovery of known amounts of sulphate added to water extracts of thirty different soils (varying widely in pH, texture and organic matter content, and including six soils containing free carbonates). Recovery ranged from 96·4 to 103·1 per cent. and averaged 101·2 per cent.

The modified method described here is about five times more sensitive than the original, thus it is particularly suitable for determining sulphate in soils where sulphate-sulphur content is low compared with that of total sulphur.[2,3] However, there is no reason why it cannot be applied to determining total sulphur in soils and plant material after the digestion procedures described by Butters and Chenery in their original method.

REFERENCES

1. Butters, B., and Chenery, E. M., *Analyst*, 1959, **84**, 239.
2. Williams, C. H., and Steinbergs, A., *Austral. J. Agric. Sci.*, 1958, **9**, 483.
3. Massoumi, A., Ph.D. Thesis, London University, 1962.

Received *November 28th*, 1962

Determination of Small Amounts of Long-chain Aliphatic Tertiary Amines in Aqueous Solution

BY M. W. DESAI AND T. K. S. MURTHY

(*Atomic Energy Establishment, Trombay, Bombay, India*)

ASHBROOK'S[1] method for determining tri-iso-octylamine in aqueous raffinates has been modified by Lloyd and Carr[2] to make it applicable for some other tertiary and secondary amines. In an attempt to evolve a more sensitive method, we observed that ferric thiocyanate is superior to the cobalt thiocyanate complex in the determination of some tertiary amines. The method consists essentially in extracting the highly coloured ferric thiocyanate - amine complex in an organic solvent for spectrophotometric determination. Cyclohexane was chosen from several solvents as the extractant. The method was found to be suitable for the two tertiary amines—tri-iso-octylamine and trinonylamine (TNA), but not so sensitive for the secondary amines Amberlite LA-1 and LA-2; no other types of amines were available.

Experimental

Development and extraction of the complex—

With known amounts of either of the tertiary amines in the aqueous solution, the extraction of the ferric thiocyanate - amine complex, as indicated by the optical density of the organic extract measured against cyclohexane at 490 mμ, was found to depend on the concentrations of ferric and thiocyanate ions in the aqueous phase, as well as on its pH. When the aqueous phase was 0·1 M in ammonium ferric sulphate and M in ammonium thiocyanate and adjusted to pH 1·5, maximum extraction was obtained at a ratio of organic to aqueous phase of not less than 1 to 5. Under these conditions the optical density of the organic extract varied linearly with concentration of the amine up to 0·8 mg in 10·0 ml.

Effect of other ions on colour formation—

During the processing of aqueous leach liquors[3] for recovery of uranium by solvent extraction, the raffinates contain, in addition to dissolved and entrained amine, several ionic impurities, such as Fe^{2+}, Fe^{3+}, Cu^{2+}, Mn^{2+}, HSO_4^- and $H_2PO_4^-$. Interference from some of these ions in the final determination of the amine was studied, and it was found that, although HSO_4^-, $H_2PO_4^-$ and Fe^{2+} could be tolerated when present up to 80, 5 and 5 g per litre, respectively, Cu^{2+} gave lower values when present to the extent of as little as 0·5 g per litre. The presence of such impurities as dissolved silica and flocculating agents,[4] such as glue or Separan, was found to cause a further decrease in the optical density of the extract. It was found, however, that by first extracting the amine with cyclohexane and then developing the colour in the organic layer by allowing it to come into contact with an aqueous solution containing ferric thiocyanate, interference from several ions likely to be present in aqueous solution could be avoided. Based on these observations, the procedure described below is recommended for determining the two tertiary amines in aqueous samples.

Method

Procedure—

By pipette, place a suitable portion of the sample solution (up to 50 ml), containing 0·05 to 0·5 mg of the amine, in a 100-ml separating funnel. Add 10·0 ml of cyclohexane, and shake vigorously for 2 minutes. Allow the phases to separate, and discard the aqueous phase. Add 1 ml of M ammonium ferric sulphate, 5 ml of 2 M ammonium thiocyanate, 0·5 ml of M sulphuric acid and about 5 ml of distilled water. Shake for 1 minute, and then allow the phases to separate. Discard the aqueous phase, and spin the organic phase in a centrifuge. Measure the optical density of the extract in a 1-cm cell at 490 mμ against cyclohexane. Read the amount of amine from a calibration graph prepared by adding known portions of a solution of the amine in methanol to 25 ml of distilled water and carrying out the same procedure as described for the samples.

