

The ANALYST

The Journal of the
Society for Analytical Chemistry

Monthly International Publication
dealing with all branches of
Analytical Chemistry

Published by the
SOCIETY FOR ANALYTICAL CHEMISTRY

Volume 97

No. 1160, Pages 841-928

November, 1972

THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

EDITORIAL ADVISORY BOARD

EXECUTIVE COMMITTEE

Chairman: H. J. Cluley (*Wembley*)

L. S. Bark (<i>Salford</i>)	R. Goulden (<i>Sittingbourne</i>)	J. M. Ottaway (<i>Glasgow</i>)
R. C. Chirnside (<i>Wembley</i>)	J. A. Hunter (<i>Edinburgh</i>)	G. E. Penketh (<i>Billingham</i>)
W. T. Elwell (<i>Birmingham</i>)	G. F. Kirkbright (<i>London</i>)	A. Townshend (<i>Birmingham</i>)
D. C. Garratt (<i>London</i>)	G. Nickless (<i>Bristol</i>)	

and the President and Honorary Officers

President of the Society: C. Whalley

Hon. Secretary of the Society:

W. H. C. Shaw

Hon. Treasurer of the Society:

G. W. C. Milner

Hon. Assistant Secretaries of the Society:

D. I. Coomber, O.B.E.; D. W. Wilson

Editor:

J. B. Attrill, M.A., F.R.I.C.

Assistant Editor:

P. C. Weston, B.Sc., A.R.I.C., M.I.Inf.Sc.

REGIONAL ADVISORY EDITORS

Professor G. Charlot, Faculté des Sciences de Paris, Laboratoire de Chimie Analytique, 10 rue Vauquelin, Paris Ve, FRANCE
Professor L. Gierst, Université Libre de Bruxelles des Sciences, Avenue F.-D. Roosevelt 50, Bruxelles, BELGIUM.
Professor R. Herrmann, Abteilung für Med. Physik. a.d. Univ.-Hautklinik, 63 Giessen, Gaffkystrasse 14, GERMANY.
Professor Axel Johansson, Institutionen for analytisk kemi, Tekniska Hogskolan, Stockholm, 70, SWEDEN.
Professor W. E. A. McBryde, Dean of Faculty of Science, University of Waterloo, Waterloo, Ontario, CANADA.
Dr. W. Wayne Meinke, Analytical Chemistry Division, Room A-309 Chemistry Building, National Bureau of Standards, Washington, D.C. 20234, U.S.A.
Professor J. Minczewski, Department of Analytical Chemistry, Institute for Nuclear Research, Warsaw-Zeran, Dorodna, POLAND.
Dr. D. D. Perrin, Department of Medical Chemistry, The John Curtin School of Medical Research, The Australian National University, Box 4, G.P.O., Canberra, A.C.T., AUSTRALIA.
Dr. A. Strasheim, National Physical Research Laboratory, P.O. Box 395, Pretoria, SOUTH AFRICA.
Professor K. Ueno, Department of Organic Synthesis, Faculty of Engineering, Kyushu University, Fukuoka, JAPAN.

OFFICES OF THE SOCIETY: 9/10 SAVILE ROW, LONDON, W1X 1AF

Telephone 01-734 9864

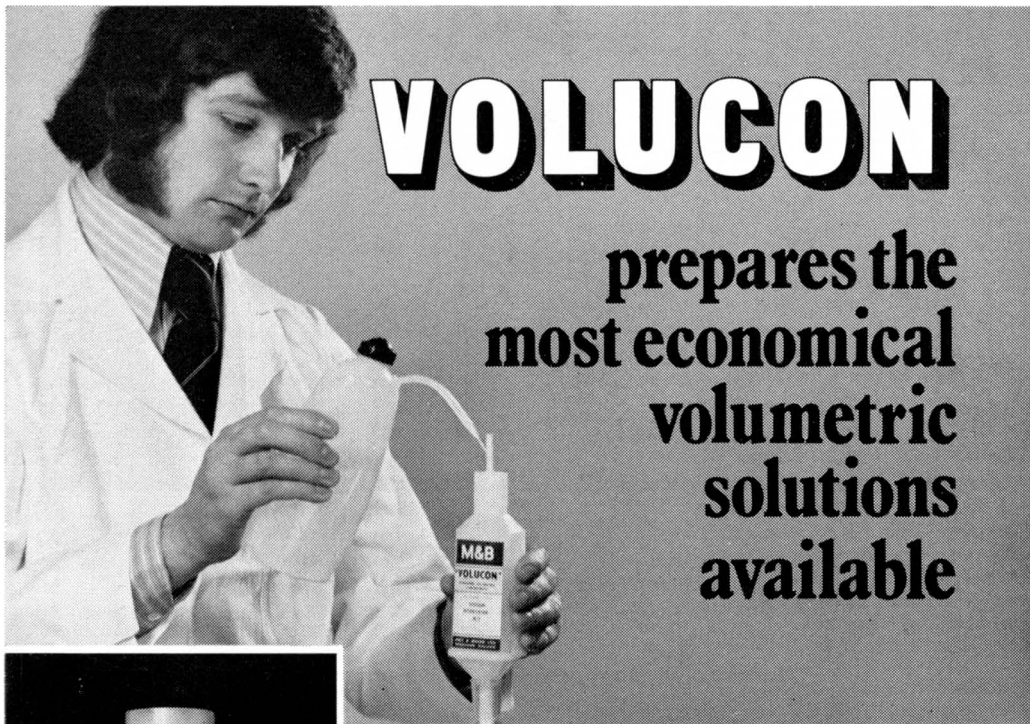
Advertisement Agent: J. Arthur Cook, 9 Lloyd Square, London, WC1X 9BA.

Telephone 01-837 6315

Published by The Society for Analytical Chemistry
Distributed by The Chemical Society, Publications Sales Office
Blackhorse Road, Letchworth, Herts., SG6 1HN

Volume 97, No. 1160

November, 1972



VOLUCON

prepares the
most economical
volumetric
solutions
available



'Volucon' saves you time, labour, space, money—and gives you peace of mind.

The easy-to-open 'Volucon' ampoules provide a simple and rapid method of preparing standard solutions of remarkable accuracy.

Most ampoules in the range make 1 litre of stated normality.

'Volucon' is a trade mark of the manufacturer, May & Baker Ltd Dagenham Essex RM10 7XS England



Send for detailed booklet today!

To May & Baker Ltd Dagenham Essex RM10 7XS

Please send me full details of 'Volucon' volumetric concentrates

Name

Address

A/11

'Volucon' volumetric concentrates for quick and sure results



M&B May & Baker

THE ANALYST

EDITORIAL ADVISORY BOARD

Chairman: H. J. Cluley (*Wembley*)

- | | |
|--|---|
| *L. S. Bark (<i>Salford</i>) | W. Kemula (<i>Poland</i>) |
| R. Belcher (<i>Birmingham</i>) | *G. F. Kirkbright (<i>London</i>) |
| L. J. Bellamy, C.B.E. (<i>Waltham Abbey</i>) | G. W. C. Milner (<i>Harwell</i>) |
| L. S. Birks (<i>U.S.A.</i>) | G. H. Morrison (<i>U.S.A.</i>) |
| E. Bishop (<i>Exeter</i>) | *G. Nickless (<i>Bristol</i>) |
| *R. C. Chirside (<i>Wembley</i>) | *J. M. Ottaway (<i>Glasgow</i>) |
| E. A. M. F. Dahmen (<i>The Netherlands</i>) | *G. E. Penketh (<i>Billingham</i>) |
| A. C. Docherty (<i>Billingham</i>) | S. A. Price (<i>Tadworth</i>) |
| D. Dyrssen (<i>Sweden</i>) | D. I. Rees (<i>London</i>) |
| *W. T. Elwell (<i>Birmingham</i>) | E. B. Sandell (<i>U.S.A.</i>) |
| *D. C. Garratt (<i>London</i>) | A. A. Smales, O.B.E. (<i>Harwell</i>) |
| *R. Goulden (<i>Sittingbourne</i>) | H. E. Stagg (<i>Manchester</i>) |
| J. Hoste (<i>Belgium</i>) | E. Stahl (<i>Germany</i>) |
| D. N. Hume (<i>U.S.A.</i>) | *A. Townshend (<i>Birmingham</i>) |
| *J. A. Hunter (<i>Edinburgh</i>) | A. Walsh (<i>Australia</i>) |
| H. M. N. H. Irving (<i>Leeds</i>) | T. S. West (<i>London</i>) |
| A. G. Jones (<i>Welwyn Garden City</i>) | P. Zuman (<i>U.S.A.</i>) |
| M. T. Kelley (<i>U.S.A.</i>) | |

* Members of the Board serving on the Executive Committee.

NOTICE TO SUBSCRIBERS

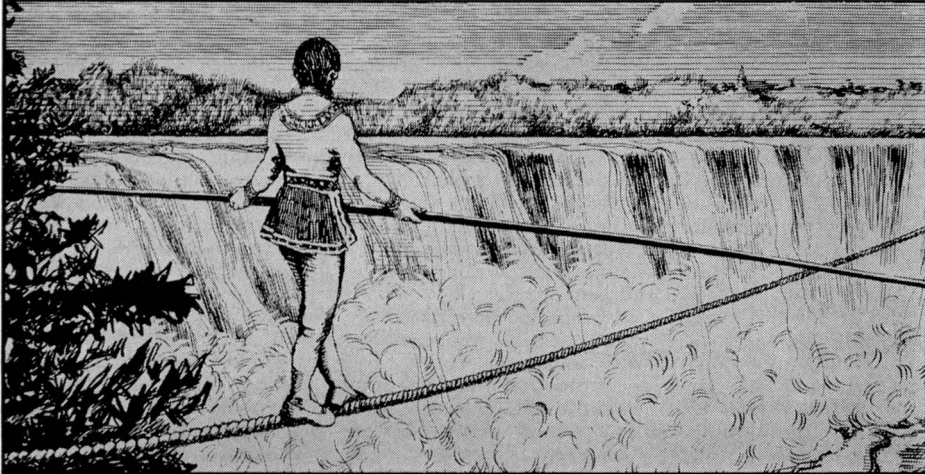
(other than Members of the Society)

Subscriptions for *The Analyst*, *Analytical Abstracts* and *Proceedings* should be sent to:

The Chemical Society, Publications Sales Office,
Blackhorse Road, Letchworth, Herts.

Rates for 1973

- | | |
|---|--------|
| (a) <i>The Analyst</i> , <i>Analytical Abstracts</i> , and <i>Proceedings</i> , with indexes | £37-00 |
| (b) <i>The Analyst</i> , <i>Analytical Abstracts</i> printed on one side of the paper (without index), and <i>Proceedings</i> | £38-00 |
| (c) <i>The Analyst</i> , <i>Analytical Abstracts</i> printed on one side of the paper (with index), and <i>Proceedings</i> | £45-00 |
| The <i>Analyst</i> and <i>Analytical Abstracts</i> without <i>Proceedings</i> — | |
| (d) <i>The Analyst</i> and <i>Analytical Abstracts</i> , with indexes | £34-00 |
| (e) <i>The Analyst</i> , and <i>Analytical Abstracts</i> printed on one side of the paper (without index) | £35-00 |
| (f) <i>The Analyst</i> , and <i>Analytical Abstracts</i> printed on one side of the paper (with index) | £42-00 |
- (Subscriptions are NOT accepted for *The Analyst* and/or for *Proceedings* alone)

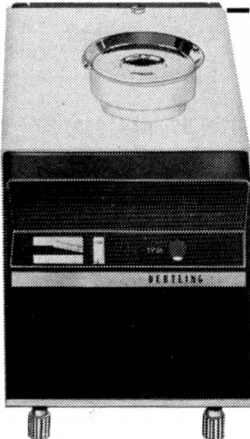


PERFECT BALANCE

Once — by Charles Blondin above Niagara in 1855. Always — by Oertling in countless universities, research laboratories, schools and factories throughout the world.

Oertling PRECISION BALANCES

L.Oertling Limited,
Cray Valley Works, St Mary Cray,
Orpington, Kent, BR5 2HA.
Telephone: Orpington 25771
Cables: Oertling, Orpington.
Telephone in Republic of Ireland:
Dublin 60272



Please send me full details of the Oertling range of top pan balances (tick those of interest)

<p>TP30 Co/D Award Winner 1970. Capacity 120 g Sensitivity 10 mg</p>	<p>TP31 (illustrated) Capacity 220 g Sensitivity 10 mg</p>	<p>TP35 Capacity 100 g + 100 g tare. Sensitivity 10 mg</p>	<p>TP40 Capacity 800 g + 100 g tare Sensitivity 10 mg</p>
<p>TP41 Capacity 1200 g + 100 g tare. Sensitivity 10 mg</p>	<p>TP45 Capacity 800 g Sensitivity 10 mg</p>	<p>TP46 Capacity 1200 g Sensitivity 10 mg</p>	<p>TP20 NEW! Capacity 1600 g + 300 g tare. Sensitivity 0.1 g</p>

TP25
NEW! Capacity 1600 g
Sensitivity 0.1 g

NAME

ADDRESS

You'll also learn about our unsurpassed team of 54 trained service men.

A5

Summaries of Papers in this Issue

Analysis of Aluminosilicate Materials by X-ray Fluorescence Spectrometry

The high precision of X-ray fluorescence spectrometric analysis is often adversely affected by various interfering factors. In the analysis of aluminosilicate materials, mineralogical, particle-size and surface-finish effects can be eliminated by fusing the sample with a flux and inserting the specimen into the spectrometer as a polished cast disc.

Inter-element effects can be overcome in several ways and in this work two methods are reported: a narrow range calibration, with slightly diluted specimens, for felspathic materials, and a multiple dilution method to cover a wide range of clays and refractories.

Several examples of felspathic materials have been analysed very carefully by a combination of chemical and spectrochemical methods, and the results are compared with those from the X-ray spectrometric method described. The X-ray spectrometric method appears to offer advantages in cost and time, and the results obtained are satisfactory for control analysis.

The multiple dilution method involves the use of standards that consist of pure compounds. This method was used to analyse a series of samples, including some commercially available standards. The results obtained indicate that the X-ray spectrometric method is considerably faster than the more commonly used combined methods, and that its accuracy is at least as good for normal control analysis.

The multiple dilution method should be applicable to a wide range of materials, but it is dependent upon a sophisticated and carefully performed specimen preparation technique.

D. G. ASHLEY and K. W. ANDREWS

Pilkington Brothers Limited, Central Analytical Laboratory, St. Helens, Lancashire, WA10 3TT.

Analyst, 1972, **97**, 841–847.

The Quantitative Determination of Uncombined Calcium Hydroxide and Oxide in Clinker and Cement by X-ray Diffraction

A quantitative X-ray diffraction method for the determination of uncombined calcium hydroxide in clinkers and cements with an interference maximum of 0.490 nm has been developed, with magnesium hydroxide as the internal standard. This method gives satisfactory results as the accuracy of determination is 0.3 per cent. *m/m*. The determination of uncombined calcium oxide was noted on the same scan with an interference maximum of 0.145 nm and was found to be inconclusive, giving unsatisfactory results.

M. BARTL

Research Institute of Binding Materials and Asbestos Cement, Brno, Czechoslovakia.

and D. PEKÁREK

Research Institute of Building Materials, Brno, Czechoslovakia.

Analyst, 1972, **97**, 848–853.

Determination of Water of Crystallisation in Gypsum by Infrared Spectroscopy in the Presence of Clay Minerals

A method is described for the determination of water of crystallisation in various types of gypsum in the presence of clay minerals with the aid of infrared spectroscopy. The water is determined by use of the 1685 cm^{-1} absorption band with an accuracy of 0.50 per cent. *m/m*.

M. BARTL

Research Institute of Binding Materials and Asbestos Cement, Brno, Czechoslovakia.

Analyst, 1972, **97**, 854–859.

Determination of Boron in Plants by Emission Spectroscopy with the Nitrous Oxide - Hydrogen Flame

Boron is readily determined in plant ash solutions, following extraction into chloroform of the chelate formed with 2-ethylhexane-1,3-diol, by means of the nitrous oxide - hydrogen flame and conventional flame photometers. Boron in concentrations as low as 8 p.p.m. in the dried plant material can be determined with a relative standard deviation of about 4 per cent. The boron oxide (BO_x) band emission intensity at 518.2 or 547.6 nm is measured with wide slits. Recorder read-out and zero suppression are desirable. Results on several samples by this and four colorimetric methods are given. The large signal strengths obtained make this method superior to atomic-absorption techniques.

J. C.-M. PAU, E. E. PICKETT and S. R. KOIRTYOHANN

Departments of Chemistry and Agricultural Chemistry, University of Missouri, Columbia, Missouri 65201, U.S.A.

Analyst, 1972, **97**, 860-865.

The Assay of Non-fat Milk Solids by the Determination of Orotic Acid in Milk Chocolate and in the Milk

When the orotic acid content of the milk that is used in the manufacture of chocolate is known, non-fat milk solids can be determined with satisfactory accuracy by the described method. The method is equally valid for chocolate products that contain milk protein that has been subjected to high-temperature heat treatment and consequently cannot be analysed satisfactorily by the method of the Association of Official Analytical Chemists.

Natural variations in the orotic acid content of milk of various origins are too great to permit the determination of milk solids, for arbitration purposes, in milk chocolate.

R. J. MOTZ

Mars Limited, Confectionery Division, Dundee Road, Slough, Bucks.

Analyst, 1972, **97**, 866-871.

Analysis of Fluoroborate Alloy Plating Solutions to Determine Iron, Cobalt and Nickel

A rapid method for the determination of iron, cobalt and nickel in fluoroborate solutions has been devised and tested. It involves an initial dichromate titration to determine both iron(II) and iron(III) followed by the simultaneous spectrophotometric determination of cobalt and nickel at wavelengths 394 and 512 nm after reduction of all the iron to the iron(II) state.

The recommended procedure, which can be completed in less than 1 hour, has been successfully applied to typical plating solutions.

T. KEILY and R. C. WOODFORD

Medway and Maidstone College of Technology, Chatham, Kent.

Analyst, 1972, **97**, 872-876.

A Colorimetric Method for the Determination of Chloral Hydrate

A specific colorimetric method for the determination of chloral hydrate is described. The method is based on the measurement of the pink colour formed when chloral hydrate is treated with 2-thiobarbituric acid. It is specific for chloral hydrate as no other related compounds tested, such as chloroform, trichloroacetic acid, trichloroethylene, carbon tetrachloride, 2,2-dichloroethanol and 1,1,2,2-tetrachloroethane, give any coloration with 2-thiobarbituric acid. The method is sensitive to concentrations as low as 50 $\mu\text{g ml}^{-1}$ of chloral hydrate with a reproducibility of ± 2 per cent.

S. S. KAMAT, V. P. BARVE and H. S. MAHAL

Forensic Science Laboratory, State of Maharashtra, Byculla, Bombay-8, India.

Analyst, 1972, **97**, 877-879.

RS for the analysis of pesticides residue

On the recommendation of international organisations such as FAO and WHO, many nations throughout the world have felt the necessity to regulate the use and the control of pesticides in foodstuffs.

The analysis of pesticide residues presents unusual problems because of the small amounts of substances to be determined and the large number of possible interfering substances which must be first eliminated. In order to determine extremely small quantities, very sensitive analytical methods are required, which however cannot be applied directly to the substances under examination. The general procedure is:

- extraction of the pesticide from the alimentary product;
- concentration of the extract by evaporation of the solvent;
- removal from the extract of naturally-occurring substances which would interfere with the pesticides;
- determination of the extracted pesticide.

In these operations large quantities of polar and non polar solvents are used. The usual Analytical Grade solvents, when subjected to a more detailed investigation (GLC e.c.d. and Na d.), show peaks due to impurities, whose positions coincide with those of the peaks of pesticides. The use of solvents from which these impurities have been eliminated is therefore indispensable.

Carlo Erba RS solvents for pesticides have been studied and developed in order to satisfy these requirements. Their main characteristic is that of having a greatly reduced quantity of any residue which may interfere with the analytical method.

This has been attained by working under special conditions, with small batches which are controlled individually, and by special choice of packing materials, cleaning methods and bottle closure procedures. For chlorinated compounds, a maximum limit of 10^{-9} % as aldrin (GLC e.c.d.) is guaranteed, and for phosphorylated compounds a maximum limit of 10^{-8} % as parathion (GLC Na d.).

All these products are available in bottles of 1000 ml.

Acetone RS
Acetonitrile RS
Benzene RS
Chloroform RS
Cyclohexane RS
Dioxane RS
Ethyl acetate RS
Ethyl ether RS

n-Hexane RS
Isooctane RS
Isopropyl alcohol RS
Methanol RS
Methylene chloride RS
Petroleum ether 40°-60° RS
n-Pentane RS
Toluene RS

CARLO ERBA

DIVISIONE CHIMICA INDUSTRIALE / VIA C. IMBONATI 24 / 20159 MILANO

THE ANALYST

Analysis of Aluminosilicate Materials by X-ray Fluorescence Spectrometry

BY D. G. ASHLEY AND K. W. ANDREWS

(*Pilkington Brothers Limited, Central Analytical Laboratory, St. Helens, Lancashire, WA10 3TT*)

The high precision of X-ray fluorescence spectrometric analysis is often adversely affected by various interfering factors. In the analysis of aluminosilicate materials, mineralogical, particle-size and surface-finish effects can be eliminated by fusing the sample with a flux and inserting the specimen into the spectrometer as a polished cast disc.

Inter-element effects can be overcome in several ways and in this work two methods are reported: a narrow range calibration, with slightly diluted specimens, for felspathic materials, and a multiple dilution method to cover a wide range of clays and refractories.

Several examples of felspathic materials have been analysed very carefully by a combination of chemical and spectrochemical methods, and the results are compared with those from the X-ray spectrometric method described. The X-ray spectrometric method appears to offer advantages in cost and time, and the results obtained are satisfactory for control analysis.

The multiple dilution method involves the use of standards that consist of pure compounds. This method was used to analyse a series of samples, including some commercially available standards. The results obtained indicate that the X-ray spectrometric method is considerably faster than the more commonly used combined methods, and that its accuracy is at least as good for normal control analysis.

The multiple dilution method should be applicable to a wide range of materials, but it is dependent upon a sophisticated and carefully performed specimen preparation technique.

THE chemical analysis of aluminosilicates by traditional methods is a highly skilled and lengthy process whereas the X-ray fluorescence technique is rapid, has a high precision, and use can be made of less experienced staff. Unfortunately, high precision does not necessarily imply high accuracy. In X-ray fluorescence there are several factors other than concentration that can influence secondary X-ray intensities and highly reproducible, but inaccurate, results may be obtained. These interfering factors must be either eliminated or corrected before the X-ray spectrometric technique can be considered satisfactory for the analysis of any material. With aluminosilicates the principal interferences are mineralogical, surface-finish or particle-size and inter-element effects.

Mineralogical effects can be avoided if the fused melt is cast as a homogeneous disc and particle-size and surface-finish effects can be avoided if the fused melt is cast as a homogeneous disc and the surface of the disc polished.

Inter-element effects are overcome in one of three ways: by their avoidance, elimination or correction. Avoidance in this context requires the use of a large number of standards so that one is available that is almost identical in composition with the sample. This method can give accurate results but is not practicable over a wide range of compositions for a comparatively small number of samples. Elimination involves the dilution of samples to a sufficient extent that any change in their composition has a negligible inter-element effect. This technique cannot always be applied: usually such a high degree of dilution or absorption by the diluent is necessary that there is a risk of considerable loss in precision because of the reduced intensities.

Corrections based on published data for absorption coefficients make no allowance for variations in the penetration of the primary beam or of enhancement effects; consequently, they are rarely satisfactory for highly accurate determinations. Empirical corrections, on the other hand, frequently require a very large number of analysed standards. The standards can be made synthetically, but the ultimate accuracy is limited by the accuracy of the determination of the correction factors and the number of factors used. In addition there

may be a tendency to transfer the error on one constituent to other constituents. In all but the simplest instances a computer is required to calculate the results from a limited mathematical model.

Felspathic aluminosilicates can be analysed by using a combination of the avoidance and elimination techniques. The diluting effect of the flux used tends to reduce the inter-element effects and, by limiting the samples to classes such as potash felspars, nepheline syenites and petalites with different standards for each class, these effects are reduced to negligible proportions. Unfortunately, when analysing a very wide range of compositions, such as is shown by aluminosilicate refractories, this method is not satisfactory.

Tertian¹ described the use of a multiple dilution technique that does not require very high dilutions or the determination of correction factors. Pure compounds can be used as standards, in which event only simple calculations are required. In the work described in this paper, Tertian's technique was applied to the analysis of aluminosilicate materials over the range from 10 to 90 per cent. of silica and aluminium oxide. All constituents in the range from 0 to 10 per cent. were determined by using graphical calibrations.

EXPERIMENTAL

FELSPATHIC MATERIALS—

The material, as received, was spread on a polythene sheet. A 10-g sample was dried at 110 °C for 30 minutes and then ground in a tungsten carbide lined disc mill (net capacity 10 ml) for 3 minutes.

A 2.75-g sample of the ground material was transferred to a 200 × 25-mm glass tube and mixed with 13.75 g of ground lithium tetraborate (Johnson Matthey Spectroflux 100, graphite free). The tube was shaken by hand for 30 s so as partially to mix the contents and the mixture was again ground for 1 minute in the disc mill.

The ground mixture was transferred to a platinum crucible and fused for 20 minutes at 1150 to 1200 °C, the melt being swirled in the crucible after 5 and 15 minutes. When fusion was complete the melt was cast in a pre-heated graphite mould as described by Ambrose, Rutherford and Muir.² When the graphite block and the cast bead were cool, the bead was sufficiently annealed and strong not to present any handling problems.