Results

Tests were carried out to verify the method in which known amounts of the amines were added to a synthetic raffinate solution, *i.e.*, an aqueous solution prepared by mixing different salts to give a final composition corresponding to a typical raffinate sample from solvent-extraction tests.

The results of triplicate analyses of two of the raffinate samples obtained in solvent-extraction tests on a laboratory mixer - settler unit are shown in Table I.

Table I

Determination of tertiary amines in simulated raffinates

The approximate composition per litre of each raffinate was 50 g of SO_4^{2-}, 5 g of Cl^-, 5 g of PO_4^{3-}, 1 g of Cu^{2+}, 2 g of Fe^{2+}, 10 g of Fe^{3+}, 3 g of Al^{3+}, 0·5 g of Ni^{2+}, 5 g of Mn^{2+} and 1·5 g of SiO_2

Sample	Amine found, mg per litre	Amine expected,* mg per litre
I (tri-iso-octylamine)	20·0, 19·7, 19·8	20·0
II (trinonylamine)	7·8, 8·0, 7·6	7·8

* Determined by the volumetric[5] and a modified cobalt thiocyanate method.

The results show that the two amines could be determined by the proposed method. The relative sensitivity of the method, compared with other methods, is shown in Table II.

TABLE II

COMPARISON OF THE METHODS WITH REFERENCE TO SENSITIVITY

Amine	Amount of amine, mg	Organic phase (10 ml)	Optical density (1-cm cell): Lloyd and Carr's method	Optical density (1-cm cell): Modified cobalt thiocyanate method	Optical density (1-cm cell): Proposed method
XE204	1	Pentanol and kerosene	0·10	—	—
Tri-iso-octylamine	1	Carbon tetrachloride	—	0·133	—
Trinonylamine ..	1	Carbon tetrachloride	—	0·133	—
Tri-iso-octylamine	1	Cyclohexane	—	—	1·67
Trinonylamine ..	1	Cyclohexane	—	—	1·70

It is clear that the advantage of the method lies in its greater sensitivity than that of Lloyd and Carr and the modified cobalt thiocyanate method.

We are grateful to Shri H. N. Sethna, Head, Chemical Engineering Division, and Shri S. Fareeduddin, Officer-in-Charge, Ore Extraction Section, Atomic Energy Establishment, Trombay, for facilities to carry out the work and permission to publish the results.

REFERENCES

1. Ashbrook, A. W., *Analyst*, **1959**, **84**, 177.
2. Lloyd, P. J., and Carr, A. D., *Ibid.*, 1961, **86**, 335
3. Pinkney, E. T., *The South African Industrial Chemist*, **1956**, **10**, No. 11, November.
4. Rosenbaum, J. B., and Clemmer, J. B., Proceedings of the International Conference on the Peaceful Uses of Atomic Energy, **1956**, **8**, 38.
5. DeSessa, M. A., "Raw Materials Development Laboratory Handbook of Analytical Methods," U.S. Atomic Energy Commission Report TID-7002, 1956.

First received *March 29th*, 1962
Amended, *November 30th*, 1962

The Determination of Sodium in Halo-phosphate Phosphors by Atomic-absorption Spectroscopy

BY J. PERKINS

(*A.E.I. Research Laboratory, Rugby, Warwickshire*)

THE phosphors currently used in the production of fluorescent lamps are of the calcium halo-phosphate type, prepared by firing together calcium hydrogen phosphate, calcium carbonate and a lesser amount of calcium fluoride. In order to study the effect of contamination by sodium on the performance of these phosphors, it was necessary to develop a method for determining the sodium content.