The bead was polished in three stages: a coarse grinding stage (with approximately 100 grit emery paper), a fine grinding stage (with British American Optical Company BM 303 polishing compound) and a final polishing stage (with British American Optical Company BM 309 WZ polishing compound). The polishing compounds are zirconium dioxide (zirconia) based materials. Contamination was not apparent, even when measuring low zirconium dioxide contents in specimens.

The X-ray intensities from the polished discs were compared directly with a standard of the appropriate type, the intensity ratios thus obtained being converted into concentrations simply by multiplying the ratio by the concentration of the element in the standard. For the majority of elements the background counts are negligible. Sodium and elements with lower atomic numbers than that of sodium were determined by alternative methods.

REFRACTORY MATERIALS—

The analysis of refractory materials is usually performed on an ignited sample. All samples of refractory material were ignited at 1100 °C for 1 hour. Two specimens of each refractory material were prepared, one with a 1 + 5 *m/m* proportion of sample to lithium tetraborate, and the other with a 1 + 10 proportion (2.75 + 13.75 g and 1.50 + 15.00 g were the actual amounts taken). The fusion, casting, annealing and polishing were carried out in the same way as for the felspathic materials.

By using a 1 + 5 bead of a refractory material (ganister) as a reference standard, intensity ratios (see under Calculation of results for the multiple dilution technique) for the 1 + 5 and 1 + 10 fusions of a sand with a high silica content (99.8 per cent. of silica) and calcined aluminium oxide (99.9 per cent. of aluminium oxide) were determined. Ratios of the 1 + 5 and 1 + 10 fusions of the samples were obtained for silica and aluminium oxide in the same way.

The reference standard was used so that the ratio method could be used for correction of long and short-term drift. The material used as a reference was a refractory selected to

be in the middle of the range, *i.e.*, composed of 50 per cent. of silica and 50 per cent. of aluminium oxide (accurate analysis was not required). By use of this ratio method it was found that there was no change in the intensity ratios for the sand and calcined aluminium oxide over a period of several months and therefore frequent checks on the standards were not necessary.

Constituents (*i.e.*, elements present expressed as oxides) at a concentration of less than 10 per cent. were determined by using the 1 + 5 fusion specimens only, the concentration being determined from calibration graphs drawn by using commercially available standards, synthetic standards or a combination of both. Constituent oxides at a concentration of more than 95 per cent. were normally determined by difference, as the statistical error in the intensity determination at these levels exceeds the cumulative error in the determination of other constituents.

The sodium content was determined by an alternative method.

EQUIPMENT—

The polishing equipment used was the normal metallurgical rotating bowl type, the specimens being mounted on steel backing plates during polishing.

X-ray spectrometric measurements were made with either a Philips PW1210 or a PW1212 automatic X-ray fluorescence spectrometer. A chromium target X-ray tube was used throughout. Ammonium dihydrogen orthophosphate, pentaerythritol and lithium fluoride (LiF 200) crystals were used to determine the intensity as appropriate for the particular radiation being measured.

CALCULATION OF RESULTS FOR THE MULTIPLE DILUTION TECHNIQUE—

Tertian¹ has derived the equation

$$\frac{C_a}{C_s} = \frac{i_2 i_1 / (i_2 - i_1)}{j_2 j_1 / (j_2 - j_1)} \dots \dots \dots \dots \dots \dots (1)$$

where C_a is the concentration of element X in the sample, C_s the concentration of element X in the standard, i_1 the intensity of radiation from element X in the first specimen of the sample, i_2 the intensity of radiation from element X in the second specimen of the sample, j_1 the intensity of radiation from element X in the first specimen of the standard, and j_2 the intensity of radiation from element X in the second specimen of the standard. This equation was obtained by eliminating the terms that represented the inter-element effects from equations relating intensity to concentration.

Substitution of the observed intensity ratios for the terms representing intensities enabled the value of C_a to be calculated, as the value of C_s was known. For silicate analysis it is usual to express the component elements of a material in terms of the oxides. This only involves the substitution of a different value for C_s (elemental Si, per cent., becomes SiO_2 , per cent.).

In some instances, depending on the equipment and excitation conditions used, it may be necessary to include a background correction. The pure aluminium oxide standards can be used to determine the silica $K\alpha$ background, and the pure silica to determine the aluminium $K\alpha$ background. With most modern equipment in which chromium target tubes are used, the background correction is probably only necessary at low concentrations of silica and aluminium oxide. With our equipment background corrections were not found to be necessary above a concentration of 10 per cent. of silica or aluminium oxide, and as constituents below 10 per cent. were determined by calibration graphs, background corrections were not used.

When fusing the sample with lithium tetraborate there are two possible causes of loss from the fusion: the loss on ignition of the sample and the loss of boron or lithium, or both, from the flux. It is possible to correct for these losses by using an internal standard, although the use of such a standard would mean extra preparation time, extra X-ray intensity measurements and additional statistical error from the counting. When comparing results of experiments with calculated values, it appears that the statistical error in the intensity determinations is the major limiting factor to the accuracy of the method and should be minimised as far as possible. The alternative to the internal standard correction method is to correct the observed oxide concentrations so that they total 100 per cent., and it was this method that was adopted.

If C_p^x is the preliminary concentration of constituent X, calculated as was C_a in equation (1), C_t^x the true concentration of constituent X, C_y^a the concentration of constituent Y, determined by a technique other than an X-ray method, and T the ideal total for the analysis (usually 100 per cent.), then

$$\int C_t^x = C_p^x \times \frac{T - \sum C_y^a}{\sum C_p^x} \quad \dots \quad \dots \quad \dots \quad \dots \quad (2)$$

Only those constituents determined by X-ray fluorescence spectrometry are corrected by use of this equation.

The use of this method of correction prevents the final total from being used as a check on the analysis so that the preliminary total is used instead. Limits have been set for the acceptable preliminary total of all constituents before correction. Analyses of samples with totals outside these limits are repeated.

RESULTS

FELSPATHIC MATERIALS—

Representative values obtained by X-ray spectrometric analysis of feldspathic materials are compared in Table I with values obtained by a combination of ultraviolet spectroscopy, flame photometry and chemical techniques (combined methods). Each sample was analysed several times and the mean values are given. The X-ray spectrometric results were each obtained from a single analysis.

TABLE I
COMPARATIVE RESULTS FOR THE ANALYSIS OF FELSPATHIC MATERIALS

Sample	SiO ₂ , per cent.	Al ₂ O ₃ , per cent.	Fe ₂ O ₃ , per cent.	CaO, per cent.	K ₂ O, per cent.	Na ₂ O, per cent.	P ₂ O ₅ , per cent.	Type of analysis
A	64.0	19.4	0.045	0.1	12.5	—	0.6	X-ray
	63.9	19.4	0.042	—	12.5	2.7	0.6	Combined methods
B	65.8	18.1	0.069	0.2	11.9	—	0.6	X-ray
	65.9	18.2	0.074	—	11.8	2.7	0.6	Combined methods
C	66.4	17.7	0.087	0.2	11.4	—	0.6	X-ray
	66.5	17.7	0.099	—	11.3	2.7	0.6	Combined methods
D	60.4	23.1	0.069	0.5	5.0	—	Trace	X-ray
	60.4	23.3	0.062	0.5	4.9	10.4	0.1	Combined methods
E	60.3	23.2	0.082	0.5	5.0	—	Trace	X-ray
	60.2	23.0	0.084	0.6	5.0	10.5	Trace	Combined methods
F	60.7	23.2	0.074	0.5	4.8	—	Trace	X-ray
	60.7	23.2	0.076	0.5	4.9	10.4	Trace	Combined methods
G	55.6	24.1	0.13	1.4	9.3	—	0.1	X-ray
	55.6	23.9	0.13	1.5	9.3	7.7	0.1	Combined methods

Twenty samples of approximately 10 g mass were taken from a bulk sample of potash feldspar. These samples were analysed by the X-ray spectrometric method to determine the precision of this method. The precisions obtained showed no significant difference from the theoretical precision calculated from the counting statistics. There is no reason to doubt that similar figures could be obtained for the other types of feldspathic materials.

REFRACTORY MATERIALS—

Initially all of the readily available commercial standards of the aluminosilicate refractories were analysed by the multiple dilution technique for aluminium oxide and silica. The results are shown in Table II.

The 1 + 5 fusion discs of the commercial standards were used to construct calibration graphs for elements other than silicon and aluminium. These graphs were used to analyse a series of refractories to determine the minor constituents and the results are shown in Table III. The aluminium oxide and silica contents of these refractories were determined by the multiple dilution technique (Table IV).

The commercial standards, the silica sand and the calcined aluminium oxide were also used to produce calibration graphs for silica and aluminium oxide. These graphs were used to obtain results for the series of refractories; these results are shown in Table IV.

TABLE II
COMPARISON OF RESULTS OBTAINED BY MULTIPLE DILUTION ANALYSIS AND
CERTIFICATE RESULTS FOR COMMERCIAL STANDARDS

Sample	SiO ₂ , per cent.		Al ₂ O ₃ , per cent.	
	Certificate	X-ray method	Certificate	X-ray method
B.C.S. 309	34.1	34.0	61.1	61.3
B.C.S. 315	51.2	51.1	42.4	42.5
B.C.S. 269	56.7	56.5	33.9	33.8
* G.U.M.R. 1.69	36.7	36.9	60.1	60.0
G.U.M.R. 1.68	55.0	55.1	38.2	38.1
N.B.S. 77	32.4	32.7	59.4	59.3

* Groupement des Utilisateurs de Matériaux Refractories (Belgium).

A series of twenty samples of a ganister refractory were prepared for analysis by the multiple dilution method. An analysis was carried out at each stage of polishing. The results obtained gave the values for the coefficient of variation that are shown in Table V. It would therefore seem that for a high-precision measurement of silica and aluminium oxide polishing is necessary.

TABLE III
COMPARISON OF MEAN COMBINED ANALYSES AND X-RAY RESULTS FOR REFRACTORIES

Sample	CaO, per cent.	MgO, per cent.	K ₂ O, per cent.	TiO ₂ , per cent.	Fe ₂ O ₃ , per cent.	Type of analysis
1	0.2	0.7	1.8	1.6	2.8	Combined methods
	0.3	0.6	1.9	1.6	2.9	X-ray
2	0.2	0.9	2.1	1.3	3.7	Combined methods
	0.3	0.8	2.2	1.4	3.6	X-ray
3	0.2	0.8	2.0	1.1	2.1	Combined methods
	0.2	0.8	2.0	1.2	2.2	X-ray
4	0.2	0.8	2.5	1.1	4.5	Combined methods
	0.2	0.7	2.6	1.2	4.4	X-ray
5	0.4	0.6	0.3	1.3	4.1	Combined methods
	0.6	0.4	0.4	1.5	4.3	X-ray
6	0.4	0.7	0.6	1.4	3.2	Combined methods
	0.5	0.5	0.7	1.4	3.2	X-ray
7	0.2	0.2	0.3	0.4	1.7	Combined methods
	0.2	0.2	0.3	0.5	1.7	X-ray
8	0.1	0.1	0.6	0.2	0.6	Combined methods
	Trace	Trace	0.6	0.2	0.6	X-ray
9	0.1	Trace	0.1	2.9	1.2	Combined methods
	0.1	Trace	0.1	2.9	1.4	X-ray

DISCUSSION

The method of sample preparation that has been described, although comparatively time consuming, must be carried out if the best accuracy and precision are to be achieved. There are other methods of sample preparation that may be considered to be suitable for control purposes: to crush and pellet, for instance, may be satisfactory when the time factor overrides the necessity for great accuracy.

Mineralogical effects may not be significant if there is only one source of a particular material. However, the comparison of a sample with a standard from a different source may introduce error. For example, with a crushed and pelleted specimen of a felspar, a value of 17.7 per cent. of aluminium oxide was obtained; the certificate value was 16.7 per cent. When the sample was analysed after fusion, a value of 16.7 per cent. of aluminium oxide was obtained by X-ray spectrometric analysis.

Fusion, followed by crushing and compacting into a pellet, improved the silica determination of a series of potash felspars, but not sufficiently to be acceptable. It appeared that the silica determination was being influenced by particle-size effects, hence the use of the polished, homogeneous, cast disc.

TABLE IV
COMPARISON OF MULTIPLE DILUTION, GRAPHICAL ANALYSIS AND MEAN COMBINED
ANALYTICAL RESULTS FOR REFRACTORIES

Sample	SiO ₂ , per cent.			Al ₂ O ₃ , per cent.		
	Combined methods	X-ray analysis (graphical)	X-ray analysis (multiple dilution)	Combined methods	X-ray analysis (graphical)	X-ray analysis (multiple dilution)
1	81.0	—	80.9	16.6	—	16.8
2	70.2	71.2	70.3	22.0	22.3	22.1
3	61.4	61.8	61.8	29.3	29.8	29.3
4	58.1	57.0	58.4	35.0	35.0	34.7
5	57.3	58.8	57.4	33.0	34.5	33.0
6	53.3	53.0	53.5	39.0	39.3	38.9
7	52.8	52.8	52.6	40.3	41.3	40.9
8	41.6	41.3	41.8	55.1	55.0	55.1
9	25.0	24.8	25.6	71.9	72.0	72.3
10	8.8	8.8	8.8*	86.1	86.0	86.1

* Determined from calibration graph.

The use of only one standard for each group of felspathic materials is equivalent to drawing a new calibration line from the origin to the standard for each determination; it is not necessary to correct back to a particular calibration. The error introduced by the implicit assumption that the calibration graph passes through the origin is negligible over the limited ranges for which this type of calibration is used. As with all calibrations, care must be taken that the standards are stable. A sample of known composition should be analysed regularly to check on this stability. With this method of calibration there is no obvious bias towards either the certificate values or the X-ray spectrometric results; hence it appears that precision and accuracy are identical in this instance. The precision, and hence the accuracy, of the X-ray spectrometric method is at least as good as that of the control method of analysis previously used.

In many situations the increased speed of analysis alone is sufficient reason to adopt a technique. In this particular situation more emphasis has been put on reliability, precision and cost, than on time. Every instance must be judged on its merits and a compromise between the requirements for speed, cost and precision must frequently be found.

On considering the use of the multiple dilution technique for the analysis of the commercial standards, the agreement between the X-ray spectrometric results (single determination) and the published values for silica and alumina was, in every instance, found to be within the 95 per cent. confidence limits determined from counting statistics.

The certificates of analysis for the G.U.M.R. standards give values for the standard deviations on the analyses used. These standard deviations for silica and aluminium oxide are presented in Table VI.

It is reasonable to accept the quoted values of 2σ as a measure of the precision of the more commonly used methods of analysis for this type of material, although it should be remembered that these figures represent inter-laboratory work.

Comparing the 2σ values for the G.U.M.R. analyses with the values obtained by the X-ray spectrometric method (Table V), it can be seen that the X-ray method gives improved precision by a factor of two or three. Even allowing for the greater variations that would be obtained for inter-laboratory work, it appears that the X-ray spectrometric method should be at least as precise as the more accurately used methods, and as there is no obvious bias between results, at least as accurate.

TABLE V
EFFECT OF SURFACE FINISH OF FUSED BEADS ON THE DETERMINATION OF SILICA AND
ALUMINIUM OXIDE IN REFRACTORIES WHEN THE MULTIPLE DILUTION PROCEDURE IS USED

Finish	Coefficient of variation		2σ	
	SiO ₂	Al ₂ O ₃	SiO ₂	Al ₂ O ₃
Rough ground	0.0138	0.0180	—	—
Fine ground	0.0076	0.0102	—	—
Polished	0.0024	0.0032	0.26	0.26

Assuming that a precision such as that shown for the X-ray spectrometric method is acceptable, the instrumental time required for each analysis is about $1\frac{1}{2}$ hours, which consists of 25 minutes for silica, 40 minutes for aluminium oxide and 25 minutes for the remainder, excluding sodium. The total time required for the preparation of the specimens is about $2\frac{1}{2}$ hours (1 hour ignition time). Thus, the total analysis time can be about 4 hours. The existing method of analysis is a much longer process, and a full control analysis may require up to 3 days from receipt of sample to reporting of results. This period can be reduced to only 1 day by using the X-ray spectrometric method.

Most of the work involved in the X-ray spectrometric method can be carried out by less experienced staff, and one person should be able to analyse four refractory samples per day. Although expensive capital equipment is required, the reduced demands on trained silicate analysts and the speed of analysis result in a cheaper method.

An alternative X-ray spectrometric method could consist in using calibration graphs for all elements. This method would have some advantages: for example, only one fusion per sample would be required, instrumental time would be reduced slightly and approximately half a man-hour would be saved per analysis. Unfortunately, the calibration graphs for silica and aluminium oxide would be curved, which would introduce the possibility of errors when drawing the calibration graph through a small number of points. The graphical method was used with the 1 + 5 dilution samples, but it appears that the results are less satisfactory than the results from the multiple dilution method (Table V). The average difference between the combined and graphical method results for silica is 0.53 per cent.; with the multiple dilution results the average difference is 0.23 per cent. For aluminium oxide the average difference between the combined and graphical results is 0.43 per cent., and with the multiple dilution method 0.17 per cent.

If the amount of a constituent present is above 95 per cent. it is determined by difference. If determined directly, the statistical error is frequently greater than the accumulated error on the other constituents; this is so with silica and aluminium oxide when the present equipment is used.

In any method of analysis there is always the possibility of an error caused by the presence of an unsuspected constituent. With most types of spectrographic analysis the total eventually achieved is a check that all constituents have been determined. However, when using the methods described here, the total is used to obtain a correction factor, so that any unfamiliar material is subject to a qualitative check by ultraviolet spectroscopy as well as the preliminary total check.

TABLE VI
PRECISION TAKEN FROM G.U.M.R. STATISTICAL DATA ON STANDARD SAMPLES

Sample	SiO ₂		Al ₂ O ₃	
	Content, per cent.	2σ, per cent.	Content, per cent.	2σ, per cent.
G.U.M.R. 1.69	36.7	0.92	60.1	0.76
G.U.M.R. 1.68	55.0	0.68	38.2	0.58

CONCLUSIONS

Analysis by X-ray fluorescence spectrometry is at least as accurate, quicker and less expensive than conventional methods for the control analysis of felspathic and refractory materials and clays consisting principally of silica and aluminium oxide. The multiple dilution technique should be suitable for the analysis of many other types of material, and work is continuing with a view to developing a general method of analysis for non-metallic materials.

The authors thank the Directors of Pilkington Brothers Limited and Dr. D. S. Oliver, Director of Group Research and Development, for permission to publish this paper.

REFERENCES

1. Tertian, R., *Spectrochim. Acta*, 1969, **24B**, 447.
2. Ambrose, A. D., Rutherford, R., and Muir, S., *Metallurgia*, 1970, **82**, No. 491, 119.

Received July 23rd, 1971
Amended May 31st, 1972
Accepted June 15th, 1972

The Quantitative Determination of Uncombined Calcium Hydroxide and Oxide in Clinker and Cement by X-ray Diffraction

By M. BARTL

(Research Institute of Binding Materials and Asbestos Cement, Brno, Czechoslovakia)

AND D. PEKÁREK

(Research Institute of Building Materials, Brno, Czechoslovakia)

A quantitative X-ray diffraction method for the determination of uncombined calcium hydroxide in clinkers and cements with an interference maximum of 0.490 nm has been developed, with magnesium hydroxide as the internal standard. This method gives satisfactory results as the accuracy of determination is 0.3 per cent. *m/m*. The determination of uncombined calcium oxide was noted on the same scan with an interference maximum of 0.145 nm and was found to be inconclusive, giving unsatisfactory results.

In the determination of uncombined calcium hydroxide in clinker and Portland cement, the 0.490-nm interference line was used with reference to the magnesium hydroxide interference line at 0.476 nm. As stated in the literature,¹ the degree of intensity of the interference line of calcium hydroxide used should be 7, this line appearing to be the most advantageous for quantitative determinations. In the vicinity of this line only the β -2CaO.SiO₂ line at a wavelength of 0.492 nm with a degree of intensity of 1, and the 6CaO.Al₂O₃.Fe₂O₃ line at a wavelength of 0.487 nm with a degree of intensity of 2, are to be found. It should be noted that the degree of intensity of an interference line, as given by Sehlke,² lies between the values 1 and 10.

In order to determine the uncombined calcium oxide in clinker and cement, the authors made use of the interference line (with copper K α radiation) at 0.145 nm and analytical-reagent grade magnesium hydroxide as the internal standard, with an interference line of 0.237 nm that did not coincide with that of uncombined calcium oxide. As suggested by Sehlke,² it was possible to use only the interference line at 0.145 nm, with a degree of intensity of 6, for the determination of uncombined calcium oxide. This interference line can, however, be influenced by interference lines of the following minerals: Ca(OH)₂ at 0.1449 nm, α' -2CaO.SiO₂ at 0.147 nm, β -2CaO.SiO₂ at 0.1448 nm, γ -2CaO.SiO₂ at 0.1457 nm, 4CaO.Al₂O₃.Fe₂O₃ at 0.145 nm, 6CaO.2Al₂O₃.Fe₂O₃ at 0.145 nm and 2CaO.Fe₂O₃ at 0.146 nm. However, in the instances with which this paper is concerned, *i.e.*, minerals that have interference lines with degrees of intensity 1 and 2, the risk of their influencing the interference line of uncombined calcium oxide is slight.

The presence of lines of α -2CaO.SiO₂ at a wavelength of 0.1488 nm with a degree of intensity of 8 and periclase MgO at 0.1489 nm with a degree of intensity of 9 could have a greater effect on the interference line of uncombined calcium oxide.

The quantitative evaluation of areas underneath the interference lines of uncombined calcium hydroxide and oxide, *i.e.*, the determination of their integral intensity, was carried out with the aid of a polar planimeter.

For the mathematical evaluation of the internal standard, the equation³

$$X_1 = k \frac{J_1}{J_s}$$

holds true, where X_1 is the mass fraction of the required component (per cent.), k is a constant, J_1 is the integral intensity of the line of the required component and J_s is the integral intensity of the line of the internal standard (magnesium hydroxide). Assuming that the concentration of the internal standard in the test mixture containing the component to be determined will be constant, then the concentration of the required component will be linearly dependent on the ratio $J_1:J_s$.

INFLUENCE OF CRYSTAL GRAIN AND PARTICLE SIZE ON REFLECTIONS—

Pulverised samples of clinkers and cements for quantitative X-ray diffraction analysis give satisfactory and reproducible results provided that they comply with certain requirements. Thus, the grains of the clinker or cement to be analysed must have a sufficiently small size for absorption to be negligible, but, at the same time, they must be large enough for the sample to be regarded as being representative of the powder as a whole.

The determination of the contents of the four main clinker minerals, tricalcium silicate (C_3S), β -dicalcium silicate (C_2S), tricalcium aluminate (C_3A) and tetracalcium aluminate/ferite (C_4AF),² and the determination of the calcium hydroxide content of hydrated calcium silicate⁴ by X-ray diffraction have shown that reproducible results can be obtained for particles smaller than $5\ \mu\text{m}$ in size. To satisfy this last requirement, Sehlke³ first screened the investigated clinker or Portland cement samples through a No. 325 sieve, following which they were ground for 2 hours in isopropyl alcohol to create a solid phase with a particle diameter of less than $5\ \mu\text{m}$, which was separated by sedimentation in isopropyl alcohol.

Copeland and Bragg⁴ screened the prepared mixtures of hydrated calcium silicates through a No. 200 sieve and then ground them for 2 hours in 1,1,1-trichloroethane, following which they again passed all the mixtures through the No. 200 sieve.

EXPERIMENTAL

Methods based on an extraction technique (the Franke method⁵) and the glycol method⁶ for the determination of free calcium oxide have been published. For measurement a Mikro 111 X-ray diffractometer (Müller, Hamburg) with recording facilities was used, while for scanning a proportional detector - counter incorporating a pulse discriminator with a channel width of 8 degrees was used. The instrument constants of the X-ray unit were: potential difference, 40 kV; current, 20 mA (the permissible maximum); sensitivity of recording equipment, 4; time constant, 8; and measuring factor, 1. The goniometer feed-rate was in every instance $0.5^\circ\ 2\theta\ \text{min}^{-1}$ and the paper speed was $400\ \text{mm}\ \text{h}^{-1}$.

PREPARATION OF SAMPLES—

After the addition of 10 per cent. *m/m* of pulverised analytical-reagent grade magnesium hydroxide (internal standard), the samples of clinker and cement that had a particle size of less than $90\ \mu\text{m}$ were ground for 40 minutes in a small porcelain ball-mill in analytical-reagent grade chloroform. After grinding and evaporation of the chloroform at 30°C , the individual samples were homogenised for a period of 20 minutes by grinding in an agate mortar and, after treatment with paraffin in glass test-tubes, were prepared for analysis.

PREPARATION OF CALIBRATION STANDARDS—

The calibration standards were prepared from the basic clinker, which contained virtually no uncombined calcium oxide or hydroxide, as determined by the X-ray diffractometer, Franke⁵ and ethylene glycol⁶ methods. Starting with this basic clinker (with a maximum particle size below $90\ \mu\text{m}$), various additions of weighed amounts of ground calcium oxide and hydroxide were made so as to give a series of standards. The purity of these additions was confirmed analytically by use of a complexometric titration method. After the addition of 10 per cent. *m/m* of pulverised magnesium hydroxide, to act as an internal standard, and grinding for 20 minutes in an agate mortar, a series of calibration samples with the following concentrations of uncombined calcium hydroxide and oxide (in per cent. *m/m*) was obtained—

Uncombined calcium oxide	..	0.50	1.00	1.50	2.00	2.50	3.00	3.50	4.00	5.00	6.00
Uncombined calcium hydroxide		0.50	1.00	1.50	2.00	2.50	3.00	3.50	4.00	5.00	6.00

Further treatment of the calibration standards by grinding in chloroform was carried out under the same conditions as those for the preparation of clinker and cement samples.