Direct flame photometry is unsatisfactory owing to the presence of strong band spectra from both calcium and phosphate ions in the region of the sodium lines at 5890 and 5896 Å. Even with an instrument having fairly high wavelength selectivity, *e.g.*, the Uvispek H700 spectrophotometer, the background level is unacceptably high. Hibbs and McDonald[1] have employed a preliminary chemical separation and then flame photometry for determining sodium in mixed alkaline-earth carbonates. The application of this method removes completely the calcium and phosphate, but owing, probably, to the gelatinous nature of the calcium phosphate precipitate, co-precipitation of sodium occurs and extremely poor recoveries of known additions are obtained. In view of the advantages in selectivity reported in the literature,[2] it was decided to investigate the possibility of using atomic-absorption spectroscopy for this determination.

EXPERIMENTAL

The apparatus used was a Uvispek H700 spectrophotometer with an H868 flame-photometer attachment. The flame chimney was modified so that light could be passed through the flame and thence into the spectrophotometer. The source of resonance radiation used was a Mazda 60-watt 250-volt sodium discharge lamp, which was under-run at 125 volts, from a Variac autotransformer, in the interest of greater stability and reduced line width.

The solutions under test were sprayed into the oxy-hydrogen flame in the normal way, and the absorption was measured at 589 mμ, the photometer being set to zero absorption (100 per cent. transmission) while distilled water was being sprayed.

CALIBRATION—

A stock solution containing 100 μg of sodium per ml was prepared by dissolving 0·051 g of sodium chloride in 200 ml of de-ionised water, and from this a series of standards containing from 10 to 50 μg of sodium per ml, in dilute hydrochloric acid (1 + 19), was prepared. These were used to calibrate the instrument, and, since the emission from the flame at these concentrations was negligible compared with the radiation from the sodium lamp, a linear response was obtained over the range 0 to 50 μg per ml; the results were—

Concentration of sodium, μg per ml	10	20	30	40	50
Absorption at 589 mμ	0·07	0·14	0·21	0·28	0·35

INTERFERENCE—

Standard additions of sodium were made to solutions containing (*a*) 1 per cent. w/v of calcium (as calcium chloride) in dilute hydrochloric acid (1 + 19), (*b*) 1 per cent. w/v of phosphate (as orthophosphoric acid) in dilute hydrochloric acid (1 + 19) and (*c*) 0·5 per cent. w/v of calcium *plus* 0·5 per cent. w/v of phosphate in dilute hydrochloric acid (1 + 19).

Determinations of sodium were carried out on these solutions by atomic absorption and by emission flame photometry with a Uvispek H700 spectrophotometer. The results in Table I show the marked superiority of the atomic absorption method in respect of interference.

TABLE I

EFFECT OF CALCIUM AND PHOSPHATE ON THE RECOVERY OF SODIUM

Interfering ions	Concentration of interfering ions, %	Sodium added, μg per ml	Sodium found by— emission flame photometry, μg per ml	Sodium found by— atomic-absorption spectroscopy, μg per ml
Calcium	1	0	8	<0·5
		20	28	18
		50	60	49
Phosphate	1	0	2	<0·5
		20	21	18
		50	52	48
Calcium + phosphate	0·5 + 0·5	0	4	<0·5
		20	23	19
		50	54	49

SENSITIVITY—

It was considered that the smallest real absorption that could be measured was 0·003, representing a sodium concentration of 0·5 μg per ml. On a 0·25-g sample of phosphor in 10 ml of solution this corresponds to a sodium content of 20 p.p.m. This was shown to be sufficiently sensitive for our purpose, as can be seen from subsequent results.

METHOD

PROCEDURE—

Dissolve a suitable weight, but not more than 0·25 g, of phosphor in the least possible amount of concentrated hydrochloric acid in a platinum crucible. Evaporate gently until salts just begin to crystallise. Dissolve in about 5 ml of de-ionised water, and add 0·5 ml of concentrated hydrochloric acid. Make up to 10 ml in a calibrated flask with de-ionised water, and return the solution at once to the platinum crucible to avoid pick-up of sodium from the glass. Spray the solution into the flame from a silica or platinum crucible, and measure the absorption at 589 mμ. Determine the sodium content of the sample by reference to a graph prepared as described under "Calibration." (This is best checked at one point, say 20 μg per ml, with each determination, as variations in the flame conditions or lamp temperature significantly affect the sensitivity.)