The samples of clinker and cement and calibration standards thus prepared were analysed on the Mikro 111 X-ray diffractometer.

Copper $K\alpha$ radiation was used for the quantitative determination of uncombined calcium oxide and hydroxide; the interference lines at $0.145\ \text{nm}$ and $0.490\ \text{nm}$ and the interference lines of the internal standard (magnesium hydroxide) at $0.237\ \text{nm}$ and $0.476\ \text{nm}$ were used.

The sample to be analysed was introduced into a cut-out in the cassette. Scanning was carried out twice for each sample, although the sample was always newly inserted into the cut-out of the sample carrier. The integral intensity of the relevant lines was determined with a polar planimeter and, in each instance, the average of the two determinations was taken.

RESULTS AND DISCUSSION

Because of the large number of X-ray diffraction records obtained in the course of this work, only the traces of the calibration standards Nos. 1, 2, 3 and 10 (Figs. 1 to 4), the traces from the samples of industrially produced clinker (Fig. 5) and of the single-component Portland cement (Fig. 6) are shown.

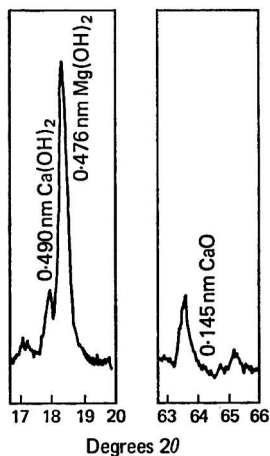


Fig. 1. X-ray diffraction traces for calibrating standard containing 0.50 per cent. *m/m* of free calcium oxide, 0.50 per cent. *m/m* of free calcium hydroxide and 10 per cent. *m/m* of additional internal standard magnesium hydroxide

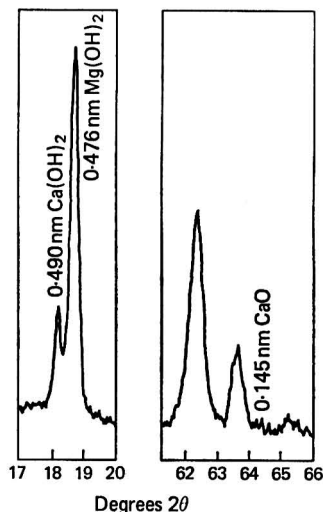


Fig. 2. X-ray diffraction traces for calibrating standard containing 1.00 per cent. *m/m* of free calcium oxide, 1.00 per cent. *m/m* of free calcium hydroxide and 10 per cent. *m/m* of additional internal standard magnesium hydroxide

It follows, from the diffraction traces of the calibration standards shown in Figs. 1 to 4, that the quantitative determination of uncombined calcium hydroxide in clinker and cement by use of the interference line at 0.490 nm was possible within the concentration range from 0.50 to 6.00 per cent. *m/m* of uncombined calcium hydroxide, and possibly also at higher concentrations. It also follows from these traces that the quantitative determination of uncombined calcium oxide in clinker and cement with an interference line at 0.145 nm is possible within the concentration range 1.50 to 6.00 per cent. *m/m*, and again possibly at higher concentrations. At concentrations of uncombined calcium oxide of 1.00 and 0.50 per cent. *m/m*, the 0.145-nm interference line is indistinct and it was therefore not possible to evaluate the integral intensity. As stated by Sehlke,² it was not possible to use a more suitable interference line for the quantitative determination of uncombined calcium oxide.

When comparing the diffraction traces of artificially prepared calibration standards with the traces of clinkers and cements from the field, both having corresponding concentrations of uncombined calcium oxide and hydroxide, it can be seen that the interference lines for uncombined calcium oxide at 0.145 nm and calcium hydroxide at 0.490 nm are narrower and higher for the calibration standards, thus indicating their higher degree of crystallinity compared with those of the samples (see Figs. 5 and 6), which are substantially wider and lower (*i.e.*, more diffuse in character), thus indicating their lower degree of crystallinity, even down to the amorphous state.

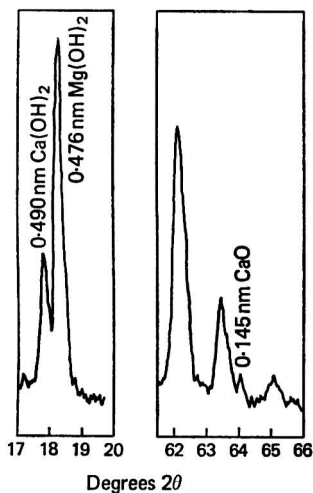


Fig. 3. X-ray diffraction traces for calibrating standard containing 1.50 per cent. *m/m* of free calcium oxide, 1.50 per cent. *m/m* of free calcium hydroxide and 10 per cent. *m/m* of additional internal standard magnesium hydroxide

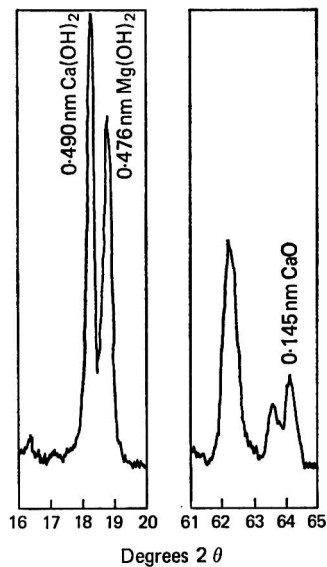


Fig. 4. X-ray diffraction traces for calibrating standard containing 6.00 per cent. *m/m* of free calcium oxide, 6.00 per cent. *m/m* of free calcium hydroxide and 10 per cent. *m/m* of additional internal standard magnesium hydroxide

It should also be mentioned that in only one instance of the seventeen samples of clinkers and cements analysed (the single-component cement), was it possible to evaluate quantitatively the interference line from uncombined calcium oxide at 0.145 nm (Fig. 6), which suggests that uncombined calcium oxide is in an amorphous state in most of the clinkers and cements, thus escaping determination by X-ray diffraction. It is, of course, also possible that the uncombined calcium oxide is present in the clinkers and cements largely in the

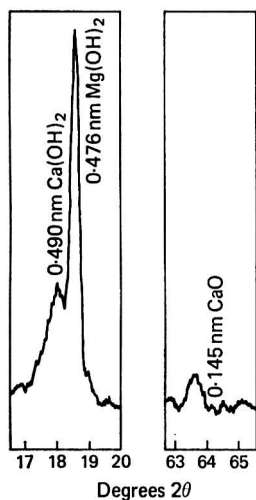


Fig. 5. X-ray diffraction traces for clinker

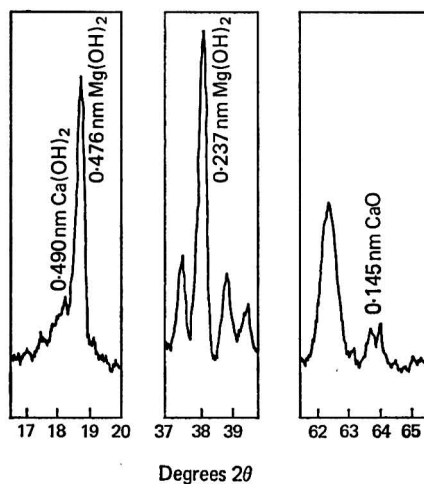


Fig. 6. X-ray diffraction traces for single-component Portland cement

hydrated form, which would make the 0.145-nm interference line indistinct and unsuitable for the determination of integral intensity. Such calcium oxide would appear as uncombined calcium hydroxide, the interference line of which at 0.490 nm is suitable for quantitative determinations in clinkers and cements.

The calibration graphs for uncombined calcium hydroxide and oxide are shown in Figs. 7 and 8. In these figures, the ratios of the integral intensities of calcium hydroxide at 0.490 nm and of the internal standard at 0.476 nm $\left(\frac{I_{0.490 \text{ nm}}}{I_{0.476 \text{ nm}}}\right)$, and the ratio of integral intensities of calcium oxide at 0.145 nm and the internal standard at 0.237 nm $\left(\frac{I_{0.145 \text{ nm}}}{I_{0.237 \text{ nm}}}\right)$, respectively, are plotted along the *y*-axis and the concentration of free calcium oxide and hydroxide in per cent. *m/m* is plotted along the *x*-axis.

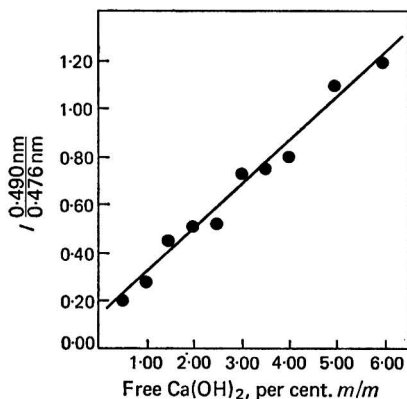


Fig. 7. Calibration graph for free calcium hydroxide

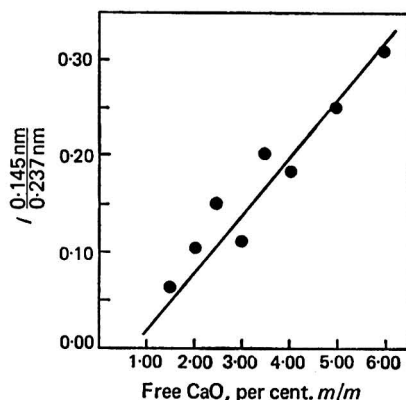


Fig. 8. Calibration graph for free calcium oxide

It follows from a comparison of the two calibration graphs that the scatter of points in the calibration graph for uncombined calcium oxide is greater than that for uncombined calcium hydroxide.

The results of determinations of uncombined calcium hydroxide in operational clinkers and cements are listed in Table I. As it was possible to determine uncombined calcium oxide only for sample No. 12 (single-component Portland cement) the result of this determination is given.

From the values given in Table I the accuracy of determination, R_p , of uncombined calcium hydroxide was calculated according to Czechoslovak Standard ČSN 01 0251. The accuracy of the determination is 0.27 per cent. *m/m* for the uncombined calcium hydroxide.

CONCLUSION

It was found, by the X-ray diffraction method, that the quantitative determination of uncombined calcium hydroxide at the interference maximum of 0.490 nm by using magnesium hydroxide as internal standard gives satisfactory results in clinker and cement. The calculated accuracy of determination, R_p , from two determinations on each sample, carried out on a total of seventeen test samples, was 0.27 per cent. *m/m* for uncombined calcium hydroxide. The determination can be carried out within the concentration range 0.50 to 6.00 per cent. *m/m* and possibly also at higher concentrations.

The determination of uncombined calcium oxide at the interference maximum of 0.145 nm by using magnesium hydroxide as internal standard proved inconclusive. The use of other interference lines for the determination is, according to information contained in the references, also problematical. Having regard to the fact that in field clinker and cement samples uncombined calcium oxide was determined in only one, single-component, cement sample

TABLE I
RESULTS OF UNCOMBINED CALCIUM HYDROXIDE DETERMINATIONS IN
CLINKERS AND CEMENTS

Number and type of sample	I $\frac{0.490 \text{ nm}}{0.476 \text{ nm}}$		Free Ca(OH) ₂ content, per cent. <i>m/m</i>			
	First deter- mination	Second deter- mination	First deter- mination	Second deter- mination	Difference	Average
1 Clinker	0.875	0.900	4.15	4.25	0.10	4.20
2 Clinker	—	—	—	—	—	—
3 PC	0.450	0.457	1.75	1.80	0.05	1.78
4 Clinker	0.469	0.510	1.85	2.10	0.25	1.98
5 PC	0.555	0.588	2.32	2.53	0.21	2.43
6 PC	0.438	0.444	1.68	1.73	0.05	1.71
7 PC	0.426	0.451	1.65	1.75	0.10	1.70
8 Clinker	0.516	0.508	2.12	2.07	0.05	2.10
9 PC	0.689	0.702	3.08	3.15	0.07	3.12
10 PC	0.549	0.569	2.30	2.42	0.12	2.36
11 PC	0.477	0.511	1.93	2.10	0.17	2.02
12 PC	0.467	0.434	1.85	1.67	0.18	1.76
13 PC	0.577	0.552	2.48	2.37	0.11	2.43
14 PC	0.618	0.611	2.70	2.62	0.08	2.66
15 PC	0.618	0.582	2.70	2.53	0.17	2.62
16 PC	0.471	0.451	1.88	1.75	0.13	1.82
17 PC	0.449	0.473	1.76	1.88	0.12	1.82

PC = Portland cement.

Samples 1 to 17 are clinkers and Portland cements in which the main mineral contents C₃S, β-C₂S, C₃A and C₄AF were determined by X-ray diffraction. The quantitative determination of clinker minerals has been described.⁷

out of the total of seventeen samples, it was not possible to calculate the accuracy of the determination on the basis of statistical methods.

The total time taken for the quantitative determination of uncombined calcium hydroxide by the X-ray diffraction method was approximately 90 minutes for one clinker or cement sample, including the preparation of the sample.

REFERENCES

1. *Mag. Concr. Res.*, March, 1957, 20.
2. Sehlke, K. H. L., *CSIRO Res. Rep.*, 1963, 1, 202.
3. Ohnemüller, W., and Zimmermann, I., *Zem.-Kalk-Gips*, May, 1970, 203.
4. Copeland, I. E., and Bragg, R. H., *Analyt. Chem.*, 1958, 30, 196.
5. Franke, B., *Z. anorg. allg. Chem.*, 1941, 247, 180.
6. Schläpfer, P., and Bukowski, R., Report of the Eidgenössische Technische Hochschule, Zurich, No. 63, 1933.
7. Šauman, Z., "The Control of Quality of the Clinker by Means of Physical Methods," Research Report, Výzkumný Ústav Stavebních Hmot, Brno, 1965.

Received January 21st, 1972

Accepted May 31st, 1972

Determination of Water of Crystallisation in Gypsum by Infrared Spectroscopy in the Presence of Clay Minerals

By M. BARTL

(Research Institute of Binding Materials and Asbestos Cement, Brno, Czechoslovakia)

A method is described for the determination of water of crystallisation in various types of gypsum in the presence of clay minerals with the aid of infrared spectroscopy. The water is determined by use of the 1685 cm^{-1} absorption band with an accuracy of 0.50 per cent. *m/m*.

THIS modified method is intended for the determination of the two molecules of water of crystallisation in gypsum by using the characteristic absorption band with a maximum at 1685 cm^{-1} . The value for water thus determined is expressed as $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$. This method was also applied to the quantitative determination of water of crystallisation in gypsum stones contaminated with clay minerals, which the methods that have been used hitherto have not been capable of achieving accurately.

TABLE I

WAVENUMBERS OF ABSORPTION BANDS OF WATER MOLECULES IN CLAY MINERALS AND OF WATER MOLECULES AND OTHER CONSTITUENTS IN VARIOUS MATERIALS

Material	Wavenumber/ cm^{-1}
Kaolinite	435, 475, 542, 700, 760, 800, 918, 940, 1012, 1038, 1108, 1640,* 3460,* 3628,* 3660,* 3672,* 3702*
Halloysite	436, 474, 545, 695, 758, 792, 918, 1020, 1040, 1109, 1640,* 3530,* 3560,* 3629,* 3702*
Montmorillonite ..	470, 525, 630, 850, 920, 1040, 1100, 1650,* 3430,* 3628*
Illite	415, 430, 475, 530, 620, 755, 800, 830, 919, 1030, 1645,* 3440,* 3630*
Silicates	900 to 1000, 3650,* 3660,* 3749*
Calcite	710, 870, 1410

The wavenumbers marked with an asterisk have been assigned to vibrations in the water molecules and hydroxyl groups by various workers.¹⁻³

As can be seen from Table I,¹⁻³ the absorption maxima of clay mineral water molecules should not interfere in the determination of gypsum water of crystallisation at 1685 cm^{-1} . In the vicinity of the dihydrate absorption band the absorption bands of the clay mineral montmorillonite, with a maximum at 1650 cm^{-1} , of kaolinite at 1640 cm^{-1} , halloysite at 1640 cm^{-1} , and illite at 1645 cm^{-1} , appear. The difference between the dihydrate absorption maximum and the maxima of the hydrated water molecules of the above clay minerals is 35 to 45 cm^{-1} , which should, according to references cited, provide sufficient latitude around the 1685 cm^{-1} absorption band for the quantitative determination of water of crystallisation in gypsum, assuming that an instrument with good resolution is used.

Gypsum, calcium sulphate dihydrate ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), which crystallises in the monoclinic system, has been the subject of numerous theoretical and practical investigations by both infrared and nuclear magnetic resonance spectroscopy.

The theoretical study of vibrational frequencies of water molecules in complexes⁴ has shown that the bond from the oxygen atom in the water molecule to the cation shifts the normal absorption at 1620 cm^{-1} towards wavenumbers that are approximately 60 to 80 cm^{-1} higher. For the dihydrate the shift is 65 cm^{-1} , as shown by the fact that the absorption at 1685 cm^{-1} corresponds to the vibration of water, the oxygen atom of which is bound to calcium.

A detailed study of the absorption spectra of gypsum crystals in the region 450 to 3800 cm^{-1} was carried out by Hass and Sutherland⁵ in an investigation of the crystallographic structure of gypsum. In this paper, the spectra of sulphate ions and water molecules in the crystals are compared. In the region 1500 to 1700 cm^{-1} , two absorption maxima for

such water molecules, 1623 and 1685 cm^{-1} , are presented. Reference should be made to the experimental investigations of Morris,⁶ who made use of the absorption maximum at 3390 cm^{-1} for the quantitative infrared analysis of calcium sulphate dihydrate.

As stated above, the method of quantitative determination of the water of crystallisation in gypsum from the absorption maximum at 1685 cm^{-1} was also applied to the analysis of gypsum stones contaminated with clay minerals, which is frequently of practical interest. Clay minerals contain a variable and sometimes considerable amount of water of crystallisation, and during the determination of the water of crystallisation in gypsum by the classical thermal analysis method at 300 °C, not only the removal of the water of crystallisation, but also the dehydration of the clay minerals occurs.

To prevent complications arising in the determination of the water of crystallisation in gypsum when using the absorption band at 1685 cm^{-1} , when there is a risk of the moisture present in the gypsum samples being determined at the same time, it is necessary first to dry the gypsum samples to constant mass at 49 to 50 °C in an electric dryer. Tests carried out by the X-ray diffraction method have shown that at this drying temperature dehydration of the dihydrate to the hemihydrate ($\text{CaSO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$) does not occur. It should be pointed out that the quantitative determination of the moisture in gypsum has so far been carried out by the thermal analysis method, *i.e.*, by drying the gypsum to constant mass at 49 to 50 °C.

EXPERIMENTAL

In the determination of the water of crystallisation in gypsum samples by infrared spectroscopy, tablets of potassium bromide *plus* the sample to be analysed were used in the measurement of absorption bands. The particle size of the standards and of the analysed gypsum samples was below 90 μm . Before the preparation of the tablets, it was necessary to dry the gypsum samples to constant mass at 49 to 50 °C (approximately 2 hours). The potassium bromide, marked "material of infrared quality," was thoroughly dried for a period of 5 hours at 105 °C *in vacuo* before the preparation of the tablets. A weighed amount (exactly 0.3 g) of the potassium bromide, of such a grain size as to pass through a 100-mesh sieve, was mixed with the dried, weighed gypsum sample. For the quantitative analysis, samples of the potassium bromide and the gypsum mixture were weighed to five decimal places. Thorough homogenisation of the mixture was achieved by mixing (not by rubbing, because of possible dehydration) for 20 minutes in an agate mortar in a dry atmosphere; the mixture was then evacuated in a tablet mould for 5 minutes and compressed by applying a pressure of 10 tonnes for 2 minutes. The compression of tablets intended for quantitative analysis was carried out with great care so as to prevent mass losses. It was not necessary to determine the thickness of the tablets, because the absorption is proportional to the mass of the potassium bromide and gypsum mixture. However, an important factor in the determination of absorption is the tablet area, which in our measurements was 1.326 cm^2 .

The optimum gypsum sample size for the preparation of tablets was determined by measuring the transmission of test samples on a Zeiss Jena UR-10 spectrophotometer. Traces were obtained for the absorption spectra of samples containing 0.001, 0.002 and 0.003 g of gypsum and 0.3 g of potassium bromide. The optimum gypsum content proved to be 0.003 g (about 1 per cent.) in a mixture with potassium bromide, giving a high transmission of between 25 and 60 per cent., which according to Kössler⁷ gives the greatest accuracy of determination. Both this determination⁷ and the measurements of standards and test samples were carried out over the wavenumber range from 1400 to 1900 cm^{-1} at a rate of 32 $\text{cm}^{-1} \text{min}^{-1}$. In these determinations, the instrument constants were: slot, No. 4; degree of amplification, 6; scale, 32 mm per 100 cm^{-1} ; and monochromator, sodium chloride prism. The accuracy of wavenumbers in the region from 1400 to 1900 cm^{-1} was checked by use of a polystyrene standard prepared in the form of a film with an accuracy of $\pm 2 \text{cm}^{-1}$.

Before proceeding with the actual determination of water of crystallisation in the gypsum test samples, it was necessary to test the extent to which the clay minerals influence the extinction of the gypsum absorption band at 1685 cm^{-1} . The contents of clay minerals in natural gypsum locations represent up to approximately 40 per cent. of the total mass (sometimes even more).

For the above reasons, three samples were prepared with a content of 80 per cent. *m/m* of high-purity gypsum and 20 per cent. *m/m* of a mixture of clay minerals (1 part by mass of illite *plus* 1 part by mass of kaolinite *plus* 1 part by mass of montmorillonite) and three

samples with a content of 80 per cent. *m/m* of high-purity gypsum and 20 per cent. *m/m* of dried potassium bromide. Next, three samples were prepared containing 60 per cent. *m/m* of high-purity gypsum and 40 per cent. *m/m* of a mixture of clay minerals with the same composition as given above, and a further three with a content of 60 per cent. *m/m* of high-purity gypsum and 40 per cent. *m/m* of dried potassium bromide.

From these mixtures, tablets were compressed and then analysed on the Zeiss Jena UR-10 instrument. The extinctions of the absorption band at 1685 cm^{-1} were evaluated from the traces obtained by the basic line method.

It was found that the difference between the average value for the extinction of samples containing 80 per cent. *m/m* of high-purity gypsum and 20 per cent. *m/m* of the mixture of clay minerals and for the extinction of samples with the same gypsum contents, but without the addition of clay minerals, represents 0.008 in favour of the samples with the addition of clay minerals. The difference between the value of the average extinction for samples containing 60 per cent. *m/m* of gypsum and 40 per cent. *m/m* of clay minerals and samples with the same gypsum content, but without the addition of clay minerals, is 0.023. It should be mentioned, however, that the average value for the extinction of samples was increased by this amount without the addition of the clay minerals mixture.

It follows from the above results that the influence of clay minerals on the extinction of the gypsum absorption band at 1685 cm^{-1} is practically negligible up to a maximum of 40 per cent. *m/m*.

The standards were prepared from high-purity crystalline gypsum that had been dried to constant mass at 49 to 50 °C and that had a water of crystallisation content of 20.62 per cent. *m/m* (determined by the thermal analysis method at 300 °C), which corresponds to 98.53 per cent. *m/m* of calcium sulphate dihydrate. The individual standards were prepared by diluting the gypsum with potassium bromide dried at 105 °C *in vacuo*.

Altogether, six standards were prepared with the following concentrations—

Gypsum concentration, per cent. <i>m/m</i>	98.53	80.00	60.00	40.00	30.00	20.00
Water of crystallisation concentration, per cent. <i>m/m</i>	20.62	16.74	12.56	8.37	6.28	4.19

Prior to determinations on standards and test samples, the infrared spectrum of a tablet compressed from pure, dried potassium bromide was determined. It was found that potassium bromide does not show an absorption maximum in the range 1400 to 1900 cm^{-1} , corresponding to adsorbed water molecules, and therefore there is no risk of the results of the determination of the water of crystallisation in gypsum being affected.

The determination of the extinction of standards and samples (by using the basic line method, as explained later) was carried out on each tablet three times, *i.e.*, in the original position, in the position obtained by rotating through $\pi/2$ rad and in that obtained by rotating through π rad. From the extinction values found the average was determined and the scatter calculated. The latter was found to be 0.0008.

The quantitative evaluation of absorption spectra for total water of the standards and gypsum samples was carried out by the basic line method,⁷ which appears to be the most advantageous method for this type of quantitative infrared analysis.

The determination of extinction is shown in Fig. 1.

For calculating the extinction (E) the relationship $E = \log \frac{I_b}{I}$ holds true, where I_b and I are as shown in Fig. 1.

The extinction values obtained for the standards were used in order to construct the calibration graph⁸ of the dependence of the extinction (plotted along the y -axis) against the concentration of the water of crystallisation in the standard (plotted along the x -axis). The calibration graph is shown in Fig. 2.

Gypsum sample numbers 1 to 13 were analysed in duplicate in order to determine the repeatability of the method.

RESULTS AND DISCUSSION

Verification tests to investigate the effect of the addition of clay minerals (1 part by mass of illite *plus* 1 part by mass of kaolinite *plus* 1 part by mass of montmorillonite) on the extinction of the absorption band of calcium sulphate dihydrate at 1685 cm^{-1} have shown

that the above minerals do not influence the extinction up to a maximum content of 40 per cent. m/m .

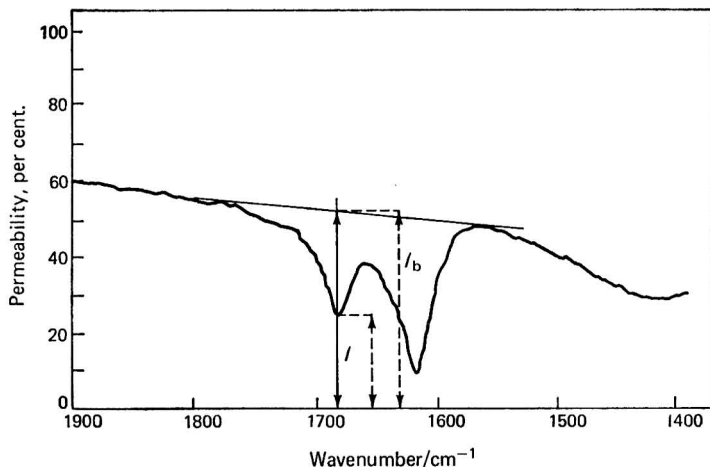


Fig. 1. Determination of extinction due to water of crystallisation in gypsum sample No. 12 by the basic line method

Comprehensive results from parallel determinations of water of crystallisation and of calcium sulphate dihydrate in gypsum stones are shown in Table II. It can be seen in Table II that in sample No. 3 an average water of crystallisation content of 2.15 per cent. m/m was found, which corresponds to 10.27 per cent. m/m of calcium sulphate dihydrate. The difference between the two determinations of dihydrate water on this sample, amounting to 0.23 per cent. m/m , is fairly large. This sample contained about 30 per cent. m/m of calcium sulphate (natural anhydrite). The absorption band of this sample at 1685 cm^{-1} was shallow and the determination of extinction could not be carried out with the same degree of accuracy as in the remaining samples. Similar difficulties were encountered in the determination of the calibration standard containing 4.19 per cent. m/m of water of crystallisation, which corresponds to a 20.00 per cent. m/m content of calcium sulphate dihydrate.

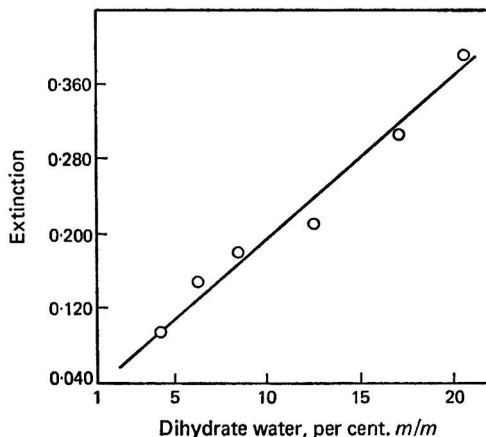


Fig. 2. Calibration graph of extinction versus concentration of water of crystallisation in per cent. m/m for standard gypsum samples

It can be seen from the above results, and from those of the tests on the effect of the addition of clay minerals on the extinction of the dihydrate absorption band at 1685 cm^{-1} , that the above method for determining water of crystallisation and calcium sulphate dihydrate in gypsum can be used successfully for gypsum samples containing from 60 to 100 per cent. *m/m* of calcium sulphate dihydrate. The method should therefore be of use in the industrial production of plaster of Paris.

The calculation of the standard deviation and the maximum permissible difference between parallel determinations of water of crystallisation in gypsum stones was carried out on the basis of the results listed in Table II according to Eckschlager.⁹ The calculated value of the standard deviation, *s*, is 0.1877 and the maximum permissible difference, R_{max} , is 0.52 per cent. *m/m*. If two results for R_{max} are obtained that differ by more than 0.52 per cent. *m/m*, a third determination of dihydrate water must be carried out, and the final result calculated from those two results which differ by a smaller amount than the permissible maximum difference.

TABLE II
DETERMINATION OF DIHYDRATE WATER AND CALCIUM SULPHATE DIHYDRATE
IN GYPSUM STONES

Sample number	First determination of dihydrate water, per cent. <i>m/m</i>	Second determination of dihydrate water, per cent. <i>m/m</i>	Difference, per cent. <i>m/m</i>	Average value for dihydrate water determination, per cent. <i>m/m</i>	Average value for calcium sulphate dihydrate determination, per cent. <i>m/m</i>
1	14.32	14.02	0.30	14.17	67.71
2	12.31	13.03	0.72	12.67	60.54
3	2.03	2.26	0.23	2.15	10.27
4	14.99	15.48	0.58	15.24	72.82
5	14.80	14.08	0.72	14.44	68.99
6	15.41	15.27	0.14	15.34	73.29
7	19.78	19.92	0.14	19.85	94.84
8	16.33	16.51	0.18	16.42	78.45
9	19.55	19.72	0.17	19.64	93.84
10	20.00	19.83	0.17	19.92	95.18
11	14.18	14.65	0.47	14.42	68.90
12	18.49	18.40	0.09	18.45	88.15

To confirm the accuracy of the results obtained by infrared spectroscopy, a comparative determination of water of crystallisation was carried out by the thermal analysis method on dried (at 49 to 50 °C to constant mass), high-purity, transparent crystalline gypsum, which was not the gypsum used in the standardisation. The thermal method, for which the results are given in Table III, could be used as a comparison method only for this high-purity gypsum, in which practically no clay minerals were present. The content of clay minerals possibly present, a maximum of 0.1 per cent. *m/m*, would represent about 10^{-2} to 10^{-3} per cent. *m/m* of water in those minerals, which can be neglected.

TABLE III
COMPARISON OF RESULTS OBTAINED BY (a) THERMAL ANALYSIS AND
(b) INFRARED SPECTROSCOPY

(a) *Results of thermal analysis—*

Dihydrate water determined at 300 °C

First determination, per cent.	Second determination, per cent.	Average value, per cent.
20.78	20.64	20.71

(b) *Results of dihydrate water determination by infrared spectroscopy—*

First determination, per cent.	Second determination, per cent.	Average value, per cent.
20.90	20.96	20.93

The difference in the determination of dihydrate water by the thermal method and by infrared spectroscopy is 0.22 per cent. *m/m*. For the sake of completeness, the dihydrate

was determined by a conventional gravimetric method as the anhydrous sulphate and the result converted into the dihydrate. A comparison of the results for the gravimetric determination of the dihydrate and determination of the dihydrate in gypsum by infrared spectroscopy is shown in Table IV.

TABLE IV
COMPARISON OF RESULTS FOR THE GRAVIMETRIC DETERMINATION OF CALCIUM
SULPHATE DIHYDRATE AND THE DETERMINATION OF THE DIHYDRATE
IN GYPSUM BY INFRARED SPECTROSCOPY

Sample number	Gravimetric determination of calcium sulphate dihydrate, per cent. <i>m/m</i>	Determination of calcium sulphate dihydrate by infrared spectroscopy, per cent. <i>m/m</i>	Difference, per cent. <i>m/m</i>
1	80.12	67.71	12.41
2	68.69	60.54	8.15
3	70.20	10.27	59.93
4	78.55	72.82	5.73
5	70.95	68.99	1.96
6	76.20	73.29	2.91
7	95.20	94.84	0.36
8	83.00	78.45	4.55
9	96.17	93.84	2.33
10	98.23	95.18	3.05
11	72.20	68.90	3.30
12	91.12	88.15	2.97

It follows from Table IV that the results for the gravimetric determination of the dihydrate are greater than those for the determination of the dihydrate in gypsum by infrared spectroscopy. With the gravimetric method not only the dihydrate, $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, but also the natural anhydrite, CaSO_4 , was determined.

CONCLUSIONS

By use of this modified method it is possible to determine the total calcium sulphate dihydrate content of dried gypsum stones, even when contaminated by clay minerals, by determining the water of crystallisation from the absorption band with a maximum at the wavenumber 1685 cm^{-1} . The value for the water of crystallisation thus determined must be converted into calcium sulphate dihydrate.

The calculated value of the standard deviation is 0.1877 and the maximum permissible difference between parallel determinations of dihydrate water, R_{max} , is 0.52 per cent. *m/m*.

The total time taken to determine the calcium sulphate dihydrate content of one gypsum sample is approximately 3 to 4 hours, including drying the gypsum at 49 to 50 °C to constant mass. Of this time, work associated with the infrared spectroscopy takes 60 to 70 minutes.

The conventional A.S.T.M. analytical method for the determination of the hydrated calcium sulphate content of gypsum has an accuracy of ± 0.5 per cent. *m/m*, but the total analysis time is 2 days.¹⁰ Also, the determination of the calcium sulphate dihydrate content in gypsum contaminated with clay minerals is not accurate.

The author thanks Ing. Zdeněk Babák, of the Research Institute of Pure Chemicals of the National Enterprise Lachema in Brno, for assistance given in the measurement of the infrared absorption spectra of gypsum samples.

REFERENCES

1. Ksandr, Z., and Holzbecher, J., *Silikáty*, 1968, **12**, 177.
2. Vazquez, I., "Espectroscopia Infraroja de Algunos Compuestos de Interés en la Química del Cemento," Madrid, July, 1969.
3. Moenke, H., "Mineralspektren," Akademie-Verlag, Berlin, 1962.
4. Sartori, G., Furlani, C., and Damiani, A., *J. Inorg. Nucl. Chem.*, 1958, **8**, 119.
5. Hass, M., and Sutherland, G. B. B. M., *Proc. R. Soc., A*, 1956, **236**, 427.
6. Morris, R. J., jun., *Analyt. Chem.*, 1963, **35**, 1489.
7. Kössler, I., "Infrared Spectroscopy in Chemical Analysis," (in Czech), SNTL, Prague, 1960.
8. Eckschlager, K., "Graphical Methods in Analytical Chemistry," (in Czech), SNTL, Prague, 1966.
9. —, "Errors of Chemical Analyses," (in Czech), SNTL, Prague, 1961.
10. A.S.T.M. Standard C22-50, "Gypsum," American Society for Testing and Materials, Philadelphia, Pa.

Received January 21st, 1972

Accepted May 31st, 1972

Determination of Boron in Plants by Emission Spectroscopy with the Nitrous Oxide - Hydrogen Flame

BY J. C.-M. PAU, E. E. PICKETT AND S. R. KOIRTYOHANN

(Departments of Chemistry and Agricultural Chemistry, University of Missouri, Columbia, Missouri 65201, U.S.A.)

Boron is readily determined in plant ash solutions, following extraction into chloroform of the chelate formed with 2-ethylhexane-1,3-diol, by means of the nitrous oxide - hydrogen flame and conventional flame photometers. Boron in concentrations as low as 8 p.p.m. in the dried plant material can be determined with a relative standard deviation of about 4 per cent. The boron oxide (BO_x) band emission intensity at 518.2 or 547.6 nm is measured with wide slits. Recorder read-out and zero suppression are desirable. Results on several samples by this and four colorimetric methods are given. The large signal strengths obtained make this method superior to atomic-absorption techniques.

BORATED fertilisers can be conveniently analysed to determine boron by measurement of the emission intensity of the boron oxide (BO_x) green bands in the oxy-hydrogen¹ or air - hydrogen² flame. The success of the method depends on the chelation of boron at low pH with 2-ethylhexane-1,3-diol and extraction of the chelate into a suitable organic solvent so as to increase sensitivity and to separate the boron from interfering substances. The emission mode was shown to be superior to atomic absorption for this purpose.²

The boron content of plants is of the order of one hundredth of that in borated fertiliser and attempts to use the above method for plant analysis were not promising. It was known that the boron oxide species is relatively stable in flames. Less than 1 per cent. of the boron is present in the atomic form even in the relatively hot nitrous oxide - acetylene flame.³ The rather cool air - hydrogen flame nevertheless was adequate for fertiliser analysis.

The nitrous oxide - hydrogen flame is almost as hot as the nitrous oxide - acetylene flame.^{3,4} By avoiding the use of solvents rich in carbon and hydrogen, the former flame might be expected to give less background than the latter (mostly C_2 and CN bands) in the region of boron oxide emission. Further work in our laboratory has shown that the high-temperature nitrous oxide - hydrogen flame, coupled with the highly selective 1,3-diol extraction with chloroform as a solvent, provides a useful means of determining boron in plants, provided that a rather large sample (2 to 5 g) can be taken. The method has been compared with several other commonly used methods.

EXPERIMENTAL

APPARATUS—

As in the work on fertilisers,² the Jarrell-Ash, 0.5-m Ebert spectrometer (No. 82-000) was used with a photomultiplier and power supply, a Keithley, No. 620, d.c. amplifier and a recorder. A lens was used to form an image of the flame on the slit. Slits were 250 μm wide, giving a 0.4-nm band pass, and 6 mm high. Even wider slits, up to 5-nm band pass, can be used to advantage. The portion of the flame just above the primary reaction zone was viewed, and the time constant was made to be about 1 s by connecting a 60- μF capacitor across the amplifier output. A Perkin-Elmer nebuliser - burner assembly was used with a single-slot nitrous oxide - acetylene burner head (50 \times 0.5-mm slot) set parallel to the optical axis so as to give a maximum emission intensity.⁵ Gas flow-rates were set at 12 l min^{-1} of nitrous oxide and 3.9 l min^{-1} of hydrogen. The pressure of nitrous oxide at the nebuliser must be about 30 lb inch^{-2} . Unlike the nitrous oxide - acetylene flame, there is very little risk of flash-back or explosion with the nitrous oxide - hydrogen flame⁴ and it is unnecessary to use air in lighting or extinguishing it.

The BO_x maxima at 518.2 and 547.6 nm are about equally useful, with a slightly greater sensitivity given by the latter. There is some likelihood of interference from mercury 546.1-nm radiation from fluorescent room lights at the 547.6-nm peak, however, and this

must be evaluated for any given laboratory set-up. The second-order spectrum of the hydroxyl radical approximately doubled the background intensity beneath both of these BO_x peaks. A piece of ordinary glass was inserted in the optical path to remove this interference.

REAGENTS—

Extraction solution—Prepare a 10 per cent. solution of 2-ethylhexane-1,3-diol in chloroform (commercial chloroform and 2-ethylhexane-1,3-diol were used without purification).

Boron stock standard solution, 1000 p.p.m.—Dissolve 2.860 g of boric acid, H_3BO_3 , in 500 ml of water.

PROCEDURE—

Weigh 2 to 5 g of ground plant material into a porcelain crucible, place the crucible in a cool muffle furnace and ash the contents by raising the temperature to 500 °C overnight. Remove the crucible from the furnace, cool it, and dissolve the ash in 10 ml of water plus 5 ml of 6 N hydrochloric acid. If undissolved matter remains, separate it by centrifuging, wash the deposit and combine the solution and washings. Transfer the solution to a plastics separating funnel and make the volume up to about 25 ml. Extract it twice with 5 ml of extraction solution and combine the two extracts. Prepare standards containing 0, 5, 10, 15, 20 and 25 $\mu\text{g ml}^{-1}$ of boron by carrying suitable stock standard aliquots, with added water and 6 N hydrochloric acid, through the extraction procedure. As the extracts are stable for at least several days, standards can be made up in larger volumes.

Measure the BO_x band plus background emission intensity on a suitable flame spectrophotometer at either 518.2 or 547.6 nm with very broad slits (up to 5.0-nm band pass), preferably by using recorder read-out. A pre-mixed flame formed at a 50×0.5 -mm slot with flows of 12 l min^{-1} of nitrous oxide and 3.9 l min^{-1} of hydrogen is recommended. Compare readings against a zero standard, that is, the extraction solution after being shaken with water and 6 N hydrochloric acid. The solution gives an appreciable background in addition to the flame background (Fig. 1). For each reading, record the signal while nebulising the zero standard, then record the sample or standard signal, and the zero standard signal again. The BO_x signal is to be measured above this average total background signal (Fig. 1, b). Set the signal for the 25 p.p.m. of boron standard, or other suitable concentration, near full scale. If zero suppression is available, suppress the background (zero standard) reading nearly to zero on the chart. The background due to flame, solvent and the 1,3-diol together is about 70 per cent. of the total reading for a $20 \mu\text{g ml}^{-1}$ of boron standard. The background signal should be stable, with fluctuations and root mean square noise of less than 2 per cent. of the BO_x signal (time constant about 1 s).

In order to obtain the best possible precision one must make frequent measurements of standards, preferably following each sample reading with a reading of the standard that has the most nearly equal analyte content. In this way the unavoidable, rapid changes in over-all response, which are generally small but common with flame instruments, can be taken into account.

COMPARISON METHODS—

(i) *Curcumin*—The colorimetric method of Dible, Berger and Truog⁶ was used with little modification.

(ii) *Quinalizarin*—The method of MacDougall and Biggs,^{7,8} which is the current official A.O.A.C. method,⁹ was used. Lime water was not added before ashing.

(iii) *Azomethine-H following extraction with the 1,3-diol*—The general method of Hofer, Brosche and Heidinger¹⁰ was used with slight changes, chiefly that required to adjust the sample size to the sensitivity of the method.

(iv) *Methylene blue fluoroborate*—The method of Weir¹¹ for determining boron in plant tissue was used without change.

RESULTS AND DISCUSSION

FLAME PROPERTIES—

The nitrous oxide - hydrogen flame is only about 150 °C cooler than the nitrous oxide - acetylene flame but it forms much more poorly free atoms of elements that have pronounced oxide-forming tendencies in flames.³ Its high temperature excites the thermally stable BO_x

bands efficiently and its background intensity is unfortunately not as low as that of the air - hydrogen flame because of the rather strong emission from "ammonia α -bands," which are actually due to the NH_2 radical.^{4,12} Thus, although the BO_x emission in nitrous oxide - hydrogen is about forty times stronger than in air - hydrogen, the BO_x to background ratio is only twice as great. However, the chloroform solvent contributes only about 20 per cent. to the total nitrous oxide - hydrogen background (Fig. 1). Unfortunately, chloroform is unsatisfactory for use in the air - hydrogen flame; the 4-methylpentan-2-one used in this flame contributed greatly to the total flame background.²

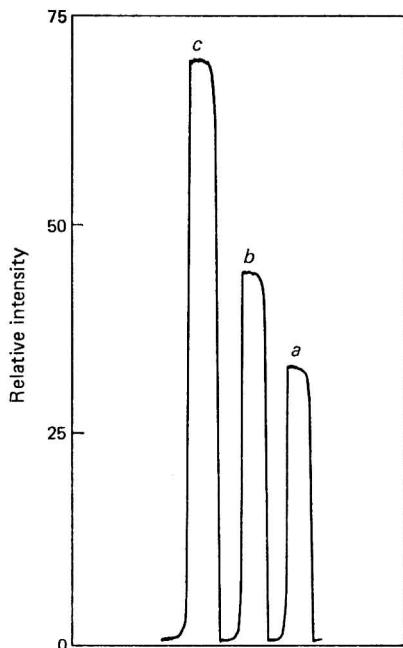


Fig. 1. Intensities of boron oxide, flame and background emission, without zero suppression: *a*, nitrous oxide - hydrogen flame only; *b*, nitrous oxide - hydrogen flame with extraction solution; and *c*, nitrous oxide - hydrogen flame with 20 p.p.m. of boron as the 1,3-diol chelate in extraction solution

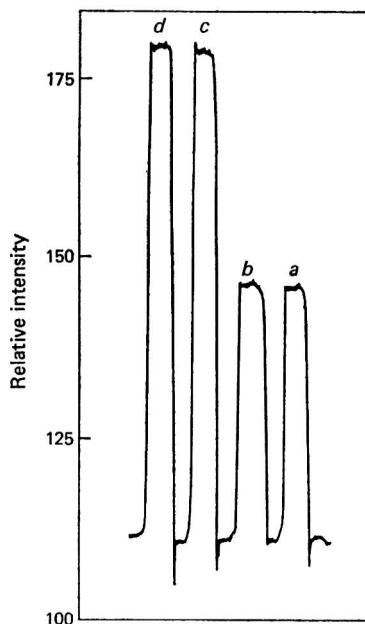


Fig. 2. Intensities of boron oxide emission at two concentrations, with zero suppression: *a* and *b*, signals from 10 p.p.m. of boron extracted as the 1,3-diol chelate in extraction solution. The base-line signal, given by the "zero standard" (extraction solution only), has been suppressed nearly full scale. Signals *c* and *d*, as *a* and *b* but for 20 p.p.m. of boron

The nitrous oxide - hydrogen flame, with chloroform used as solvent for the chelate extraction, appears to give the improved reproducibility that was needed to make the determination of boron in plants by flame emission practical. The magnitude and reproducibility of readings, with zero suppression, are demonstrated in Fig. 2. The duplicated signals for the 10 p.p.m. of boron standard solution (*a* and *b*) give about 4 inches of deflection above the zero standard signal. The latter signal was suppressed nearly full scale so that the zero of intensity is well beyond the edge of the recorder chart. Signals due to boron are measured above this zero standard signal base-line, which was adjusted in this instance to about 110 intensity units. (The recorder tracings were made with 40- μF capacitance across the amplifier output, giving a time constant of about 0.7 s.) The signals are large enough to allow the use of low amplifier gains and photomultiplier potentials. With 1-mm slits (1.6-nm band pass), the 1 p.p.m. of boron standard signal can be expanded to 1 inch or more with no increase in noise, the detection limit thus approaching 0.05 p.p.m.

The need to measure and subtract the background intensity is often cited as a disadvantage in flame-emission work. Actually, if the background intensity is constant, as it is in this procedure, background measurement is only slightly more difficult (by using the zero standard) than the measurement of "incident" intensity, that is, the transmitted intensity of the flame without sample atoms, in atomic-absorption spectroscopy.

Without the use of a solvent, the nitrous oxide - hydrogen flame has a pale pink - yellow (peach) colour throughout, with a somewhat more intense, but very narrow, thread-like zone between primary and secondary reaction zones. When chloroform is aspirated, the primary zone expands and becomes brighter and pink, the interzonal thread becomes blue and the plume remains peach coloured. Solvents rich in C-H groups give bluer colours. The green BO_x emission shows a gradual decrease in intensity on ascending vertically above the thread but so does background intensity, so that vertical location of measurement of intensity above the primary zone is not critical.

PRECISION OF THE METHOD—

Large amounts of two samples, one of lucerne and the other of maize stover, were ashed and the ash was brought into solution by the procedure described above. Ten aliquots were taken for analysis, with results given in Table I. Ten 5-g portions of two other samples were ashed separately and carried through the procedure, with results given in Table II. There is no significant difference between the two sets of errors and they are not large.

TABLE I
REPETITIVE EXTRACTIONS

Sample*	Boron/ μg per gram of sample	Relative standard deviation, per cent.
Lucerne	37.4	2.2
Maize	4.65	6.8

* Boron was determined in ten aliquots of a solution of each sample.

Presumably no significant losses or gains of boron occurred during ashing. No base or other fixative was used. Addition of lime water⁹ may be desirable in ashing certain kinds of samples. The dishes were not covered although, in some furnaces, it may be desirable to cover them so as to prevent contamination with boron.¹³

TABLE II
REPETITIVE ASHINGS

Sample*	Boron/ μg per gram of sample	Relative standard deviation, per cent.
Lucerne	33.4	2.1
Maize	8.5	5.9

* Boron was determined in ten separate ashings of each sample (not the same samples as in Table I).

COMPARISON WITH OTHER METHODS—

Initial comparisons of analyses by this method with those by certain other methods on plant samples were rather disappointing. Of the very many methods proposed for colorimetric determination of boron, it became important to make comparative analyses with one or, preferably, several that are commonly considered to be reliable for plant analysis. It would also be desirable to be able to obtain favourable comparisons among several methods that depend on the use of distinctly different chemical reactions.

Most colorimetric methods for determining boron might be classified among four types, primarily according to the following reaction conditions:

- (i) Chelation under anhydrous conditions, by evaporation of water.
- (ii) Chelation under nearly anhydrous conditions, in concentrated sulphuric acid.
- (iii) Chelation in aqueous solution without strong mineral acid.
- (iv) Ion-pair complex formation between fluoroborate anions and a cationic dye.

The four methods chosen [comparison methods (i), (ii), (iii) and (iv)] correspond to these four types. Some colour-forming substances can be used in at least two of the first three ways. The coloured boron-containing product may or may not require extraction

with an organic solvent. Other methods could just as well have been chosen. All but method (iii) are commonly used for plant analysis, however.

The results of the comparison for several plant samples are presented in Table III. Samples were ashed singly and analysed by each method on five successive days.

TABLE III
COMPARISON OF RESULTS (p.p.m.) FOR THE DETERMINATION OF BORON
WITH FLAME AND COLORIMETRIC METHODS

Standard deviations are given after each value and percentage standard deviations in parentheses

	Nitrous oxide - hydrogen flame	Curcumin*	Quinalizarin†	Azomethine-H‡	Methylene blue§
Lucerne 1	21.0 ± 0.67 (3.2 per cent.)	21.5 ± 0.84 (3.9 per cent.)	21.6 ± 1.55 (2.5 per cent.)	15.2	20.2 ± 1.95 (9.6 per cent.)
Lucerne 2	42.6 ± 1.39 (3.0 per cent.)	42.5 ± 1.75 (4.1 per cent.)	42.3 ± 1.94 (4.6 per cent.)	40.5	41.8 ± 3.2 (7.6 per cent.)
Maize	8.35 ± 0.22 (2.6 per cent.)	8.08 ± 0.49 (6.1 per cent.)	8.44 ± 0.20 (2.5 per cent.)	8.0	8.93 ± 0.80 (9.0 per cent.)

* Method of reference 6.

† Method of reference 7.

‡ Method of reference 10.

§ Method of reference 11.

|| Only one run was made on each sample by this method. By all other methods, the samples were ashed and analysed on five different days.

Except for the azomethine-H method, the results show no significant bias and the flame method can be said to be about as accurate as the three commoner colorimetric methods.