Results

The sodium content of three phosphors was determined, and three further determinations were made after known amounts of sodium had been added to each. A 0·25-g sample was used for each determination; the results were—

	Sample A		Sample B		Sample C	
Sodium added, μg	Nil	50	Nil	200	Nil	200
Sodium found, μg	20, 19	69	150, 150	345	170, 160	365

To test the reproducibility of the method, replicate determinations were carried out on two samples, one of low and one of high sodium content and standard deviations were calculated; the results were—

	Sample D	Sample E
Sodium found, μg	25, 24·5, 22·5, 22·5 24·5, 23, 24, 22·5	275, 282, 278, 284 284, 280, 283, 283
Standard deviation, μg ..	1·1	3·3

Conclusion

The method described has proved satisfactory for determining sodium in halo-phosphate phosphors, and many samples have been successfully analysed. It would, with suitable modifications, be applicable to other materials, where for reasons of interference from other species, emission flame photometry is impracticable.

The time taken for a single determination is approximately 15 minutes.

References

1. Hibbs, J. M., and McDonald, G. W., *Analyst*, 1960, **85**, 846.
2. David, D. J., *Ibid.*, 1960, **85**, 779.

Received *August 3rd*, 1962

Elimination of the Water Effect on Argon Ionisation Detectors fitted to Pye Chromatographs

By J. L. MARTIN

(*Glaxo Laboratories Ltd., Greenford, Middlesex*)

To obtain quantitative results for small amounts of alcohols in aqueous solutions from a gas chromatograph fitted with an argon ionisation detector, it is necessary to prevent water passing into the detector. If this is not done the drop in sensitivity, which is proportionate to the amount of water and the temperature of the detector, reduces the peak areas of components subsequently eluted. The back-flushing technique[1] is not conveniently applied at temperatures approaching 100° C if the substance to be measured has a larger retention volume than water (even when the extremely polar diglycerol is used as stationary phase), and the device described was introduced primarily for determining 0·02 to 0·25 per cent. of 2-phenylethanol in aqueous solutions. However, the problem of determining small amounts of relatively non-volatile substances in aqueous solution is one that is frequently met in pharmaceutical analysis, and the modification has proved of general use.

It was thought desirable temporarily to divert the gas flow during the time that it was carrying water vapour, so that the detector would be by-passed. It is difficult to contrive a tap at the lower end of the column that can be manipulated during elution, owing to the particular geometry of the Pye instrument used; the column itself was therefore used as a tap control as described below.

The adaptor consists of as short a length of Pyrex-glass tubing as possible (to reduce dead space to a minimum), fitted with a B7 socket at one end (the top) and a B7 cone at the other (see Fig. 1 (*a*)). The B7 socket is grooved lengthwise internally, and a hole is also drilled in it 180° from the groove. The lower B7 cone of the chromatographic column to be used is modified so that the only exit for gas is a hole drilled in the side of the cone: the hole coincides, when column and adaptor are assembled, with the hole in the adaptor socket.

When the two holes are juxtaposed in use (Fig. 1 (*b*)), the argon, together with any water separated on the standard 4-foot column, is vented to the atmosphere, leaving the detector unaffected. When the argon gas flow has to be diverted again into the ionisation cell, the column is turned through 180° without stopping the flow, and the hole in the column cone then corresponds with one end of the groove leading to the detector (Fig. 1 (*c*)). A preliminary chromatogram must be prepared, to indicate at what time the column should be turned.

Benzyl alcohol, chlorocresol, trichloroethanol and the esters of *p*-hydroxybenzoic acid have been determined by means of the modification; it has also proved useful during the conditioning of chromatographic columns by removal of the volatile impurities from them.