The azomethine-H method of Hofer, Brosche and Heidinger¹⁰ is the most recent of the colorimetric methods. In earlier efforts to use this reagent, the method of Basson, Böhmer and Stanton,¹⁴ in which the preliminary separation by extraction with 2-ethylhexane-1,3-diol was not used, was found to give results that were much too high. The method of Hofer *et al.* used here gave results that were significantly lower than those given by the other methods.

Percentage standard deviations are given for each method, but comparisons among them are of doubtful value. Attempts were made to gain enough experience with each method so that standard deviations in our results would eventually become about as small as those given for the methods when published, or at least would become no smaller with continued use of the methods. This aim was only partially successful. The results with methylene blue remained about one half as precise as those presented by Weir.¹¹ The results with curcumin were consistent with those obtained during our long-term experience with this reagent; the original paper⁶ does not give adequate estimates for precision. For the quinalizarin method, the estimate for precision calculated from the inter-laboratory A.O.A.C. trial (12.6 per cent. average⁸) was easily improved upon, as might be expected of any one laboratory. No other results for precision were found for this method. Perhaps all that should be said is that the flame method is about as precise as the common colorimetric methods.

The flame method was further compared with the curcumin method on a group of twenty samples of maize with rather low concentrations of boron, and the results are given in Table IV. The average difference was 4.38 per cent. and the average bias was -0.87 per cent.; the flame values were higher on average but not to a significant extent.

The average percentage standard deviation in the results of the atomic-absorption method of Melton, Hoover and Howard¹⁵ appears to be about 11.5 per cent., which probably results chiefly from the relatively low atomic-absorption signal afforded by boron, even in the nitrous oxide - acetylene flame. The much larger BO_x flame-emission signals obtained with wide slits can be measured at low electronic gain.

The use of chloroform in the present method gives a more efficient extraction of the boron - 1,3-diol complex than 4-methylpentan-2-one² (about 97 per cent.) and is more convenient to handle in the extraction process.

TABLE IV
ANALYSIS OF MAIZE VEGETATION BY FLAME AND CURCUMIN METHODS

Sample*	Boron, p.p.m.		Difference, per cent.†
	Curcumin method	Flame method	
1	7.90	7.90	0.00
2	9.18	10.0	-8.55
3	8.29	9.40	-12.52
4	8.40	9.10	-8.00
5	8.10	8.25	-1.83
6	5.71	5.75	-0.70
7	6.18	6.50	-5.05
8	5.20	5.00	3.92
9	5.05	5.30	-4.83
10	7.65	8.00	-4.47
11	5.73	5.50	4.09
12	4.63	4.50	2.84
13	13.0	13.0	0.00
14	13.0	13.3	-2.28
15	13.0	12.0	8.00
16	11.4	11.0	3.57
17	11.5	12.0	-4.25
18	20.0	19.8	1.00
19	16.3	14.8	9.62
20	15.0	14.7	2.02

* The first twelve samples are maize stover (leaves plus stalks); the last eight are maize ear-leaves.

† Average difference is 4.38 per cent. The average bias is -0.87 per cent.

Interferences have been found to be nearly non-existent.² The interference of fluoride with the formation of the 1,3-diol chelate and its extraction has been re-examined. At 10 p.p.m. of boron in the aqueous phase, 50 p.p.m. of fluoride still permit complete extraction of the complex, while 100 p.p.m. of fluoride suppress extraction by about 6 per cent. and 250 p.p.m. suppress it by about 45 per cent. A ten-fold excess of fluoride over boron is seldom encountered in plants.¹⁶ Agazzi¹ showed that interference at high levels of fluoride could be prevented by addition of zirconium nitrate and heating the mixture briefly. We have confirmed his results.

The proposed flame method has been found to be faster and easier to perform than the colorimetric methods with which it was compared.

REFERENCES

1. Agazzi, E. J., *Analyt. Chem.*, 1967, **39**, 233.
2. Pickett, E. E., Pau, J. C.-M., and Koirtyohann, S. R., *J. Ass. Off. Analyt. Chem.*, 1971, **54**, 796.
3. De Galan, L., and Samaey, G. F., *Spectrochim. Acta*, 1970, **25B**, 245.
4. Willis, J. B., Fassel, V. A., and Fiorino, J. A., *Ibid.*, 1969, **24B**, 157.
5. Koirtyohann, S. R., and Pickett, E. E., *Appl. Spectrosc.*, 1969, **23**, 597.
6. Dible, W. T., Berger, K. C., and Truog, E., *Analyt. Chem.*, 1954, **26**, 418.
7. MacDougall, D., and Biggs, D. A., *Ibid.*, 1952, **24**, 556.
8. Brown, R. S., *J. Ass. Off. Agric. Chem.*, 1958, **41**, 304.
9. "Official Methods of Analysis of the Association of Official Analytical Chemists," Eleventh Edition, Association of Official Analytical Chemists, Washington, D.C., 1970, p. 48.
10. Hofer, A., Brosche, E., and Heidinger, R., *Z. analyt. Chem.*, 1971, **253**, 117.
11. Weir, C. C., *J. Sci. Fd Agric.*, 1970, **21**, 545.
12. Pearse, R. W. B., and Gaydon, A. G., "The Identification of Molecular Spectra," Third Edition, John Wiley & Sons Inc., New York, 1963, p. 223.
13. Williams, D. E., and Vlamis, J., *Analyt. Chem.*, 1961, **33**, 967.
14. Basson, W. D., Böhmer, R. G., and Stanton, D. A., *Analyst*, 1969, **94**, 1135.
15. Melton, J. R., Hoover, W. L., and Howard, P. A., *J. Ass. Off. Analyt. Chem.*, 1970, **53**, 682.
16. Brewer, R. F., in Chapman, H. D., Editor, "Diagnostic Criteria for Plants and Soils," University of California, Riverside, 1965, Chapter 12.

Received October 11th, 1971
Accepted March 17th, 1972

The Assay of Non-fat Milk Solids by the Determination of Orotic Acid in Milk Chocolate and in the Milk

By R. J. MOTZ

(Mars Limited, Confectionery Division, Dundee Road, Slough, Bucks.)

When the orotic acid content of the milk that is used in the manufacture of chocolate is known, non-fat milk solids can be determined with satisfactory accuracy by the described method. The method is equally valid for chocolate products that contain milk protein that has been subjected to high-temperature heat treatment and consequently cannot be analysed satisfactorily by the method of the Association of Official Analytical Chemists.

Natural variations in the orotic acid content of milk of various origins are too great to permit the determination of milk solids, for arbitration purposes, in milk chocolate.

It has been shown¹ that the method of the Association of Official Analytical Chemists is unsatisfactory for characterising accurately the milk content of chocolate that has been produced by a crumb process involving heating or which contains milk protein that has been exposed to heat, such as that applied during the manufacture of milk powder.

In the presence of moisture, milk proteins and lactose will undergo a Maillard reaction, thus making parts of them unavailable for analysis. In addition, the proteins will be denatured to an extent that depends upon the degree of heating, thus reducing their recovery further. This effect makes the method equally unsuitable for production control within chocolate factories.

In the search for a more suitable method, undertaken on behalf of the Technical Committee of the Office International du Cacao et du Chocolat, the possibility was examined of basing a determination on the orotic acid (uracil-4-carboxylic acid) content of milk. Unlike lactose and milk protein in chocolate crumb, orotic acid remains unchanged by heating.

The method for the determination of orotic acid, as described by Wallrauch^{2,3} and based on that by Tsuji,⁴ was developed further for specific application to chocolate and chocolate crumb and, in particular, with a view to simplifying the procedure.

A survey was made of the orotic acid contents of a number of British and foreign commercial milk powders. The effects of variations in experimental conditions (ruggedness test), the interference of chocolate constituents and the influence of the high-temperature treatment of milk solids on the recovery of orotic acid were investigated.

Owing to the natural variation in the orotic acid content of milk (see Table III), the proposed method can be used for the accurate determination of milk solids in chocolate only when the orotic acid content of the milk used is known.

EXPERIMENTAL

PRINCIPLE—

When saturated bromine water is added to orotic acid, dibromobarbituric acid is formed. The excess of bromine is removed by the addition of ascorbic acid and, when *p*-dimethylaminobenzaldehyde in pentanol is added, a yellow dye is produced by a coupling reaction. This dye is extracted with butyl acetate and its extinction is measured at 461 nm with a spectrophotometer. An Optica CF spectrophotometer was used in this work.

REAGENTS—

Orotic acid, for calibration graph.

Saturated bromine water—Shake bromine with water, retaining excess of bromine in the reagent bottle. Use the saturated aqueous solution, and dispense it from a burette.

Ascorbic acid, approximately 10 per cent. solution in water.

p-Dimethylaminobenzaldehyde, analytical-reagent grade, 3 per cent. solution in propanol.

Butyl acetate.

Sodium sulphate, anhydrous.

Light petroleum, boiling range 40 to 60 °C.

Carrez reagent I—Dissolve 22 g of crystalline zinc acetate and 3 ml of glacial acetic acid in water and make the volume up to 100 ml.

Carrez reagent II—Dissolve 11 g of potassium hexacyanoferrate(II) in water and make the volume up to 100 ml.

APPARATUS—

Separating funnels, capacity approximately 50 ml (or glass-stoppered test-tubes, see Note on alternative procedure on p. 868).

Water-bath, thermostatically controlled at 40 ± 0.5 °C.

Stop-clock.

Pipettes, 2 ml, 3 ml and usual sizes.

Visible-light spectrophotometer.

PROCEDURE—

Construction of calibration graph—Prepare a “strong” standard solution, containing 50 mg l^{-1} of orotic acid, *i.e.*, of $50 \text{ } \mu\text{g ml}^{-1}$ concentration. For the construction of a calibration graph, prepare dilute solutions containing 1 to $6 \text{ } \mu\text{g ml}^{-1}$ of orotic acid.

Transfer 5 ml of each solution by pipette into a separating funnel (Note). Add, from a burette, 1.5 ml of saturated bromine water to the first separator and start the stop-clock. After 30 ± 2 s, add 2 ml of ascorbic acid solution from a pipette. Continue in the same way with the additions of the reagents to the other separators. Stopper the separators and transfer them to the water-bath, which is maintained at 40 °C. (It was found useful to suspend the separators in the water-bath by means of wire hooks attached to their necks.) After about 10 minutes, when the temperature of the contents of the separators has reached that of the water-bath, start the stop-clock and run 3 ml of *p*-dimethylaminobenzaldehyde reagent into the first separator from a pipette and mix. Make similar additions to the other separators at 1-minute intervals. As the blank, use one of the standard solutions, but add 3 ml of propanol instead of 3 ml of *p*-dimethylaminobenzaldehyde reagent.

Ten minutes (± 30 s) after having made the addition to the first separator, remove the separator from the water-bath and suspend it in a beaker containing cold tap-water. Cool the remaining separators in a similar manner at intervals of 1 minute.

When the contents of the separators have reached room temperature, remove the separators from the cold water, add exactly 10.0 ml of butyl acetate from a pipette to each and shake them for 5 s. Allow the phases to separate, which takes only 1 to 2 minutes, and run the bottom layer to waste (Note). Stopper each separator and, while holding it in a horizontal position, turn it slowly around its axis, which movement should dislodge most of the adhering drops of water from the wall. Allow a few minutes for further separation to occur, then discard the bottom layer. Place a small funnel with a short stem in the neck of each separator and add approximately 0.5 g of anhydrous sodium sulphate. It may be necessary to shake the separators at this stage.

Repeat the addition of about the same amount of sodium sulphate after a few minutes. The solution should now be absolutely clear and can be decanted, through the neck, into a small test-tube. Measure the solution spectrophotometrically in a 10-mm cell at 461 nm. Construct the calibration graph by plotting optical density against the amount of orotic acid in micrograms in a 5-ml sample. The graph should be a straight line that passes through the origin.

It is convenient to express the ratio of the amount of orotic acid in micrograms in a 5-ml sample to the optical density, as read off from the graph, as a numerical factor. When the optical density obtained with samples is multiplied by this factor, the amount of orotic acid in micrograms in a 5-ml sample can be calculated without further reference to the calibration graph.

Determination of orotic acid in milk chocolate—Weigh the chocolate into a centrifuge bottle or tube, warm it in a water-bath to facilitate dissolution, add light petroleum and break up lumps of chocolate with a glass rod. De-fat the chocolate by centrifuging and decant the clear solution. Dry the residue in the bottle by means of a current of air and transfer it, with hot water, into a calibrated flask (*e.g.*, use a 200-ml flask for a 5-g chocolate sample).

Add sufficient water to fill about 80 per cent. of the volume of the flask. Place a thermometer in the flask, immerse the flask in a boiling water bath, heat the contents of the flask to a temperature of 98 °C or above and continue to heat for at least 15 minutes. Then add Carrez I reagent from a pipette (corresponding to 3 ml for a 200-ml flask) and the same amount of Carrez II reagent. Cool the flask to room temperature with running tap-water before making it up to volume. Filter a portion of the mixture into a dry flask through a fast-filtering paper (*e.g.*, Whatman No. 114). Use 5.0 ml of the filtrate for the test and for the blank, to which, however, propanol is added instead of *p*-dimethylaminobenzaldehyde reagent. Continue the procedure as for the construction of the calibration graph.

NOTE—An alternative procedure is to use glass-stoppered test-tubes of more than 25-ml capacity instead of separators. With this procedure, the aqueous bottom layer is removed by means of a bulb pipette. Small losses of the coloured upper phase have no influence on the determination as only about 3 ml are needed for measurement in a 10-mm cell.

TABLE I

RECOVERY OF OROTIC ACID ADDED TO A SOLUTION OF FULL CREAM MILK POWDER

Orotic acid per 5 ml of solution			Recovery, per cent.
Present/ μ g	Added/ μ g	Found/ μ g	
14.0	10.0	23.6	94
55.4	10.0	65.2	98

Determination of orotic acid in milk powder—Weigh accurately about 1.5 g of milk powder into a beaker, cover the sample with cold distilled water and immediately stir the mixture with a small glass rod. Allow it to stand for a few minutes before transferring it to a 200 or 250-ml calibrated flask by using hot water. Add sufficient water to fill about 80 per cent. of the volume of the flask, heat the flask in a water-bath and continue the procedure as for the analysis of milk chocolate.

Results for the recovery of orotic acid added to a 1.69 per cent. *m/V* solution of full cream milk powder are given in Table I.

Determination of orotic acid in crumb—Grind crumb very finely, if possible by means of a coffee grinder or another mill of similar efficiency. If the grinding is carried out by hand with a mortar and pestle, pass the ground material through a sieve (30-mesh B.S.S. or 500 μ m). Re-grind the particles retained by the sieve and repeat the sieving. (This operation is necessary so as to obtain sufficiently small particles, thus permitting the complete extraction of orotic acid.) Weigh accurately about 2.5 g of crumb into a beaker and continue the procedure as for the analysis of milk powder.

TABLE II

OPTICAL DENSITIES OBTAINED ON OROTIC ACID SOLUTIONS BY VARYING THREE EXPERIMENTAL PARAMETERS

Parameter varied	Parameters kept constant		
	$T = 40\text{ }^{\circ}\text{C};$ $t(\text{DAB}) = 10\text{ minutes}$	$T = 40\text{ }^{\circ}\text{C};$ $t(\text{Br}) = 30\text{ s}$	$t(\text{Br}) = 30\text{ s};$ $t(\text{DAB}) = 10\text{ minutes}$
$t(\text{Br})/\text{s}$ —			
15	0.598		
30	0.615		
45	0.614		
60	0.606		
$t(\text{DAB})/\text{minutes}$ —			
5		0.614	
10		0.621	
15		0.642	
$T/^{\circ}\text{C}$ —			
35			0.590
40			0.612
45			0.626

RUGGEDNESS TEST—

The effects of the variation of three experimental parameters on the optical density obtained with orotic acid solutions were examined. The parameters examined were the reaction time with bromine water, $t(\text{Br})$, the reaction time with *p*-dimethylaminobenzaldehyde reagent, $t(\text{DAB})$, and the temperature of the water-bath, T . The results are given in Table II.

STATISTICAL DEVIATIONS OF REPRODUCIBILITY—

A series of ten solutions was prepared from one full cream milk powder and the orotic acid content of each solution was determined separately by the proposed method. The replicateresults were 453.3, 456.0, 457.5, 460.3, 459.9, 459.6, 458.2, 460.6, 453.5 and 458.2 p.p.m. The mean value was 457.7 p.p.m. and the standard deviation was 2.7 p.p.m.

STABILITY OF COLOUR—

The colour intensity of the ten solutions used above decreased by an average of 8.8 per cent. after 44 hours.

OROTIC ACID CONTENT OF COMMERCIAL SAMPLES OF FULL CREAM MILK POWDER—

Results on samples from Britain and six foreign countries are given in Table III.

TABLE III

OROTIC ACID CONTENT FOUND IN COMMERCIAL SAMPLES OF FULL CREAM MILK POWDER

Sample	Orotic acid content, p.p.m.	Sample	Orotic acid content, p.p.m.
Austrian	507	British	395
	553		510
			503
Danish	520		605
			458
German	437		533
	541		514
	384		533
			517
Swedish	577		514
			495
Swiss	578		481
			494
Belgian	515		470
			585

EFFECT OF CHOCOLATE CONSTITUENTS ON THE DETERMINATION OF OROTIC ACID IN FULL CREAM MILK POWDER—

The colour produced by orotic acid, present in a 1.69 per cent. m/V solution of full cream milk powder gave an optical density of 0.379. The colour produced by orotic acid present in milk chocolate, in a solution containing 1.69 per cent. m/V of full cream milk powder, gave an optical density of 0.379.

The milk chocolate used in this experiment had the following composition: 33.8 per cent. of full cream milk powder, 29.3 per cent. of sugar, 22.5 per cent. of cocoa mass, 13.5 per cent. of added cocoa butter and 0.9 per cent. of emulsifier. As the full cream milk powder contained 494.8 p.p.m. of orotic acid, the expected orotic acid content of the chocolate was 167.7 p.p.m., while 163.3 p.p.m. was actually found.

TABLE IV

INFLUENCE OF THE TOTAL VOLUME, IN WHICH PROTEINS ARE PRECIPITATED, ON THE RECOVERY OF OROTIC ACID FROM A SOLUTION OF SKIM MILK POWDER

Sample No.	Volume/ml	Orotic acid, p.p.m.
1	75	626.3
2	115	603.8
3	155	625.5
4	185	616.5
Mean		618.0

TABLE V

RESULTS FOR OROTIC ACID, LACTOSE, A.O.A.C.-NITROGEN AND TOTAL NITROGEN IN FULL CREAM MILK POWDER BEFORE AND AFTER HEATING IN THE PRESENCE OF MOISTURE AT ABOUT 140 °C AND ATMOSPHERIC PRESSURE UNTIL BROWNING OCCURRED (ABOUT 1½ HOURS)

	Orotic acid, p.p.m.		Lactose, per cent.		A.O.A.C.-nitrogen, per cent.*		Total nitrogen, per cent.	
	On actual samples	On dry samples	On actual samples	On dry samples	On actual samples	On dry samples	On actual samples	On dry samples
Before heating	473	485†	37.0	37.9†	3.70	3.80†	4.02	4.12†
After heating	—	490	—	34.0	—	2.86	—	4.12
Loss	..	Nil	..	10.3	..	24.7	..	Nil

* Determined according to the A.O.A.C. method for milk protein.
 † Value calculated from the results on the actual sample, which contained 2.5 per cent. of moisture.

TABLE VI

COMPARISON BETWEEN THEORETICAL AND ACTUAL RESULTS FROM OVER-ROASTED CRUMB

Sample	Total nitrogen, per cent.		A.O.A.C.-nitrogen, per cent.		Factor, A/B		Lactose, per cent.		Sucrose, per cent.		Orotic acid, p.p.m.	
	Theoretical	Found	Theoretical (A)	Found (B)	Theoretical	Found	Theoretical	Found	Theoretical	Found	Theoretical	Found
Full cream milk powder	—	3.94	—	3.64	1.082	37.2	—	—	—	—	—	509.0
Skim milk powder	..	5.32	..	4.93	1.079	49.2	727.9
Dried crumb paste	..	1.80	..	1.49	1.072	15.0	..	48.0	49.3	214.1	217.7	217.7
Roast crumb	..	0.20†	..	0.07	—	11.4	..	48.0	42.8	214.1	212.9	212.9
						(6.5, invert sugar)						

* From milk.
 † From mass.

RECOVERY OF OROTIC ACID FROM A SOLUTION OF SKIM MILK POWDER—

The influence of the total volume, in which proteins are precipitated, on the recovery of orotic acid from a solution of skim milk powder is shown in Table IV.

INFLUENCE OF DARK CHOCOLATE ON THE RECOVERY OF OROTIC ACID FROM SKIM MILK POWDER—

A sample of 3.055 g of skim milk powder (the same material as was used in the previous experiment) was analysed to determine orotic acid in the presence of 4.0 g of dark chocolate. The powder containing the dark chocolate gave a similar value for the orotic acid content, 625.0 p.p.m., to that given by the skim milk powder alone.

INFLUENCE OF HEAT TREATMENT—

The unique advantage of orotic acid as an analytical measure of milk solids is that its recovery is not influenced by heat treatment of the milk proteins, as shown in Table V. No loss of either orotic acid or total nitrogen occurred, while losses of lactose and A.O.A.C.-nitrogen were of the expected magnitude.

OROTIC ACID AS A MEASURE OF FAT-FREE MILK SOLIDS IN ROASTED CRUMB—

The milk content of crumb and therefore of crumb chocolate cannot be determined reliably from determinations of protein or lactose. The figures for an over-roasted crumb demonstrate how unsatisfactory these methods are, while those for orotic acid remain unaffected.

Several analytical parameters were determined on two milk powders and a crumb that had been produced from them and intentionally over-roasted.

The crumb mixture was made in the laboratory by one of the usual procedures involving the preparation of a "crumb paste." One portion of the crumb paste was dried at room temperature in a vacuum. The analytical results obtained with the roasted crumb were compared with those with the dried crumb paste. This served as an independent control for the changes that had taken place in the material as a consequence of the roasting process.

The main bulk of the crumb paste was dried in several portions at atmospheric pressure in a heating cabinet at temperatures up to 135 °C (not all portions were, in practice, roasted to the same extent, and thus had different residual moistures). The finished product was finely ground and mixed until a homogeneous powder was obtained. The results are given in Table VI.

DISCUSSION

The proportion of the orotic acid present before roasting that was recovered was 97.8 per cent., corresponding to a recovery of 98.6 per cent. calculated on dry substances.

Less than 5 per cent. of the milk protein present in the dried crumb paste before roasting was accounted for by the A.O.A.C. method after roasting, although the total nitrogen content had not decreased.

About 25 per cent. of the lactose present in the dried crumb paste was not found again after roasting, probably because it had taken part in a Maillard reaction.

The much extended drying period caused some inversion of the sucrose, which does not normally occur.

CONCLUSIONS

The method described permits the amount of orotic acid found in a milk chocolate to be used as a measure of the proportion of milk in the chocolate when the proportion of orotic acid in the milk has been determined by the same method. As orotic acid occurs only in the non-fat milk solids and as the use of additional cow butter is permissible in the making of chocolate, the results obtained must be interpreted accordingly.

REFERENCES

1. Motz, R. J., *Analyst*, 1968, **93**, 116.
2. Wallrauch, S., "The Determination of Orotic Acid for the Detection of Added Milk in Foodstuffs," Dissertation, Würzburg University, 1967.
3. Brieskorn, C. H., and Wallrauch, S., *Z. Lebensmittelunters. u. -Forsch.*, 1968, **138**, 154.
4. Tsuji, K., *J. Pharm. Soc. Japan*, 1961, **81**, 1655.

Received April 27th, 1971
Amended March 2nd, 1972
Accepted July 6th, 1972

Analysis of Fluoroborate Alloy Plating Solutions to Determine Iron, Cobalt and Nickel

BY T. KEILY AND R. C. WOODFORD

(Medway and Maidstone College of Technology, Chatham, Kent)

A rapid method for the determination of iron, cobalt and nickel in fluoroborate solutions has been devised and tested. It involves an initial dichromate titration to determine both iron(II) and iron(III) followed by the simultaneous spectrophotometric determination of cobalt and nickel at wavelengths 394 and 512 nm after reduction of all the iron to the iron(II) state.

The recommended procedure, which can be completed in less than 1 hour, has been successfully applied to typical plating solutions.

DURING operation of an alloy plating bath containing iron, cobalt and nickel fluoroborates, the pH of the solution is controlled between 3 and 4 and the concentrations of total iron, cobalt and nickel are 6, 10 and 12 g l⁻¹, respectively. It was expected that no individual metal concentration would at any time exceed 20 g l⁻¹. Frequent analyses of the electrolyte are necessary for the correct operation of the plating bath.¹ A rapid method was therefore required for the determination of these metals present in the plating bath.

Previous analytical methods used involved three separate determinations: iron(II) and iron(III) were determined titrimetrically, cobalt colorimetrically and nickel gravimetrically. These methods are time consuming and co-precipitation errors occurred in the gravimetric determination of nickel.²

Methods involving the separation of the individual metal ions, *e.g.*, by ion exchange,³⁻⁵ were too lengthy and this approach was rejected. Simultaneous titration methods are available,⁶⁻⁸ but, in general, require the use of metal-ion concentrations that are too low to enable the present problem to be solved. Also, the presence of large amounts of iron gives rise to inaccurate results for cobalt and nickel by titration with EDTA solution. Cobalt and nickel can also be determined spectrophotometrically after forming their EDTA complexes⁹ at pH 4.6 and a similar method is available for determining iron,¹⁰ but preliminary measurements showed that the pH values of the solutions required to be used in the two methods were not compatible and that the dilution needed for the iron was many times greater than those required for the cobalt and nickel. The presence of iron at a concentration comparable with those of the cobalt and nickel also caused considerable interference. Other spectrophotometric methods available for the simultaneous determination of cobalt and nickel¹¹⁻¹³ are either too sensitive or subject to similar interference from iron.