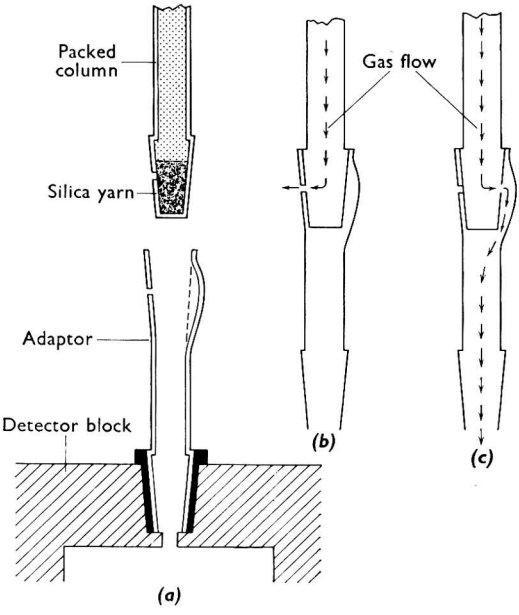


Fig. 1. Diagram of adaptor

REFERENCE

1. Swoboda, P. A. T., *Chem. & Ind.*, 1960, 1262.

Received *August 31st*, 1962

Book Reviews

ABSORPTION SPECTROSCOPY. By ROBERT P. BAUMANN. Pp. xiv + 611. New York and London: John Wiley & Sons Inc. 1962. Price 94s.

The most impressive single feature of this book is its wide scope. As explained in the author's preface, it is intended to be of value in three distinct types of educational courses in molecular spectroscopy. The first is designed for the specialist worker concerned with fundamental studies of molecular structure and dynamics, the second for the "applied" spectroscopist, whose major interests are qualitative and quantitative analysis, and the third is the provision of background information on molecular spectroscopy for the industrial analyst with no previous formal training in the subject. This classification is a valid one. Undergraduate instruction in the fundamentals of molecular spectroscopy is necessarily limited to the consideration of selected aspects of theory and application, if only because of time limitations and the demands of other important topics for attention. Conversely, "applied" molecular spectroscopy has often been dealt with on an essentially empirical basis, so that both the power of the underlying theory and the great value of relatively simple qualitative concepts have not always been emphasised. The demand of the

general analyst for an introduction to molecular spectroscopy arises from the fact that present-day commercial spectroscopic equipment, with high standards of performance, is now sufficiently simple to operate that, in principle at least, obtaining reliable results no longer requires a long apprenticeship in spectroscopic techniques.

To a notable extent this book does cover the field of absorption spectroscopy in a manner that meets, in large measure, two of the three stated objectives. Since these aims involve the exposition of the subject both in breadth and depth they account for a certain unevenness in the treatment. Thus the problems that occur at the end of each chapter range from trivial numerical calculations, through problems in the physics of radiation and the optics of spectrometers, to examples of quantum theory and molecular dynamics. The problems provided clearly demonstrate the way in which the subject is being developed in depth, but the "general analyst" reader may have been tempted to give up before reaching Chapter 8, "Qualitative Analysis" (pp. 314 to 363). Here the treatment of qualitative analysis by electronic (visible and ultraviolet) and infrared absorption spectroscopy, although lucid, is probably too brief to be of real value and has been done better elsewhere; even so the 19 problems to this chapter provide a useful test of competence in this field and are well worth looking at. Chapter 9, "Quantitative Analysis" (pp. 364 to 433), contains useful sections on transmittance error functions in relation to minimum concentration errors, expanded scale operation, the examination of ideal and non-ideal multi-component systems (including a detailed matrix treatment for a 4-component case), and a useful series of problems. Possible errors due to stray radiation effects in electronic absorption spectra are not dealt with, however, and the treatment of this important topic in Chapter 3, "Spectrometer Design and Performance," is too brief to be of direct practical value.

The remainder of the book comprises a long section, Chapter 10, "Principles of Molecular Spectroscopy" (pp. 434 to 544), supplemented by appendixes on matrix methods, character tables, etc., and selected answers to many of the problems. In this Chapter the theoretical aspects of the subjects dealt with earlier in Chapters 5 to 7 (pp. 202 to 313) are developed in detail, with sections on group theory and molecular symmetry, electronic spectra, vibrational spectra, rotational spectra and interactions between rotational, vibrational and electronic transitions. The discussion of these topics is concise and formal, without much explicit reference to illustrative spectral data.

These remarks should be sufficient to indicate that this book covers a large proportion of the basic theory of molecular spectroscopy, and thus goes a considerable way towards satisfying the demands of potential specialist workers. The discussion of the instrumental, practical and applied aspects is also excellent, despite some deficiencies. The chapter on "Spectrometer Components" is noteworthy in providing an outline of the physical principles on which instrument design and performance are based.

It may be doubted, however, if the book is suitable as an introduction to molecular spectroscopy for the newcomer with no previous experience of the subject. The wide scope of the book and the depth to which particular topics are explored would make it heavy reading for this type of student. But for the other classes of readers at which it is aimed, it constitutes an impressive combination of theoretical exposition and practical instruction. G. H. BEAVEN

METHODS OF BIOCHEMICAL ANALYSIS. Volume X. Edited by DAVID GLICK. Pp. x + 399. New York and London: Interscience Publishers, a division of John Wiley & Sons. 1962. Price 109s.

This volume opens with an account by C. T. Bishop (of Ottawa) on separation of carbohydrate derivatives by gas - liquid partition chromatography. The widest use of the procedures described is in analysing methanolysis products from methylated polysaccharides, but other possible applications are noted. The method is, however, limited to compounds having the necessary volatility and supports rather than replaces other techniques.

R. and Ruth E. Benesch (of Columbia University) contribute a critical account of the determination of –SH groups in proteins and conclude that all existing methods have limitations and that several methods should be used on each protein.

The measurement of sodium and potassium by glass electrodes is discussed by S. M. Friedman (of Vancouver). This is a rather new field with considerable possibilities. The selectivity of cation-specific glasses for one alkali metal cation relative to another is reproducibly dependent on the composition of the glasses and appears to depend on the Na (or Li)/Al (or B) ratio of the lattice. Commercial glass electrodes of the desired type are now appearing, and the instrumentation needed is "not particularly difficult," either for experiments *in vitro* or *in vivo*.

H. G. Pontis and Leloir (of Buenos Aires) describe the measurement of uridine diphosphate (UDP) - enzyme systems. The various enzyme systems connected with UDP compounds are outlined, and the reliability of the assay procedure "has been checked by several investigators." This is a clear and authoritative contribution.

C. S. Vestling (of Urbana, Illinois) deals with the determination of dissociation constants for two-substrate enzyme systems, such as alcohol dehydrogenase, lactic dehydrogenase and malic dehydrogenase, which are DPN^+-linked. Here the DPN^+ (or nicotine adenine dinucleotide NAD) may properly be regarded as a second substrate. Various experimental approaches are discussed (ultracentrifugation, dialysis, spectrophotometry, fluorescence) and there is a section covering the theoretical and experimental aspects of kinetic determinations of dissociation constants.

L. C. Craig and T. P. King (of the Rockefeller Institute) contribute an article on dialysis in which, in the main, seamless cellulose tubing is used. The porosity can be decreased by acetylation for definite times or it can be increased by treatment with zinc chloride solution. The use of a series of cells permits counter-current dialysis. The same authors in another chapter also discuss counter-current distribution in a masterly way with examples of many biochemical applications.

P. A. Albertson (of Uppsala) has an interesting chapter on partition methods for fractionation of cell particles and macromolecules. The methods used are polymer phase systems, such as dextran - polyethylene glycol - H_2O or dextran - methylcellulose - H_2O, in which the viscosities are not too high. The materials studied are viruses, proteins, nucleoproteins and nucleic acids. Among the examples of purifications described are those of bacteriophage T and polio virus.

The determination and microscopic localisation of cholesterol is the subject of a chapter by J. J. Kabara (of Detroit). This is an excellent and fully documented account of an important and difficult problem.

K. Yagi (of Nagoya, Japan) surveys the chemical methods for the determination of flavins. There is first a good account of the background of the subject and this is followed by details of extraction methods from animal and plant tissues. Estimations of total flavins by absorption or fluorescence spectrophotometry are then described and a further section discusses clearly the separation and determination of individual flavins.

This volume maintains the standard set in the earlier volumes. The international character of the publication is noteworthy, as is the absence this time of any contributor from this country.