Several examples occur in which cobalt and nickel have been determined directly on the sample solutions by spectrophotometry. Electroplating solutions,¹⁴ soap solutions¹⁵ and salt solutions¹⁶ have been studied. Initial investigation showed that direct measurements might be successful in the present instance.

EXPERIMENTAL

REAGENTS—

None of the fluoroborates were available commercially and were therefore prepared by adding stoichiometric amounts of the appropriate metal, oxide or basic carbonate to 42 per cent. *m/V* fluoroboric acid solution, the proportions used being indicated below. The iron solution was stirred for 12 hours at about 40 °C and all solutions were filtered before being transferred to closed containers. The nickel solution was boiled to remove carbon dioxide before storage.

Iron(II) fluoroborate solution, 150 g l⁻¹ of iron—Dissolve 1 g of iron powder in 7.5 ml of fluoroboric acid solution.

Cobalt fluoroborate solution, 140 g l⁻¹ of cobalt—Dissolve 1 g of cobalt(II) oxide in 5.6 ml of fluoroboric acid solution.

Nickel fluoroborate solution, 140 g l⁻¹ of nickel—Dissolve 1 g of basic nickel carbonate [NiCO₃·2Ni(OH)₂·4H₂O] in 7.0₅ ml of fluoroboric acid solution.

Analytical grade chemicals were used whenever possible.

Buffer solution, pH 10—Add 570 ml of concentrated ammonia solution to an aqueous solution containing 70 g of ammonium chloride and dilute the mixture to 1 litre.

Eriochrome black T indicator solution—Dissolve 0.2 g of the dye in 15 ml of triethanolamine plus 5 ml of ethanol.

EDTA solution, 0.1 M.

Magnesium sulphate solution, 0.1 M.

STANDARDISATION OF FLUOROBORATE SOLUTIONS—

The magnesium sulphate solution was standardised by titrating it against 20 ml of EDTA solution diluted to 200 ml, after adding 4 ml of buffer solution and 4 drops of indicator solution. The indicator changes from blue to red at the end-point.

The cobalt and nickel fluoroborate solutions were standardised separately after dilution, 10 ml of each solution being diluted to 250 ml. To 10 ml of the diluted solutions, 200 ml of water, 20 ml of standard EDTA solution, 4 ml of buffer solution and four drops of indicator solution were added in that order and the mixture was titrated with standardised magnesium sulphate solution.

The iron fluoroborate solution was standardised with 0.1 N dichromate reagent, with *N*-phenylanthranilic acid as indicator, and its iron(III) content was determined by difference after passing the solution through a silver reductor column at the rate of 25 ml min⁻¹. Analyses over a period of 5 months showed no change in the iron(II) content of the stock solution.

SPECTROPHOTOMETRY—

Preliminary measurements with a Unicam SP800 recording spectrophotometer showed that a cobalt peak occurred at 512 nm and a nickel peak at 394 nm. The spectrum of solutions containing both nickel and cobalt was the sum of the spectra of the individual ions, and a small amount of overlap existed between the spectra of the two ions. The presence of iron(II) fluoroborate did not cause spectral overlap with the cobalt peak and only slight overlap occurred at the nickel peak; the absorption of iron(II) was very intense and occurred below 320 nm. These studies were carried out on synthetic solutions containing 6 to 40 g l⁻¹ of iron(II), 13 to 15 g l⁻¹ of cobalt and 8 to 19 g l⁻¹ of nickel and on bath electrolyte solutions the compositions of which approximated to those of mixtures of these metal ions with concentrations towards the lower end of the above ranges; 10-mm silica cells were used throughout with distilled water as reference.

EFFECT OF pH—

The pH of solutions containing iron, cobalt and nickel fluoroborates together was varied over the range 1 to 4 when using 1 N sulphuric acid or saturated borax solution. When sodium hydroxide was used local precipitation of iron(II) hydroxide occurred. Table I shows that changes in pH seriously affect the absorption of the iron at 394 nm but that the absorption of nickel and cobalt is virtually unaffected. Measurements were made with an SP500 spectrophotometer with 10-mm silica cells. Because of the overlap of the iron and nickel absorption spectra at 394 nm the pH of the sample solution must be fixed. The value chosen was 3.3, the normal pH of the bath electrolyte.

TABLE I
EFFECT OF pH ON THE ABSORBANCE OF IRON, COBALT AND NICKEL
FLUOROBORATE SOLUTION (10-MM CELL)

pH	Iron (30 g l ⁻¹)		Cobalt (7 g l ⁻¹)		Nickel (6 g l ⁻¹)	
	at 394 nm	at 512 nm	at 394 nm	at 512 nm	at 394 nm	at 512 nm
1.0	0.300	0.043	0.036	0.538	0.510	0.006
1.5	0.100	0.042	0.038	0.537	0.510	0.007
2.0	0.075	0.042	0.039	0.540	0.509	0.006
2.5	0.080	0.043	0.039	0.540	0.512	0.007
3.0	0.090	0.043	0.039	0.538	0.510	0.007
3.5	0.100	0.042	0.038	0.540	0.510	0.007
3.8	0.100	0.042	—	—	—	—
4.0	Precipitation		0.039	0.540	0.510	0.007

EFFECT OF THE STATE OF OXIDATION OF IRON—

Cobalt and nickel are almost invariably present in their bivalent states but iron may be present as iron(II) or iron(III). Solutions obtained by diluting the stock iron fluoroborate solution were studied with the iron in the oxidised and reduced condition, and as originally prepared. Oxidation of the iron was effected by adding an excess of 100-volume hydrogen peroxide solution and its reduction by heating with an excess of 5 per cent. hydrazinium sulphate solution. The absorbances of various solutions treated as above are shown in Table II with reference to distilled water. All solutions were adjusted to pH 3.3. The results show that iron(II) ions cause less interference than iron(III) ions at 394 nm and only with iron(II) at a concentration of up to 15 g l⁻¹ was a linear absorbance - concentration relationship demonstrated at 394 and 512 nm. Solutions containing all three ions were studied in the presence of an excess of hydrazinium sulphate, which produced no change in absorbance. In order that its state of oxidation should be known with certainty, the iron in all of the solutions used was reduced.

TABLE II

ABSORBANCE OF IRON(II) AND IRON(III) FLUOROBORATE SOLUTIONS (10-MM CELL)

Total iron concentration/g l ⁻¹	Iron(III) (oxidised)		Iron(II) (as prepared)		Iron(II) (reduced)	
	at 394 nm	at 512 nm	at 394 nm	at 512 nm	at 394 nm	at 512 nm
	1.58	0.007	0.002	0.004	0.003	0.004
3.15	0.014	0.004	0.008	0.005	0.008	0.005
6.30	0.029	0.008	0.017	0.010	0.017	0.011
12.60	0.058	0.017	0.033	0.019	0.034	0.021
20.20	0.101	0.027	0.053	0.030	0.054	0.034
25.20	0.136	0.034	0.067	0.037	0.069	0.042

PREPARATION OF CALIBRATION GRAPHS—

A calibration graph for completely reduced iron(II) fluoroborate at 394 and 512 nm can be constructed from the results shown in Table II. Those for cobalt and nickel were constructed as follows: appropriate aliquots of the cobalt and nickel fluoroborate stock solutions were heated with sufficient hydrazinium sulphate to reduce 20 g l⁻¹ of iron (1 g of iron(III) requires 0.58 g of hydrazinium sulphate or 12 ml of 5 per cent. *m/V* solution). After cooling, the solutions were diluted nearly to final volume, the pH of each was adjusted to 3.3 and the solutions were finally made up to volume in calibrated flasks. The absorbances of the separate cobalt and nickel solutions were measured in 10-mm cells at 394 and 512 nm, and are shown in Table III. Both cobalt and nickel gave linear calibration graphs passing through the origin. The slopes obtained from the graphs were checked by the least squares method and are shown in Table IV.

TABLE III

STANDARD GRAPHS FOR COBALT AND NICKEL FLUOROBORATES

Metal concentration/g l ⁻¹	Absorbance (1-cm cell)	
	at 394 nm	at 512 nm
<i>Cobalt</i> —		
1.37	0.008	0.017
2.74	0.016	0.218
6.86	0.040	0.547
10.98	0.064	0.869
13.73	0.080	1.096
<i>Nickel</i> —		
2.06	0.175	0.003
4.13	0.350	0.005
8.27	0.700	0.011
12.40	1.050	0.016
16.54	1.400	0.022

The simultaneous equations required for the determination of the cobalt and nickel concentrations in sample solutions can be established as follows.

A_{394} and A_{512} are the net absorbances of sample solutions at 394 and 512 nm; E_{394} and e_{512} the absorptivities of nickel(II) at 394 and 512 nm; and E_{512} and e_{394} the absorptivities of cobalt(II) at 512 and 394 nm.

By using the additivity of individual ion absorptions $A_{394} = [\text{Ni}] E_{394} + [\text{Co}] e_{394}$ at 394 nm; and $A_{512} = [\text{Co}] E_{512} + [\text{Ni}] e_{512}$ at 512 nm, from which

$$[\text{Co}] = \frac{A_{512}E_{394} - A_{394}e_{512}}{E_{512}E_{394} - e_{512}e_{394}} \quad \dots \quad (1)$$

and

$$[\text{Ni}] = \frac{A_{394}E_{512} - A_{512}e_{394}}{E_{512}E_{394} - e_{512}e_{394}} \quad \dots \quad (2)$$

Equations (1) and (2) can be applied to concentrations in moles or grams per litre provided the absorptivities are in the appropriate units. The error produced by ignoring $e_{512}e_{394}$ with respect to $E_{512}E_{394}$ is about 0.15 per cent. Equations (1) and (2) become equations (3) and (4) (with concentrations in grams per litre), respectively, by using the values given in Table IV:

$$[\text{Co}] = 12.6 A_{512} - 0.194 A_{394} \quad \dots \quad (3)$$

and

$$[\text{Ni}] = 11.8 A_{394} - 0.862 A_{512} \quad \dots \quad (4)$$

TABLE IV
SLOPES OF CALIBRATION GRAPHS FOR COBALT AND NICKEL FLUOROBORATES

	Cobalt		Nickel	
	at 394 nm	at 512 nm	at 394 nm	at 512 nm
$e \left\{ \begin{array}{l} 1 \text{ g}^{-1} \text{ cm}^{-1} \\ 1 \text{ mol}^{-1} \text{ cm}^{-1} \end{array} \right.$	0.00582	—	—	0.00131
$E \left\{ \begin{array}{l} 1 \text{ g}^{-1} \text{ cm}^{-1} \\ 1 \text{ mol}^{-1} \text{ cm}^{-1} \end{array} \right.$	0.343	—	—	0.0767
	—	0.0798	0.0847	—
	—	4.70	4.97	—

PROCEDURE FOR THE DETERMINATION OF IRON, COBALT AND NICKEL—

Iron—A suitable aliquot of the sample solution, which is chosen to give a reasonable titre, is acidified with concentrated hydrochloric acid and titrated with standard 0.10 N dichromate solution, with diphenylamine sulphate (sodium salt) solution as indicator, thus giving the iron(II) content (1.0 ml of 0.10 N dichromate solution \equiv 0.005585 g of iron). A further aliquot, acidified as above, is passed through a silver reductor column¹⁷ at the rate of 25 ml min⁻¹. The total iron content is determined by titrating this reduced aliquot with dichromate solution and the iron(III) content calculated from the difference between the above titrations.

Cobalt and nickel—The nickel and cobalt concentration in the final solution to be measured should be in the range 2 to 10 g l⁻¹ and the total iron concentration should not exceed 15 g l⁻¹, above which concentration the calibration graph is non-linear. For normal bath electrolyte samples the iron(III) in a 25.0-ml aliquot is reduced by adding 10 ml of neutral 5 per cent. *m/V* solution of hydrazinium sulphate and heating the mixture nearly to boiling for 5 minutes; it is then cooled, its pH adjusted to 3.3 with 1 N sulphuric acid or saturated borax solution and the solution is made up to a final volume of 50 ml. The absorbance of this solution is measured in 10-mm cells at 394 and 512 nm against distilled water. The absorbance due

TABLE V
DETERMINATION OF IRON

Solution	Iron added/g l ⁻¹	Iron recovered/g l ⁻¹	Determined absorbance	
			at 394 nm	at 512 nm
A	2.00	2.08	0.006	0.004
B	10.0	10.3	0.028	0.018
C	10.0	10.3	0.028	0.018
D	6.00	5.90	0.016	0.010
E	10.0	10.1	0.029	0.018
F	2.00	2.05	0.006	0.004

to the total iron, as determined above, is interpolated from the results given in Table II, column 3, at 394 and 512 nm and these values are subtracted from the measured absorbances to give the net absorbances due to cobalt and nickel. Appropriate dilution factors are applied when determining the iron absorbance. The net absorbances are substituted in equations (3) and (4), which give the cobalt and nickel contents of the solution.

TABLE VI
DETERMINATION OF COBALT AND NICKEL

Solution	Net absorbance		Added/g l ⁻¹		Recovered/g l ⁻¹			
	at 394 nm	at 512 nm	Cobalt	Nickel	by proposed method		by previous methods	
					Cobalt	Nickel	Cobalt	Nickel
A	0.894	0.797	10.0	10.0	9.83	9.88	10.5	10.4
B	0.862	0.167	2.00	10.0	1.93	10.1	2.1	10.0
C	0.232	0.812	10.0	2.00	10.14	2.04	10.8	2.4
D	0.534	0.488	6.00	6.00	6.02	5.89	6.3	6.1
E	0.911	0.802	10.0	10.0	9.88	10.1	10.3	10.8
F	0.184	0.166	2.00	2.00	2.05	2.03	2.1	2.1

CONCLUSIONS

The above method has been tested on six synthetic solutions made up by diluting standard stock solutions, and the results, which are recorded in Tables V and VI, are compared with those obtained by the three separate methods used previously. Similar tests on actual samples of bath electrolytes are shown in Table VII.

The proposed method is faster, less than 1 hour being required for a complete analysis once the method is ready for use, and is more reliable than the methods previously used. It is possible that the total iron content could be determined by spectrophotometry at wavelengths lower than 390 nm, but the titration method is simple and accurate and gives both iron(II) and iron(III) contents.

TABLE VII
ANALYSIS OF SOME PLATING BATH SOLUTIONS

Sample	Iron/g l ⁻¹	Cobalt/g l ⁻¹		Nickel/g l ⁻¹	
		by proposed method	by colorimetric method	by proposed method	by gravimetric method
1	10.4	13.0	13.4	10.0	10.7
2	12.5	6.70	6.00	3.60	3.00
3	7.50	12.9	13.0	20.3	19.5
4	27.5	—	—	45.0	40.0
5	3.90	62.0	60.2	3.68	3.60

REFERENCES

- Smith, J. F., *Trans. Inst. Metal. Finish.*, 1970, **48**, 208.
- McDowell, B. L., and Schweitzer, G. K., *Analytica Chim. Acta*, 1956, **14**, 115.
- Hazan, I., and Korkisch, J., *Ibid.*, 1965, **32**, 46.
- Lewis, L. L., and Straub, W. A., *Analyt. Chem.*, 1960, **32**, 96.
- Quinche, J. P., and Sax, S., *J. Chromat.*, 1966, **22**, 149.
- Schwarzenbach, G., *Translated and revised by Irving, H.*, "Complexometric Titrations," Methuen & Co. Ltd., London, 1957, p. 82.
- Přibil, R., "Chelometry Basic Determinations," Chemapol, Prague, 1961.
- Přibil, R., and Veselý, V., *Chemist Analyst*, 1963, **52**, 5.
- Welcher, F. J., "The Analytical Uses of Ethylenediaminetetraacetic Acid," D. van Nostrand Co. Inc., Princeton, N. J., New York, Toronto and London, 1958.
- Usumasa, Y., and Nishimura, M., *Bull. Chem. Soc. Japan*, 1955, **28**, 88.
- Burke, R. W., and Yoe, J. H., *Analyt. Chem.*, 1962, **34**, 1378.
- Přibil, R., *Talanta*, 1966, **13**, 515 and 821.
- Berge, H., and Messenga, Z. H., *Z. analyt. Chem.*, 1965, **213**, 346.
- Vidler, J. A. G., *Electroplg Metal Finish.*, 1964, **17**, 122.
- Nalik, W., and Rizwanul, H., *Z. analyt. Chem.*, 1962, **189**, 179.
- Trúchly, T., and Šramko, T., *Chemické Zvesti*, 1965, **19**, 767; *Analyt. Abstr.*, 1967, **14**, 709.
- Vogel, A. I., "A Textbook of Quantitative Inorganic Analysis, Theory and Practice," Second Edition, Longmans, Green & Co., London, 1951, p. 224.

Received July 15th, 1971
Accepted May, 19th 1972

A Colorimetric Method for the Determination of Chloral Hydrate

By S. S. KAMAT, V. P. BARVE AND H. S. MAHAL

(Forensic Science Laboratory, State of Maharashtra, Byculla, Bombay-8, India)

A specific colorimetric method for the determination of chloral hydrate is described. The method is based on the measurement of the pink colour formed when chloral hydrate is treated with 2-thiobarbituric acid. It is specific for chloral hydrate as no other related compounds tested, such as chloroform, trichloroacetic acid, trichloroethylene, carbon tetrachloride, 2,2-dichloroethanol and 1,1,2,2-tetrachloroethane, give any coloration with 2-thiobarbituric acid. The method is sensitive to concentrations as low as $50 \mu\text{g ml}^{-1}$ of chloral hydrate with a reproducibility of ± 2 per cent.

SEVERAL methods have been described for the determination of chloral hydrate, most of which are based upon the measurement of the pink or red colour developed when it is treated with a mixture of pyridine and alkali (Fujiwara's reaction¹). Although several modifications²⁻⁴ of this method have been suggested, none of them is specific for chloral hydrate as other related organic compounds containing the CCl_3 group, such as chloroform, trichloroacetic acid, carbon tetrachloride and 2,2,2-trichloroethanol, also give the test. Stehwen and Kühmstedt⁵ have reported a more specific colorimetric method for the determination of chloral hydrate, which is based upon the red pyrisatin dye that it gives when treated with hydroxylammonium chloride and 2,6-diaminopyridine. This method has the disadvantages that the reaction conditions are critical, sensitivity is low and the reagent 2,6-diaminopyridine is not readily available. Archer and Haugas⁶ have suggested another colorimetric method

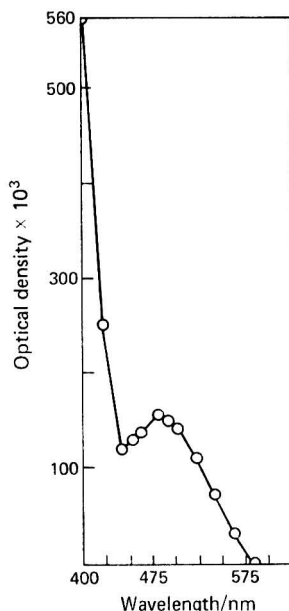


Fig. 1. Absorption spectrum of the reaction product of chloral hydrate and 2-thiobarbituric acid

based upon the stable cyanine dye that chloral hydrate forms with quinaldine and ethanolamine, which is reported to be very specific with a recovery of about 93 to 107 per cent.⁷

In this paper a simple specific method for the determination of chloral hydrate is described. In our earlier paper⁸ it was reported that chloral hydrate forms a pink-coloured compound with 2-thiobarbituric acid. This compound has an absorption maximum at 480 nm in the visible wavelength range (Fig. 1). The reaction is so sensitive that even a trace amount of chloral hydrate will give a pink colour that is stable for over 24 hours. It was therefore thought desirable to develop a colorimetric method for the determination of chloral hydrate based upon its reaction with 2-thiobarbituric acid. The method is highly specific for chloral hydrate as related compounds tested, *viz.*, chloroform, trichloroacetic acid, trichloroethylene, carbon tetrachloride, 2,2-dichloroethanol and 1,1,2,2-tetrachloroethane, did not give any coloration with 2-thiobarbituric acid. A concentration as low as $50 \mu\text{g ml}^{-1}$ of chloral hydrate could be determined by this method, the reproducibility being ± 2 per cent.

EXPERIMENTAL

Chloral hydrate stock solution—Dissolve 1.00 g of chloral hydrate in distilled water and dilute the solution to 1 litre.

2-Thiobarbituric acid reagent, 0.2 M—Dissolve 2.12 g of anhydrous sodium carbonate in about 50 ml of distilled water. Add to this solution 2.88 g of 2-thiobarbituric acid and dilute the resulting solution to 100 ml.

GENERAL PROCEDURE—

Add 5 ml of 2-thiobarbituric acid reagent to 5 ml of the sample under test and maintain the temperature of the mixture at 32°C for 100 minutes. Measure the optical density of the pink colour developed in a photoelectric colorimeter at 480 nm against a blank prepared by the above procedure with 5 ml of distilled water instead of the test solution.

CALIBRATION GRAPH—

A series of working standards of chloral hydrate solution were prepared by diluting the stock solution to give concentrations of 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 and $1000 \mu\text{g ml}^{-1}$. By using the above procedure the optical density of the colour produced with each standard was measured. The graph obtained by plotting optical density against concentration gave a straight line passing through the origin (Fig. 2). Beer's law is obeyed at concentrations between 50 and $1000 \mu\text{g ml}^{-1}$ of chloral hydrate.

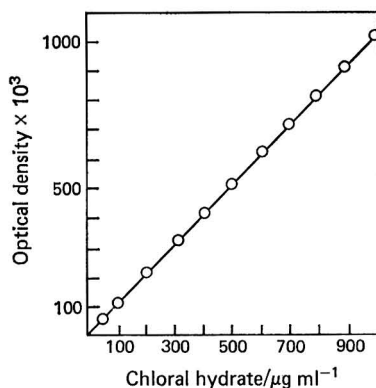


Fig. 2. Standard graph

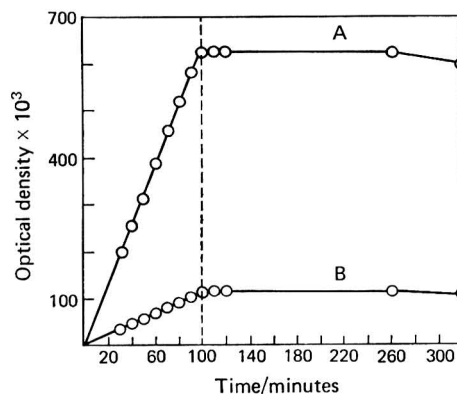


Fig. 3. Rate of reaction at 32°C : A, $600 \mu\text{g ml}^{-1}$; and B, $100 \mu\text{g ml}^{-1}$

DISCUSSION

The rate of the reaction between chloral hydrate and 2-thiobarbituric acid has been found to increase with increase in temperature. In a boiling water bath the reaction is very rapid and is completed within 5 minutes. It was, however, found more practical to carry out the reaction at 32°C , at which temperature it reaches completion within 100 minutes, the colour remaining stable for a further 150 minutes (Fig. 3).

The intensity of the colour developed depends upon the amount of the 2-thiobarbituric acid reagent used (Fig. 4). The amount of the reagent chosen was therefore such that the concentration of chloral hydrate in the range 50 to 1000 $\mu\text{g ml}^{-1}$ gave an absorption in the region 0 to 1000 (optical density $\times 10^3$). As shown in Fig. 4, addition of 5 ml of 0.2 M 2-thiobarbituric acid reagent may be insufficient for maximum colour development. However, full colour development was found to occur within 60 minutes with 6 ml of 0.4 M 2-thiobarbituric acid reagent when added to 0.5 ml of 0.05 per cent. *m/V* chloral hydrate solution and the mixture finally diluted to 10 ml with distilled water. Adoption of the latter conditions would also increase the sensitivity two-fold, but was, however, found not to be practicable because of the instability of the colour developed under these conditions.

Development of the colour was found to be dependent upon the pH of the 2-thiobarbituric acid reagent. It was observed that the reaction was slow and the colour developed was orange instead of pink at pH values lower than 9.5 (Fig. 5). The pH of the reagent was therefore maintained at 9.5 by using the requisite amount of sodium carbonate in preparing the reagent.

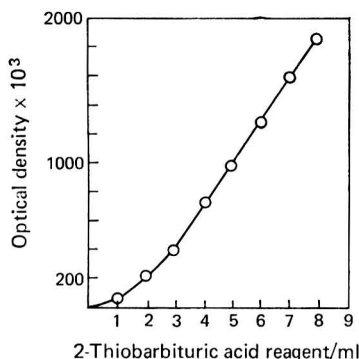


Fig. 4. Effect of the amount of 2-thiobarbituric acid reagent (0.2M) on the intensity of the colour when added to 0.5 ml of 1 per cent. *m/V* chloral hydrate solution and finally diluted to 10 ml with distilled water

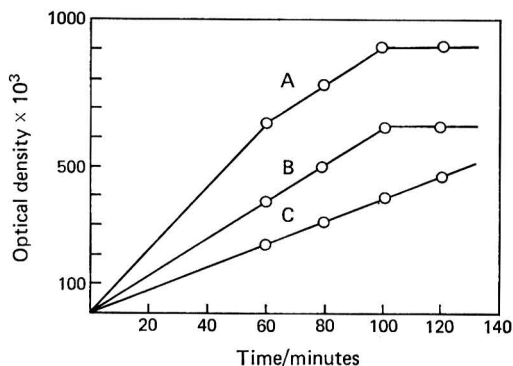


Fig. 5. Effect of pH of 2-thiobarbituric acid reagent on the rate of reaction: A, with reagent at pH 9.9; B, with reagent at pH 9.5; and C, with reagent at pH 8.7

The standard solutions of chloral hydrate with concentrations in the range 50 to 1000 $\mu\text{g ml}^{-1}$ have a pH of about 6.8. If an unknown sample of chloral hydrate has a lower pH, it should be brought to pH 6.8 by the addition of sodium carbonate before the test.

We thank Dr. B. N. Mattoo, Director, for his interest and many helpful suggestions.

REFERENCES

1. Fujiwara, K., *Sber. Abh. naturf. Ges. Rostock*, 1916, 6, 33.
2. Ross, J. H., *J. Biol. Chem.*, 1923, 58, 641.
3. Seto, T. A., and Schultze, M. O., *Analyt. Chem.*, 1956, 28, 1625.
4. Meyer, A. E., and Lee-Motter, R. B., *Arzneimittel-Forsch.*, 1957, 7, 194.
5. Stehwien, D., and Kühmstedt, H., *Pharmazie*, 1955, 10, 482.
6. Archer, E. W., and Haugas, E. A., *J. Pharm. Pharmac.*, 1960, 12, 754.
7. Davidson, A. W., *J. Ass. Off. Analyt. Chem.*, 1968, 51, 626.
8. Kamat, S. S., Barve, V. P., and Mahal, H. S., *Curr. Sci.*, 1971, 40, 190.

Received January 3rd, 1972

Accepted April 28th, 1972

The Determination of Polycyclic Aromatic Hydrocarbons in Mineral Oils by Thin-layer Chromatography and Mass Spectrometry

By C. A. GILCHRIST, A. LYNES, G. STEEL AND B. T. WHITHAM
(Shell Research Ltd., Thornton Research Centre, P.O. Box 1, Chester, CH1 3SH)

A procedure involving column chromatography and preparative thin-layer chromatography, with high-resolution mass spectrometry, has been developed for the determination of polycyclic aromatic hydrocarbons in mineral oils. The approximate content of these hydrocarbons can be determined by preparative thin-layer chromatography and a more accurate result obtained by analysing certain fractions from this separation by mass spectrometry. The mono- and dicyclic aromatic hydrocarbon contents can also be determined from the analysis of these fractions and considerable additional information is obtained about the types of polycyclic aromatic hydrocarbons present, as designated by Z number in the hydrocarbon formula C_nH_{2n+2-Z} .

Limitations of the procedure result from the presence of sulphur compounds in the thin-layer chromatographic fractions, although assumptions can be made that permit certain of these compounds to be determined in the mass-spectrometric analysis. Also, the latter method is not applicable to oils containing appreciable amounts of compounds with relative molecular masses higher than 500.

In recent years there has been an increasing interest in the polycyclic aromatic hydrocarbons occurring in mineral oils and improved procedures have been required in the petroleum industry for determining the total amount of these hydrocarbons and also for obtaining detailed information about the various types that are present. Although considerable information has been published on the determination of polycyclic aromatic hydrocarbons, much of the recent work is related to studies of atmospheric pollution in which the problem is rather different. On the other hand, use has been made of various combinations of separation techniques and spectroscopy in the study of mineral oil composition, but such procedures are often complex and useful only for special limited applications; liquid - solid chromatography in columns has been used extensively as a separation procedure prior to the spectroscopic examination of polycyclic aromatic hydrocarbon concentrates. The simple adsorption chromatographic method with a fluorescent indicator¹ has been adapted for the determination of dicyclic *plus* polycyclic aromatic hydrocarbons in lubricating oils but the main emphasis is now on the tricyclic and higher polycyclic compounds so that this method is of limited value for present-day requirements. Thin-layer chromatography has been widely used in the examination of mineral oils^{2,3} and also, in conjunction with high-resolution mass spectrometry, for the determination of polycyclic aromatic hydrocarbons in air pollution studies.⁴ Mass spectrometry is probably now the most powerful single technique available for classification of petroleum fractions in terms of hydrocarbon type. The procedure developed by Gallegos, Green, Lindeman, Le Tourneau and Teeter⁵ will give a measure of the three, four and five-ring polycyclic aromatic hydrocarbons, but this excludes the important six-ring compounds; also the method is restricted because it is based entirely on a complex instrumental technique.

It was evident that a fair degree of flexibility was required in any procedure that was developed. The total polycyclic aromatic hydrocarbon content was of major importance and the emphasis here was on methods of reasonable simplicity that could be applied in a wide range of laboratories, from research laboratories to those attached to small refineries. The more detailed information on the types of these aromatic hydrocarbons present in mineral oils was likely to be a less frequent requirement and one confined more especially to research and development laboratories so that complex procedures could be used in any such extension.

It seemed that some suitable combination of thin-layer chromatography and mass spectrometry would have the required flexibility and yet be capable of giving considerable detailed information about the polycyclic aromatic hydrocarbons present in mineral oils.

EXPERIMENTAL

SEPARATION PROCEDURE—

Preliminary studies were carried out on the direct separation of oils into hydrocarbon types by thin-layer chromatography. A variety of developing solvents and the adsorbents silica gel, alumina and cellulose were examined. The best system of separation found for mineral oils was with silica gel - light petroleum. However, even with this system the separation of aromatic hydrocarbon types was unsatisfactory, particularly with oils of low aromatic content. Consequently, the separation of the aromatic hydrocarbons was investigated after the saturated hydrocarbons had first been removed from the oil by column chromatography. Various thin-layer chromatographic systems were studied by using aromatic hydrocarbon fractions from several oils and, again, the best separations were obtained with silica gel - light petroleum. The separation of the hydrocarbon types from the aromatic hydrocarbon fractions was much more efficient than that from the whole oils. When this system was used on a preparative scale three zones were obtained by development of a silica gel coated plate with light petroleum; it was subsequently shown that generally the upper zone contained all the monocyclic with some dicyclic aromatic hydrocarbons, the middle zone contained nearly all the polycyclic together with the remaining dicyclic aromatic hydrocarbons, and the base zone contained the highly polar material.

The oil separation procedure finally adopted for the subsequent investigation work was as follows: 5 g of mineral oil dissolved in 5 ml of light petroleum (boiling range 30 to 40 °C) is percolated over activated silica gel (Davisons 923, activated for at least 16 hours at 190 ± 5 °C; column size 75×2.5 cm) and the saturated hydrocarbons are removed from the oil by elution with 200 ml of light petroleum. The aromatic hydrocarbons and other polar constituents are eluted from the column with 250 ml of chloroform - methanol (3 + 1), which is evaporated to obtain the aromatic hydrocarbons - polar portion of the oil. In order to avoid any oxidation or photochemical effects, or both, the column containing the silica gel is purged with nitrogen before use and wrapped with dark paper.

A sample of the aromatic hydrocarbons - polar portion (about 0.3 g) is then applied directly, without dilution, to a preparative-scale thin-layer plate of pre-coated silica gel,* 2 mm thick; the silica gel contained the inorganic fluorescent indicator F_{254} . The sample is applied as a narrow band 2 to 3 mm wide across the plate about 1 cm from the bottom edge and to within about 1 cm from each side. The plate is developed with light petroleum (30 to 40 °C) in a chromatographic tank protected from daylight. In most instances a double development improves the separation; for this purpose the plate is removed from the tank after the first development and the light petroleum is allowed to evaporate from it; the plate is then replaced in the tank to develop for a second time. After the second development the light petroleum is again evaporated from the plate, which is viewed under ultraviolet light at a wavelength of 254 nm in order to determine the demarcations between the various zones. A line is drawn across the plate just above the brown-coloured base (polar) material. All the silica gel below this line is collected as the first preparative-scale thin-layer chromatographic band. A second line is drawn across the plate at the end of the blue fluorescent zone, which is usually about halfway up the plate (R_f 0.5). In most instances there is no gap between this fluorescent zone and the non-fluorescing zone above, and care has to be taken when the line is drawn. The silica gel between the lines is collected as the second band and all the silica gel above the upper line as the third band. Each silica-gel band is scraped off the plate into a separate beaker and stirred with about 100 ml of hot methanol. The methanolic solutions are then passed through sintered-glass filters (porosity 3) and the silica gel retained on the filters is washed with hot methanol, corresponding filtrates and washings being combined. The methanolic solutions are evaporated on a rotary evaporator to obtain the fractions, which are weighed prior to spectroscopic analysis. Over-all recoveries of greater than 97 per cent. m/m are usually obtained with the separations. Several oils were examined in replicate and the results of the thin-layer separations are shown in Table I. Agreement between the replicate determinations of percentage of material recovered in the second and third fractions can vary to some extent because the precise demarcation between these bands is sometimes indistinct. However, the total polycyclic aromatic hydrocarbon contents calculated from the subsequent mass-spectrometric analyses of the fractions from

* Merck, Darmstadt, Germany.

these replicate determinations are in good agreement (as shown later in Table IV, column 2, in which the replicate results are given in parentheses).

A range of typical mineral oils, which have been designated oils A to K, was subjected to the chromatographic procedure described and the fractions obtained were examined in detail by high-resolution mass spectrometry and other spectroscopic techniques.

TABLE I
PREPARATIVE THIN-LAYER CHROMATOGRAPHIC FRACTIONS OBTAINED FROM
THE AROMATIC HYDROCARBON - POLAR PORTIONS OF MINERAL OILS ISOLATED BY
REPLICATE COLUMN-CHROMATOGRAPHIC SEPARATIONS

Preparative thin-layer chromatographic separations of
aromatic - polar portions of mineral oil

Mineral oil sample	Preparative thin-layer chromatographic separations of aromatic - polar portions of mineral oil		
	1st fraction, per cent. <i>m/m</i> of original oil	2nd fraction, per cent. <i>m/m</i> of original oil	3rd fraction, per cent. <i>m/m</i> of original oil
Oil C	12.3	10.4	30.0
	13.7	9.9	27.3
	12.4	12.1	26.6
Oil H	1.0	0.7	7.7
	0.8	1.2	7.7
	0.9	0.6	8.5
Oil I	1.4	14.8	18.0
	1.7	10.5	24.6

SPECTROSCOPIC EXAMINATION OF THE FIRST PREPARATIVE-SCALE THIN-LAYER CHROMATOGRAPHIC FRACTIONS FROM A RANGE OF OIL SAMPLES—

The first fractions (polar material) from the oils examined varied in amount from 0.1 per cent. *m/m* for oil J to 12.3 per cent. *m/m* for oil C. All of the first fractions contained appreciable amounts of sulphur as determined by the oxygen-flask procedure. Ultraviolet spectroscopic analysis indicated that any aromatic hydrocarbon material present was mainly monocyclic with some dicyclic (method based on occurrence of major absorption differences for different polycyclic aromatic hydrocarbons).

The fractions were introduced into an AEI MS9 high-resolution mass spectrometer via the hot-box inlet system at temperatures between 260 and 280 °C and a heated line temperature of approximately 250 °C. The spectra were recorded at an electron accelerating potential of 70 V with a trap current of 100 μ A, and also at a potential of 10 V with a trap current of 20 μ A and an ion repeller potential of zero. This analysis showed that the apparent parent ions present in the fractions covered a wide range of *Z* numbers,* which suggested that the samples were gross mixtures. Possible hydrocarbon parent ions mainly corresponded to mono- and dicyclic aromatic material, agreeing with the evidence obtained by ultraviolet spectroscopy. The presence of peaks in the mass spectra corresponding to CS_2^+ , H_2S^+ and SO_2^+ confirmed the presence of sulphur compounds and also suggested that sulphur - oxygen material was present.

Infrared spectroscopic analysis of the fractions showed evidence of the presence of carbonyl and sulphoxide groups. Thus, although precautions to prevent oxidation were taken, the possibility of oxidation of some of the mono- and dicyclic aromatic compounds to polar compounds during the separation procedures cannot be excluded.

Analysis of material in the first fractions was not continued further as the evidence indicated that the fractions did not contain tricyclic or higher polycyclic aromatic compounds.

SPECTROSCOPIC EXAMINATION OF SECOND AND THIRD FRACTIONS FROM THE OIL SAMPLES—

Examination of the fractions by ultraviolet spectroscopy showed that the third fractions contained mono- and dicyclic aromatic hydrocarbons and that the second fractions contained tricyclic and higher aromatic hydrocarbons.

The second and third fractions from the separated oils were analysed by high-resolution mass spectrometry in the same way as the first fractions. The results were calculated from

* *Z* number is a measure of hydrogen deficiency in hydrocarbons, according to the formula, $\text{C}_n\text{H}_{2n+Z}$. Alkanes have the formula $\text{C}_n\text{H}_{2n+2}$ and therefore $Z = 2$; olefins of formula C_nH_{2n} have a *Z* number of 0 and so on.

spectra obtained at an electron accelerating potential of 10 V, which was that electron voltage at which low-mass fragment ions were no longer detected. Series of parent ions were then observed at even mass numbers, as is usual for petroleum fractions, and the general formula, C_nH_{2n+z} , was assigned to each mass number. The intensities of the parent ions appearing on the same Z number series were expressed as a percentage of the total.

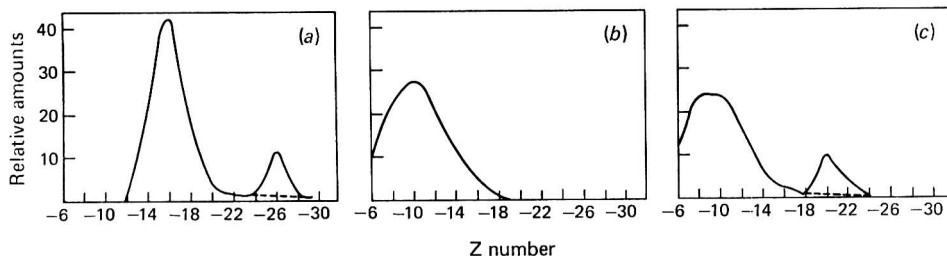


Fig. 1. Mass-spectrometric Z number distribution curves for some of the fractions obtained by preparative thin-layer chromatography from oil samples A, C and D: (a) oil A, fraction 2; (b) oil C, fraction 3; and (c) oil D, fraction 3

A Z number distribution curve was plotted for each fraction and some typical examples of curves obtained for the mineral oils investigated are shown in Fig. 1 (a) to (c). The curve [Fig. 1 (b)] obtained for the third fraction of oil C is seen to be a fairly smooth curve similar to the hydrocarbon distribution curves encountered for many mineral oils; the distribution curve for oil H was also of this type. An enhancement of the $Z = -20$ and $Z = -22$ series can be seen in the distribution curves of the other third fractions [Fig. 1 (c)]; the smooth hydrocarbon distribution is seen from $Z = -6$ to -18 and would be expected to continue smoothly through $Z = -20$, -22 and -24 . The apparent excess of $Z = -20$ and $Z = -22$ is attributed to benzothiophenes of general formula $C_nH_{2n-10}S$, and to naphthenobenzothiophenes, $C_nH_{2n-12}S$, respectively. The third fractions of oils A and F also show an additional enhancement of the $Z = -26$ series, which was attributed to dibenzothiophenes of general formula $C_nH_{2n-16}S$.

The distribution curves plotted for the second fractions were observed to show an enhancement of the $Z = -26$ and $Z = -28$ series [Fig. 1 (a)]. The apparent excess of the $Z = -26$ series is attributed to the presence of dibenzothiophenes of general formula $C_nH_{2n-16}S$, and of the $Z = -28$ series to the presence of naphthenodibenzothiophenes.

In the mass region for which the calculations are made on parent ions, the resolving power of the mass spectrometer is sufficient to separate hydrocarbon peaks at the same nominal mass number but on different Z number series, e.g., alkylbenzenes, $Z = -6$, and acanthrenes, $Z = -20$, requiring a resolution of about 3700 at m/e 344. However, the resolving power is not sufficient to separate, for example, a peak of formula C_nH_{2n-20} and one of formula $C_mH_{2m-10}S$ occurring at the same nominal mass number, as this separation would require a resolving power of about 101 000 at m/e 344. The assumptions made that the enhancements of certain Z number series are due to the presence of aromatic sulphur compounds is not, therefore, unreasonable, particularly as it was found that these chromatographic fractions had significant sulphur contents.

Corrections for the aromatic sulphur compounds detected were made by continuing the smooth curve through the distribution graphs as illustrated in Fig. 2 and taking the excess at $Z = -20$, -22 , -26 and -28 as being due to the relevant sulphur compounds. The amounts of aromatic sulphur compounds detected do not usually account for the total sulphur contents of the fractions. This discrepancy could result from the use of inappropriate relative sensitivities for the sulphur compounds (see later) but it is more likely that other sulphur compounds are present, which fragment readily in the mass spectrometer and have weak parent ions, and are therefore not detected in the measurements involved in these calculations.

Further analyses were performed to check the attribution of the enhanced intensities of the $Z = -20$ and $Z = -26$ series to sulphur compounds, by adding 0.3 per cent. m/m each of butylbenzothiophen and dibenzothiophen separately to the two oils (oils C and H). Increased benzothiophen contents were detected in the third fractions at the relative molecular

mass corresponding to butylbenzothiophen; also, additional dibenzothiophens were detected in the second fractions, as was expected from observations made in the mineral oil analyses. These blend analyses were also useful in assessing the reliability of the sensitivity corrections for the sulphur compounds. Sensitivities of 1.73 for dibenzothiophens and related sulphur compounds and 1.23 for benzothiophens and related sulphur compounds, relative to a sensitivity of 1 for hydrocarbons, were used. These sensitivities are given by Reid, Mead and Bowen,⁶ and were measured on an AEI MS9 mass spectrometer for benzothiophens and dibenzothiophens relative to a sensitivity of 1 for naphthalene.

Because the exact nature of the wide range of hydrocarbon parent ions is difficult to deduce, it was decided to assume equal parent sensitivities for them.

Table II shows the results of the analyses of the oils C and H after the addition of dibenzothiophen and butylbenzothiophen. In both oils the amounts of sulphur compounds determined, by using the sensitivity factors given by Reid *et al.*,⁶ were higher than those added. It would be necessary to study the addition of sulphur compounds to a wider range of mineral oils with varying sulphur contents in order to try and find an explanation for this effect. It may well be that the sensitivity figures of Reid *et al.* should be re-assessed.

Table III shows the relative amounts of hydrocarbons of different *Z* number series in the second and third fractions for the mineral oils examined. The series including *Z* numbers -6, -8 and -10 can be considered to be largely monocyclic aromatic compounds and, similarly, the series including *Z* = -12, -14 and -16 to be bicyclic aromatic compounds. The *Z* = -18, -20, -22, etc., hydrocarbons can be considered to be mainly tricyclic and higher polycyclic aromatic compounds but some overlap of the series can occur.

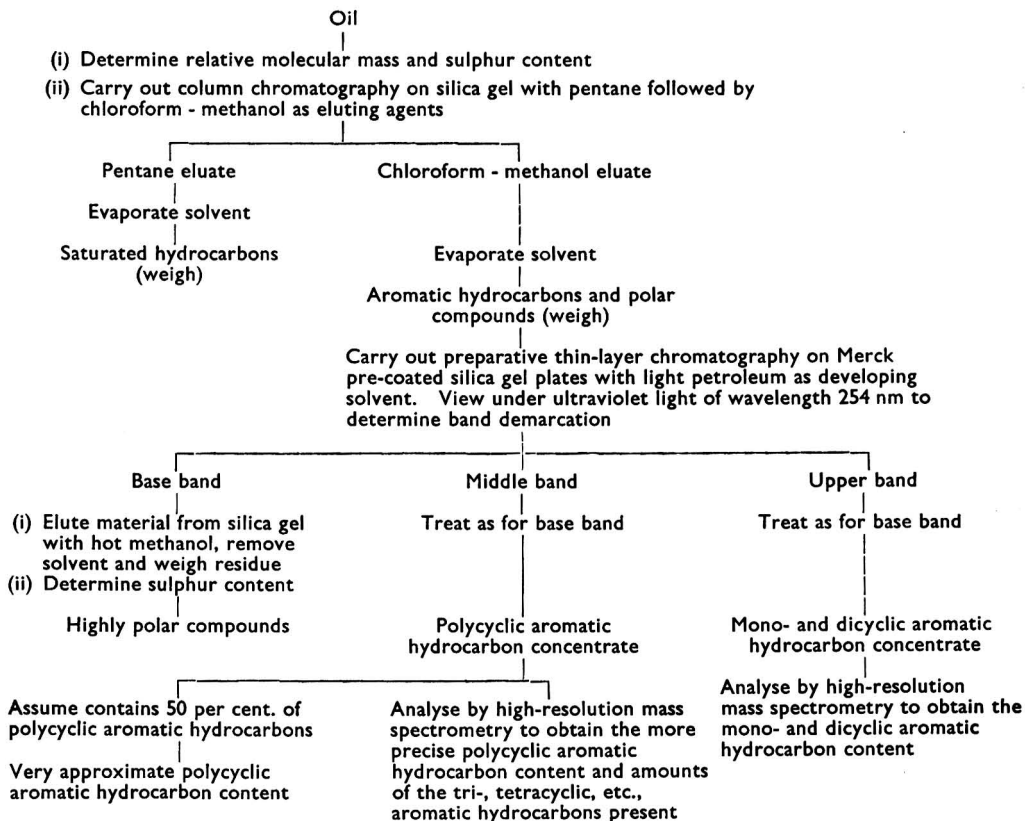


Fig. 2. Summary of the analytical procedure developed for the analysis of polycyclic aromatic hydrocarbons in mineral oils

It was found that the second fractions contained the major proportion of the polycyclic together with some of the dicyclic aromatic hydrocarbons; in some instances monocyclic aromatic hydrocarbons were also detected. In the ultraviolet examination mentioned earlier, dicyclic aromatic hydrocarbons were not detected in the second fractions, owing to the fact that the more intense absorption of polycyclic aromatic hydrocarbons, which also extends to longer wavelengths, masks the absorption of the dicyclic compounds; it would be necessary to calculate the relatively small absorption of the latter as the difference of two large absorptions, thus introducing potentially large errors. The third fractions contained mainly mono- and dicyclic with some polycyclic aromatic hydrocarbons.

TABLE II
ANALYSIS OF OILS C AND H AFTER THE ADDITION OF DIBENZOTHIOPHEN
AND BUTYLBENZOTHIOPHEN

Compound type	Analytical results, per cent. <i>m/m</i> of original oil					
	Oil C	Oil C <i>plus</i> 0.3 per cent. <i>m/m</i> of di- benzothiophen	Oil C <i>plus</i> 0.3 per cent. <i>m/m</i> of butyl- benzothiophen	Oil H	Oil H <i>plus</i> 0.3 per cent. <i>m/m</i> of di- benzothiophen	Oil H <i>plus</i> 0.3 per cent. <i>m/m</i> of butyl- benzothiophen
Monocyclic aromatic hydrocarbons	17.3	16.1	15.8	5.5	5.2	5.6
Dicyclic aromatic hydrocarbons	14.0	12.0	12.9	1.7	2.2	2.1
Polycyclic aromatic hydrocarbons	3.0	3.4	3.3	0.2	0.4	0.2
Dibenzothiophens	0.6	1.3	0.7	<0.1	0.5	<0.1
Benzothiophens	None detected	None detected	0.8	None detected	0.1	0.6

RESULTS AND DISCUSSION

From all the fraction recoveries and the mass-spectrometric results the over-all polycyclic aromatic hydrocarbon contents were calculated. The concentrations of these hydrocarbons obtained by this procedure ranged from 0.3 per cent. *m/m* for oil H to 15.2 per cent. *m/m* for oil D. The approximate concentration of their different types can be obtained by taking *Z* numbers of -18 and -20 as corresponding to tricyclic, -22, -24 and -26 to tetracyclic, -28 and -30 to pentacyclic and -32 to hexacyclic aromatic hydrocarbons; however, some overlap will occur. By this method, only oil D was shown to contain any of these last hydrocarbons; several oils were shown to contain pentacyclic aromatic hydrocarbons. All the polycyclic aromatic hydrocarbon portions of the oils were shown to contain mainly tri- and tetracyclic compounds.

The limit of detection for any polycyclic aromatic hydrocarbon type, as specified by a particular *Z* number, is largely dependent on the percentage of the second fraction in the whole sample. Provided this percentage is not so low that insufficient oil is isolated from the chromatographic separation for mass-spectrometric analysis, then it is possible to detect any polycyclic aromatic hydrocarbon type down to 1 per cent. *m/m* in the fraction by mass spectrometry. Thus, the limits of detection have varied from 0.01 to 0.28 per cent. *m/m* in the oils that have been examined as the percentage of the fraction has varied between 1 and 28 per cent. *m/m*.

A very approximate indication of the polycyclic aromatic hydrocarbon content of the oils can often be obtained by assuming that the second fraction contains all of these compounds and that half of this fraction consists of other aromatic hydrocarbons. Because it avoids the use of mass spectrometry, this procedure is considerably less time consuming than the full procedure. Results obtained in this way are shown in Table IV and are compared with the polycyclic aromatic hydrocarbon contents calculated in conjunction with the mass-spectrometric analysis of the fractions. The general applicability of this rapid procedure would have to be established by the examination of a greater number of oils.