R. A. MORTON

THE PROPERTIES AND TESTING OF PLASTICS MATERIALS. By A. E. LEVER, A.I.R.I., and J. RHYS, M.Sc., A.K.C. Second Edition. Pp. x + 321. London: Temple Press Books. 1962. Price 55s.

"Plastics" is a word embracing an ever-growing variety of polymeric materials that may have little in common other than their man-made origin. After a basic polymer has been made it must often go through a complex series of operations to produce a material that can be used in the manufacture of every-day things, and the manufacturer of plastics must be prepared to test this material at all stages of manufacture for its suitability for a particular purpose, for it often happens that he is, in fact, selling physical properties to his customers. Although chemical analysis is required at some stages in the manufacture of plastics, by far the larger amount of testing carried out is physical in character.

As in other branches of chemical technology, information about the testing of plastics is widely scattered in the literature; the object of the book under review is to summarise this information. The value of the book lies in its classification of these tests so that, without much difficulty, the reader may quickly discover sources of information about any particular one. The first six chapters cover general principles and physical, thermal, optical, electrical and sundry chemical properties, a short chapter deals with identification and chemical analysis, twenty-one classes of industrial materials are then discussed in some detail, and the last two chapters deal with the testing of finished mouldings and the efficiency of plasticisers. Although the chapter on chemical analysis is short, further analytical information is to be found in other chapters. Intending readers should appreciate that the book is, in effect, a literature review covering over three thousand references appearing up to 1961 with, usually, only brief remarks about any particular test; no attempt is made to give the reader a critical evaluation of the methods discussed.

A. G. JONES

Publications Received

ANORGANISCHE ULTRAMIKROANALYSE. By I. P. ALIMARIN and M. N. PETRIKOVA. Pp. 169. Berlin: Veb Deutscher Verlag Der Wissenschaften. 1962. Price DM 27.

ABSORPTION SPECTRA IN THE ULTRAVIOLET AND VISIBLE REGION. Volume III. Edited by Dr. L. LÁNG, with the collaboration of Dr A. BARTECKI, Dr. J. SZÖKE, Dr. G. VARSÁNYI and M. VIZESY. Pp. 424. (loose leaf). Budapest: Akadémiai Kiadó, 1962. Price $16.00.

THIN FILM CHROMATOGRAPHY. By E. VERNON TRUTER, Ph.D., B.Sc., A.R.C.S., D.I.C. Pp. xii + 205. London: Cleaver-Hume Press Ltd. 1963. Price 37s. 6d.

GERMAN-ENGLISH CHEMICAL TERMINOLOGY: AN INTRODUCTION TO CHEMISTRY IN ENGLISH AND GERMAN. By ALEXANDER KING, C.B.E., D.Sc., A.R.C.S., D.I.C., and Prof. Dr. HANS FROMHERZ. Fourth Edition. Pp. xx + 588. Germany: Verlag Chemie. 1963. Price DM 48; $12.00.

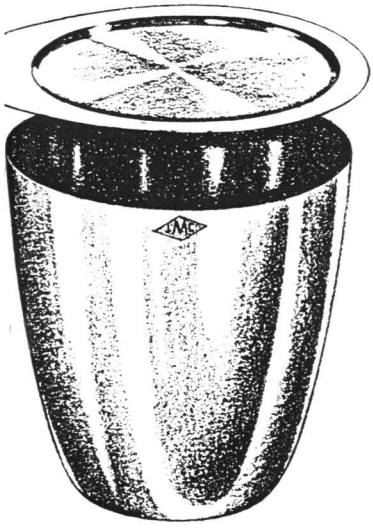
ION FLOTATION. By FELIX SEBBA. Pp. viii + 154. Amsterdam, London and New York: Elsevier Publishing Company. 1962. Price Dfl. 15.00; 30s.; DM 17.

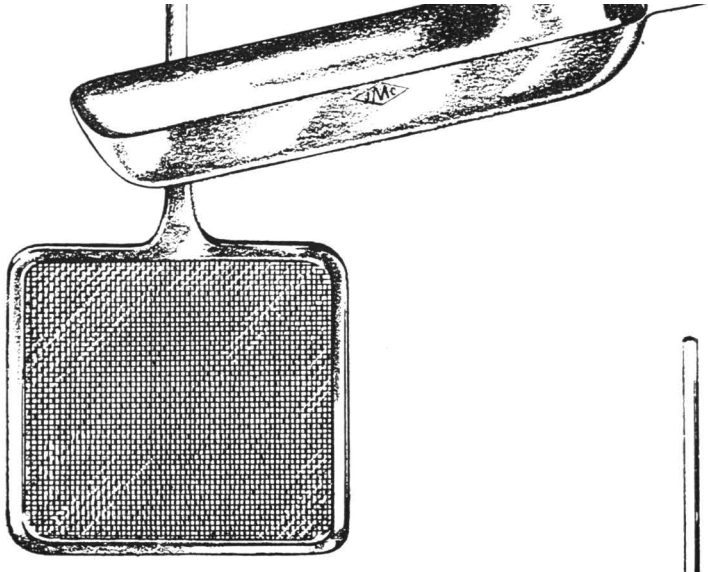
PHYSICAL AND CHEMICAL METHODS OF SEPARATION. By EUGENE W. BERG, Ph.D. Pp. xiv + 366. New York, San Francisco, Toronto and London: McGraw-Hill Book Company Inc. 1963. Price 97s.

CHEMICAL BONDING AND THE GEOMETRY OF MOLECULES. By GEORGE E. RYSCHKEWITSCH. Pp. x + 116. New York: Reinhold Publishing Co.; London: Chapman and Hall Ltd. 1963. Price 16s.

Errata

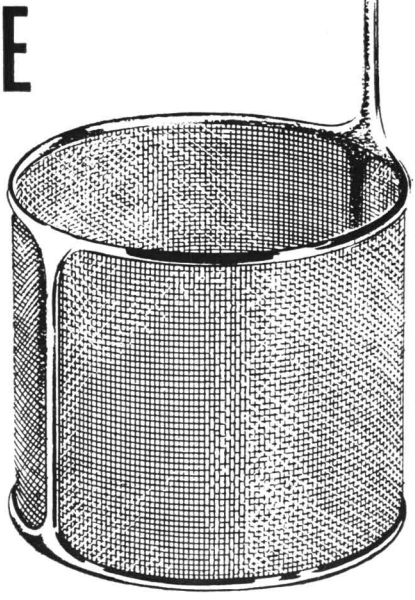
JANUARY (1963) ISSUE, p. 28, 25th line. *For* "0·1 N" *read* "0·01 N."
IBID., p. 61, 8th line. *For* "130·13 *VC*/178" *read* "130·13 *VC*/356."
MARCH (1963) ISSUE, p. 215, 13th line. *For* "$<$ 6 litres" *read* "$<$ 6 changes."





Pt

THE BASIS OF ACCURATE ANALYSIS



ıch of the laboratory apparatus used for
ical and electrochemical analysis, platinum
ential because of the unique combination
perties it provides.

MC standard range of platinum equipment
oth macro- and micro-chemical analysis
cribed in booklet 1700, free on request.
types of standard apparatus are now
ble from stock, while others are supplied
the minimum of delay. To meet unusual
rements apparatus can be fabricated to
designs.

ever the need—standard or special—JMC
ium apparatus is produced by precision
ods to combine maximum life with
ute efficiency in use.

ıhnson Matthey

NSON, MATTHEY & CO., LIMITED, 73-83 HATTON GARDEN, LONDON, E.C.1. Telephone: Holborn 6989

ia Street, Birmingham, 1. Telephone: Central 8004 *75-79 Eyre Street, Sheffield, 1. Telephone: 29212.*

Volume 88. No. 1045, Pages 249 - 330 April, 1963

THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

CONTENTS

Printed and Published for the Society for Analytical Chemistry by W. Heffer & Sons Ltd., Cambridge, England. **Communications to be addressed to the Editor, J. B. Attrill, 14 Belgrave Square, London, S.W.1.** Enquiries about advertisements should be addressed to Walter Judd Ltd., 47 Gresham Street, London, E.C.2.

Entered as Second Class at New York, U.S.A., Post Office