TABLE III

RELATIVE AMOUNTS, PER CENT. *m/m*, OF Z NUMBER SERIES IN THE SECOND AND THIRD PREPARATIVE THIN-LAYER CHROMATOGRAPHIC FRACTIONS

Z number	Compound type	Oil A		Oil B		Oil C		Oil D		Oil E		Oil F		
		Fraction 2	Fraction 3	Fraction 2	Fraction 3	Fraction 2	Fraction 3	Fraction 2	Fraction 3	Fraction 2	Fraction 3	Fraction 2	Fraction 3	
-4														
-6	Monocyclic aromatic hydrocarbons		11		7	Trace	10		10		15		8	
-8			27		17	Trace	23	Trace	25		15		14	
-10			29		18	1	28	1	25	Trace	16		15	
-12	Dicyclic aromatic hydrocarbons	1	14	4	19	2	21	6	17	7	15		15	
-14			25	12	19	13	17	12	14	6	10	12	9	15
-16			44	4	18	5	30	5	14	3	13	6	3	8
-18	Tricyclic aromatic hydrocarbons	18	1	21	1	19	1	16	1	15	Trace	62	3	
-20			3	Trace	9	1	11	Trace	12	1	16	Trace	9	2
-22	Tetracyclic aromatic hydrocarbons	1	Trace	7	1	7	Trace	11	Trace	14	Trace		2	
-24			1	Trace	3	1	2		5	Trace	8	Trace		2
-26			1	Trace	3		1		3		5			1
-28	Pentacyclic aromatic hydrocarbons	Trace		2		1		2		2				
-30							Trace		2					
-32	Hexacyclic aromatic hydrocarbons							2						
	Benzothiophens and related sulphur compounds				17				12	4	21		8	
	Dibenzothiophens and related sulphur compounds	6	2	14		9		12		6		17	7	
Z number	Compound type	Oil G		Oil H		Oil I		Oil J		Oil K				
		Fraction 2	Fraction 3	Fraction 2	Fraction 3	Fraction 2	Fraction 3	Fraction 2	Fraction 3	Fraction 2	Fraction 3			
-4					Trace						Trace			
-6	Monocyclic aromatic hydrocarbons		14	4	22		9		25		Trace		10	
-8			18	6	32		24		19		23		23	
-10			2	15	7	25	2	27	6	15	4	24		24
-12	Dicyclic aromatic hydrocarbons	4	15	11	13	9	19	8	13	10	18			
-14			19	11	22	5	17	10	16	9	21	11		
-16			23	6	21	3	17	5	18	4	25	7		
-18	Tricyclic aromatic hydrocarbons	17	2	15	Trace	14	3	15	2	19	3			
-20			10	1	6		11	2	12	1	10	2		
-22	Tetracyclic aromatic hydrocarbons	7	1	3		11	Trace	7	1	5	1			
-24			3	1	1		9		3		2			
-26			2	1			5		2		1			
-28	Pentacyclic aromatic hydrocarbons					1		2						
-30			2											
-32	Hexacyclic aromatic hydrocarbons													
	Benzothiophens and related sulphur compounds		15				1		11		1			
	Dibenzothiophens and related sulphur compounds	11		4		4		11		3				

The contents of mono- and dicyclic aromatic hydrocarbons, benzothiophens and dibenzothiophens can also be calculated from the fraction recoveries and mass-spectrometric results. However, as already indicated, the mass-spectrometric analysis does not always account for all the sulphur compounds in the second and third fractions but a correction to the aromatic hydrocarbon concentrations based on the relative molecular mass of the oil can be made by assuming that only one atom of sulphur is present in each molecule of sulphur compound.* From this assumption, and taking the average relative molecular mass of the sulphur compounds present to be the same as that of the oil, the average sulphur content of the sulphur compounds can be calculated. From this figure and the sulphur content of the second and third fractions the percentage of sulphur compounds in these fractions can be obtained. If this calculated sulphur compound content is in excess of the amount of sulphur compounds detected by the mass-spectrometric analysis of the fraction, then the results of this analysis are re-normalised to take into account the additional sulphur compounds that are apparently present. Table V gives the aromatic hydrocarbon content of the oils corrected for sulphur material not detected by mass spectrometry. This same sulphur correction has been made to the first fractions and it was found on this basis that, in oil K, all the polar material contained sulphur. The other oils examined contained additional polar material, ranging in concentration from less than 0.1 per cent. *m/m* for oil J to 9.3 per cent. *m/m* for oil C.

One major restriction in the application of the mass-spectrometric method should be mentioned: it is difficult or impossible to introduce materials of relative molecular mass higher than 500 through the hot-box inlet of the mass spectrometer and the method cannot therefore be applied to bright stocks (refined non-distillate mineral oil) or to any other type of heavy oil.

The over-all analytical procedure, together with the information obtained at the various stages, is outlined in Fig. 2.

TABLE IV
POLYCYCLIC AROMATIC HYDROCARBON CONTENT OF A RANGE OF MINERAL OILS

Mineral oil sample	Polycyclic aromatic hydrocarbon content, per cent. <i>m/m</i>	
	Chromatography only: 50 per cent. <i>m/m</i> of fraction 2	Chromatography and high-resolution mass spectrometry (Results uncorrected for sulphur compounds not detected by mass spectrometry)
Oil		
A	2.6	1.5
B	8.7	8.8
C	5.2 (5.0) (6.0)	4.5 (4.1) (5.1)
D	13.9	15.2
E	5.4	6.5
F	2.1	5.0
G	2.8	3.6
H	0.3 (0.6) (0.3)	0.3 (0.4) (0.3)
I	7.4; 5.3†	8.5, 8.5†
J	2.1	2.3
K	1.1	1.6

The results in brackets are repeat analyses of the samples to which 0.3 per cent. *m/m* of dibenzothiophen or butylbenzothiophen had been added (see Table II). Corrections have been made for the presence of these particular sulphur compounds so that the results are comparable with those for the oils with no added compounds.

† Duplicate results.

CONCLUSIONS

The combination of preparative thin-layer chromatography and high-resolution mass spectrometry gives a procedure with considerable flexibility in the determination of polycyclic aromatic hydrocarbons in mineral oils. In a number of instances an approximate indication of their content (tricyclic and higher) can be obtained directly from the chromatographic

* Although CS_2^+ was detected in the mass-spectrometric analyses this finding does not necessarily indicate the presence of compounds containing two sulphur atoms per molecule because it is known that CS_2^+ occurs in the spectra of thiols, sulphides, etc.,⁷ which contain one atom of sulphur per molecule.

TABLE V

AROMATIC HYDROCARBON CONTENTS (PER CENT. m/m) OF OIL SAMPLES CORRECTED FOR SULPHUR COMPOUNDS NOT DETECTED BY MASS SPECTROMETRY

Compound type	Oil A	Oil B	Oil C	Oil D	Oil E	Oil F	Oil G	Oil H	Oil I	Oil J	Oil K
Monocyclic aromatic hydrocarbons	20.4	11.5	17.3	10.7	6.1	6.8	9.4	5.5	9.2	7.9	6.7
Dicyclic aromatic hydrocarbons	11.7	14.1	14.0	9.1	5.5	7.2	7.4	1.7	7.9	4.0	4.6
Total polycyclic aromatic hydrocarbons	1.2	5.4	3.0	7.4	2.2	3.2	2.1	0.2	4.1	1.1	1.0
Tricyclic	1.0	3.5	2.2	4.1	1.2	3.0	1.3	0.1	2.2	0.7	0.8
Tetracyclic	0.2	1.7	0.7	2.5	0.9	0.2	0.7	<0.1	1.8	0.3	0.2
Pentacyclic	<0.1	0.2	<0.1	0.5	0.1	N.D.	0.1	N.D.	0.1	<0.1	N.D.
Hexacyclic	N.D.	N.D.	N.D.	0.3	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Benzothiophens and dibenzothiophens	0.8	6.0	0.6	3.7	3.2	3.0	3.2	<0.1	0.4	1.6	0.1
Other sulphur compounds*	3.5	11.8	9.2	22.2	13.7	8.0	5.9	2.2	11.8	3.5	3.6
Other polar compounds†	4.9	3.0	9.3	4.2	1.7	4.8	1.5	0.1	0.8	<0.1	Nil

* Calculated from the sulphur content of the three fractions and the relative molecular mass of the oil by assuming only 1 atom of sulphur is present in each molecule of sulphur compound.

† Taken as the mass of first fraction after subtraction of the sulphur compounds.

N.D. denotes none detected.

separation. A more accurate value for the content of these hydrocarbons can be calculated from the mass-spectrometric analysis of fractions obtained from the separation; this analysis will give additional information about the types of polycyclic aromatic hydrocarbons present and also the mono- and dicyclic aromatic hydrocarbon contents can be determined.

The limitations of the procedure are related to the presence of sulphur compounds in the aromatic fractions recovered from the chromatographic separation. Reasonable assumptions that are supported by experimental evidence can be made about the benzothiophens and dibenzothiophens detected by mass-spectrometric analysis, but in any future work it would be necessary to investigate in greater detail the rôle of naphthenodibenzothiophen, which coincides with the Z number (-28) of the important polycyclic aromatic hydrocarbon benzo[a]pyrene. The main problem, however, is that a considerable proportion of the sulphur compounds is often unaccounted for and only arbitrary corrections for them can be applied to the polycyclic aromatic hydrocarbon contents. Thus, except for oils of low sulphur content, the results obtained cannot be considered as an absolute measure of the content of these compounds. Nevertheless, the analytical results obtained give a good indication of the relative detailed polycyclic aromatic hydrocarbon characters of various mineral oils.

The assumption of equal parent ion sensitivity for the different aromatic hydrocarbons could lead to slight inaccuracies. It has been observed by some workers⁶ that the more aromatic the character of a hydrocarbon compound, the more enhanced is its sensitivity.

In order further to increase the reliability of the analysis it will be necessary to consider procedures for the complete removal of the sulphur compounds or to develop a more satisfactory way of correcting for their presence.

REFERENCES

1. Knight, H. S., and Groennings, S., *Analyt. Chem.*, 1956, **28**, 1949.
2. Killer, F. C. A., and Amos, R., *J. Inst. Petrol.*, 1966, **52**, 315.
3. Peurifoy, P. V., O'Neal, M. J., and Woods, L. A., *J. Chromat.*, 1970, **51**, 227.
4. Majer, J. R., Perry, R., and Reade, M. J., *Ibid.*, 1970, **48**, 328.
5. Gallegos, E. J., Green, J. W., Lindeman, L. P., Le Tourneau, R. L., and Teeter, R. M., *Analyt. Chem.*, 1967, **39**, 1333.
6. Reid, W. K., Mead, W. L., and Bowen, K. M., in Mead, W. L., Editor, "Advances in Mass Spectrometry," Volume 3, Elsevier Publishing Co., Amsterdam, 1966, p. 731.
7. McLafferty, F. W., *Adv. Chem. Ser.*, No. 40, 1963, 33.

Received November 9th, 1971

Accepted May 31st, 1972

Specific Detection of Aromatic Compounds Containing Sulphur and Thiazole Compounds on Thin-layer Chromatographic Plates

By M. S. BHATIA, K. L. BAJAJ, SIMRAN SINGH AND I. S. BHATIA
(Department of Chemistry and Biochemistry, Punjab Agricultural University, Ludhiana, India)

Thin-layer chromatographic procedures involving the use of silica gel G plates have been developed for the specific detection of aromatic sulphur-containing and thiazole compounds in amounts of 4 μ g. A mixture of chloroform - methanol (9 + 1) was used as the developing system. Thiazole compounds with a free *ortho* position with respect to sulphur and aromatic sulphur-containing compounds (benzene derivatives) have been detected by using ninhydrin, and all aromatic sulphur-containing compounds can be detected by using copper(II) acetate containing hydroxylammonium chloride as the spray reagent. A method is described for distinguishing between the benzene-derived and heterocyclic sulphur-containing compounds.

In the present investigation the sulphur-containing compounds that are usually the starting materials in the synthesis of thiazole compounds are distinguished from the thiazole compounds on thin-layer chromatographic plates by using different spray reagents. Vernin and Metzger¹ have previously reported on the detection of thiazole compounds on thin-layer chromatographic plates by using 5 per cent. ethanolic molybdophosphoric acid, which is a comparatively less sensitive reagent than that used in the present work.

EXPERIMENTAL

APPARATUS AND REAGENTS—

Thin-layer plates—A number of 20 \times 20-cm glass plates were coated with a silica gel G slurry, which was prepared by mixing thoroughly one part by mass of silica gel G with two parts by volume of water, spreading being carried out by the method of Davidek and Prochazka.² After drying in air the plates were stored and baked in an oven for 30 minutes at 110 °C when required for use.

Solvent system—A mixture of chloroform - methanol (9 + 1) was used as the solvent system in a chamber in which the atmosphere was unsaturated. A 12.5-cm run on the thin-layer chromatographic plates took 45 minutes.

Standard compounds—Different aromatic and heterocyclic sulphur-containing compounds and thiazole compounds (Table I) were spotted on to thin-layer chromatographic plates in 4- μ g amounts with a micropipette.

Spray reagent I (for thiazole and aromatic sulphur-containing compounds)—A 1 per cent. solution of ninhydrin dissolved in cold concentrated sulphuric acid was used.

Spray reagent II (for aromatic and heterocyclic compounds)—This reagent was prepared as follows. A solution was made up by dissolving 0.75 g of copper(II) acetate and 1.5 g of ammonium chloride in a small volume of water, treating the mixture with 1.5 ml of concentrated ammonia solution and making the volume up to 25 ml in a calibrated vessel. Next, a 20 per cent. aqueous solution of hydroxylammonium chloride was prepared. Finally, equal volumes of these solutions were mixed before spraying.

RESULTS AND DISCUSSION

When ninhydrin was used the thin-layer chromatographic plates were heated for 1 to 2 minutes at 105 °C in order to make the spots visible, while with copper(II) acetate - hydroxylammonium chloride reagent, the spots were observed at room temperature. The R_f values, colour reactions with these spray reagents and the colour in ultraviolet light are given in Table I.

Spray reagent I gives a characteristic colour with thiazole derivatives that have a free *ortho* position with respect to sulphur (compounds 1 to 4) and gives negative results with compounds 8 to 11, in which the *ortho* position with respect to sulphur is not free. This behaviour is in accordance with the mechanism proposed by Feigl³ concerning the condensation of 1,2-diketones (isatin, ninhydrin and benzil) with thiophene derivatives having free *ortho* positions. Ninhydrin also gives a colour reaction with aromatic sulphur-containing compounds (compounds 5 to 7) that have a free methine (=CH-) group adjacent to the carbon atom to which a substituted or free -SH group is attached, but does not give a colour reaction with sulphur-containing derivatives of pyrimidines, quinazoline, isoquinoline and imidazole (compounds 16 to 23). The reaction of ninhydrin with compounds 14 and 15 is due to the presence of an amino group rather than of an -SH group as ninhydrin does not give a reaction with aliphatic sulphur-containing compounds containing only the -SH group (compounds 12 and 13).

TABLE I
THIN-LAYER CHROMATOGRAPHIC RESULTS

Number	Compound	R _F value	Colour with spray reagent I	Colour* with spray reagent II	Fluorescence in ultra-violet light
1	2-Chloro-4- <i>p</i> -methoxyphenylthiazole ..	0.97	Intense green	—	Brown
2	2-Chloro-4-phenylthiazole	0.98	Pink	—	Brown
3	2-Amino-4-phenylthiazole	0.67	Orange	—	Brown
4	2-Amino-4- <i>p</i> -methoxyphenylthiazole ..	0.91	Intense green	—	Blue
5	Benzenethiol	0.98	Intense green	Yellowish brown	Brown
6	<i>o</i> -Mercaptobenzoic acid	0.12	Intense green	Yellowish brown	Blue
7	2-(Phenylthio)cyclohexanone	0.97	Intense green	—	Brown
8	2-Amino-6-methoxybenzothiazole ..	0.58	—	—	Brown
9	2-Amino-6-chlorobenzothiazole ..	0.78	—	—	Blue
10	2-Amino-6-ethoxybenzothiazole ..	0.50	—	—	Brown
11	2-Amino-6-methylbenzothiazole ..	0.49	—	—	Brown
12	Mercaptoacetic acid	0.99	—	Yellow†	—
13	β -Mercaptopropionic acid	0.91	—	Green†	—
14	Cysteine	0.76	Purple	Green†‡	—
15	Methionine	0.06	Purple	Brown†	—
16	2-Mercapto-1,4,5,6-tetrahydropyrimidine	1.00	—	Yellowish brown	Blue
17	2-Mercapto-4,4,6-trimethyl-1,4-dihydro-pyrimidine	0.96	—	Yellowish brown	Brown
18	1-Phenyl-2-mercapto-4,4,6-trimethyl-1,4-dihydropyrimidine	0.96	—	Yellowish brown	Brown
19	Quinazoline-4-thiol	0.90	—	Yellowish brown	Brown
20	4-Oxa-3 <i>H</i> -quinazoline-2-thiol	0.91	—	Yellowish brown	Brown
21	Isoquinoline-1-thiol	0.78	—	Yellowish brown	Brown
22	Benzimidazole-2-thiol	0.90	—	Yellowish brown	Brown
23	5-Chlorobenzimidazole-2-thiol	0.63	—	Yellowish brown	Brown

* Colours described are final colours after 1 minute.

† Colours observed for an amount of 10 μ g.

‡ Colour observed after 5 minutes.

Spray reagent II is capable of detecting aliphatic, aromatic and heterocyclic sulphur-containing compounds (compounds 12 to 23) and does not give a colour reaction with compounds lacking the -SH group (compounds 1 to 4 and 8 to 11).

With the application of ninhydrin as spray reagent, it is therefore possible to determine whether or not thiazole compounds contain a free or substituted *ortho* position. By using both spray reagents, it is possible to distinguish between the aromatic and heterocyclic sulphur-containing compounds.

REFERENCES

1. Vernin, G., and Metzger, J., *Chim. Analyt.*, 1964, **46**, 487.
2. Davidek, J., and Prodchazka, Z., *Colln Czech. Chem. Commun.*, 1961, **26**, 2947.
3. Feigl, F., "Spot Tests in Organic Analysis," Elsevier Publishing Company, New York, 1956.

Received September 13th, 1971

Accepted May 16th, 1972

Separation and Thin-layer Chromatographic Determination of Denatonium Benzoate and Other Quaternary Ammonium Denaturants in Spirituous Preparations

BY M. J. GLOVER AND A. J. BLAKE

(Department of Trade and Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, SE1 9NQ)

Denatonium benzoate is an effective partial denaturant at 2 mg l⁻¹ concentration in alcoholic preparations such as toiletries. While its presence is easily detected by tasting the preparation, an analytical procedure for its identification and determination is desirable. Denatonium salts are separated by ion exchange on carboxymethylcellulose and are determined by using thin-layer chromatography, which also separates other quaternary ammonium compounds. Some other methods that have been applied to solve this problem are briefly described.

WHEN duty-free ethanol is used for industrial and commercial purposes other than as a beverage, it is required to be denatured. For many purposes it is desirable that the odour and residue left after evaporation should be minimal, *e.g.*, when the ethanol is used in the preparation of toiletries. Denatonium benzoate¹ (benzyl-diethyl-N-2,6-xylyl-carbamoylmethyl-ammonium benzoate) is an effective partial denaturant at a concentration of 2 mg l⁻¹ and is preferred for the denaturing of ethanol used in toiletries and similar articles. This concentration is considerably below those of quinine, quassin or brucine which would be required to cause the same degree of bitterness. The analytical requirement is, firstly, for effective separation of this quaternary ammonium compound from ethanolic (*e.g.*, industrial methylated spirit) solutions of perfume essential oils, fixatives, hair lacquer resins, plasticisers, emulsifiers, etc., and from other quaternary ammonium compounds such as cetrimide, which may be present at 0.1 per cent. *m/V* concentration. Secondly, a method of determination is required that can be used to distinguish samples containing the required 2 mg l⁻¹ concentration from those containing significantly lower concentrations. Two specific difficulties arise from the type of sample material involved. As with the denaturant, many of the likely ingredients are more soluble in ethanol than in water or in chloroform. This fact limits the efficacy of the commoner liquid-liquid separations, results in the precipitation of some ingredients when treated with some otherwise useful extraction solvents and encourages the formation of emulsions in chloroform-water separations. If precipitation is allowed to occur, denatonium is frequently partially or fully adsorbed on the precipitate. The second difficulty arises from the possible presence of other quaternary ammonium compounds at perhaps 500 times the concentration at which denatonium is normally used. The methods most widely used for the determination of small amounts of these compounds are based on their property of forming complexes (ion pairs) with water-soluble dyes or other coloured anionic reagents, which are soluble in organic solvents. Of these reagents the most commonly used is bromophenol blue (see, for example, reference 2) but Orange II³ and picric acid⁴ have also been advocated. These procedures do not, of course, differentiate between quaternary ammonium compounds without their preliminary separation. Also, in our experience interference is caused by a wide range of organic substances as well as inorganic salts and solvents that disturb the equilibrium in the final separation.

A procedure developed in this laboratory (C. Scott, unpublished work) involved the use of solvent separations to strip interfering components from the denatonium benzoate, followed by thin-layer chromatographic determination. The lengthy separation procedure, with frequent emulsification difficulties and the incomplete separation from cetrimide sometimes encountered, led to an investigation of alternative procedures.

Quaternary ammonium compounds are involatile and decompose on heating to about 100 °C so that the use of direct gas-liquid chromatography is not possible. Several workers

have reported analyses based on determination by this method of products of pyrolysis or elimination reaction at the quaternary nitrogen atom of these compounds,⁵⁻⁷ or of oxidation of specific parts of the molecule.⁸ It was envisaged that, additionally, the amide linkage in the side-chain of denatonium salts might be hydrolysed, thus releasing 2-amino-1,3-dimethylbenzene (xylidine), the determination of which would be specific for denatonium. If a 25-ml sample containing 2 mg l⁻¹ of denatonium were concentrated to 1 ml, the denatonium concentration would be 50 mg l⁻¹ and the theoretical yield of xylylidine 13.5 mg l⁻¹. It was shown that the application of gas-liquid chromatography to this concentration of the xylylidine in toluene or in chloroform was practicable. However, hydrolysis with strong acids or alkalis at temperatures between 100 and 200 °C gave poor yields. The maximum yield, which could not be repeated in successive experiments, was 40 per cent. Further, there was interference from breakdown products of cetrimide as well as gross interference from other constituents of typical samples. The hoped for specificity and development of a quantitative gas-chromatographic method appeared unlikely to be achieved in these circumstances. Polarography was also tried and, while a quantitative response was obtained with pure denatonium at suitably low concentrations, interferences from a variety of possible perfumery or toiletry ingredients were such as to render the method ineffective for practical mixtures.

DESCRIPTION OF METHOD—

As quaternary ammonium compounds are readily ionised, initial separation by ion exchange was investigated. Use of sulphonated polystyrene resins failed to give reasonable recovery; it has been reported that these ion exchangers adsorb quaternary ammonium compounds irreversibly.^{9,10} Some success was achieved with adsorption of denatonium on to the methacrylic type of cation exchanger and subsequent desorption. However, significant amounts of interfering material were leached from the resin by the acidic 95 per cent. ethanol used for elution. The use of carboxymethylcellulose for the separation of quaternary ammonium compounds has been described by Metcalfe.¹¹ We found that denatonium benzoate was incompletely adsorbed from 95 per cent. ethanolic solution. When converted into the hydroxide by passage over anion-exchange resin, in the hydroxide form, denatonium was efficiently adsorbed by carboxymethylcellulose. It is then possible to pass the sample directly down a pair of columns and wash with ethanol, only strong bases being retained. Problems of emulsification are, of course, avoided.

For identification purposes, none of the existing paper or thin-layer chromatographic methods have been found very satisfactory. The degree of separation obtained with the quaternary ammonium compounds is often poor and excessive diffusion occurs in some methods. The wide variety of acidic, basic, aqueous and non-aqueous solvent mixtures recommended suggests that these compounds are too polar to be chromatographed satisfactorily on the usual media (such as those of Bregoff, Roberts and Delwiche,¹² Garcia and Couerbe,¹³ Holness and Stone,¹⁴ Cross¹⁵ and Covello and Schettino¹⁶). Gordon¹⁷ illustrated another approach by chromatographing different salts of quaternary ammonium compounds and showed that the R_f values are not characteristic of the quaternary cation alone but of the salt which, in certain instances, is sufficiently associated to be described as an ion pair; in which case the quaternary ammonium compound has a higher R_f value in non-aqueous eluting solvents. A range of these compounds of toiletry interest has been examined by thin-layer chromatography by using silica gel plates loaded with salts the anions of which give high values (see below). This system gives well shaped and well defined spots for the compounds examined; denatonium was found to have the highest R_f value of the substances tested. As a result it is possible to identify denatonium in the presence of some of these substances without further clean-up and, by applying selective precipitation, of all of the quaternary ammonium compounds used in preparations in which denatonium is approved as a denaturant.

Although the system allows denatonium to be separated from as much as 500 times its concentration of cetrimide, an improvement is obtained by partial precipitation of the latter as the dichromate before thin-layer chromatography. Extraction of the mother liquor gives the denatonium with only a small percentage of the original cetrimide, which is more easily chromatographed. The recoveries obtained in the examination of a series of toiletry ingredients spiked with standard denatonium solution, which are based on visual comparison

with directly applied standard solutions, are given below. For identification and visual assessment purposes, 1 μg of denatonium gives a sufficient depth of colour when the chromatoplate is sprayed with potassium bismuth iodide solution. A reasonably compact spot can still be obtained with 50 μg of denatonium, streaking occurring with larger amounts on a layer 0.25 mm thick. An attempt was made to obtain more quantitative results by chromatographing 50- μg amounts of pure denatonium, locating the spots with iodine vapour and removing the appropriate area of thin-layer adsorbent. The denatonium was extracted from the silica gel by using a syringe filter¹⁸ (cellulose or glass-fibre filter discs) with two portions of chloroform-methanol mixture and then determined by using a picric acid colorimetric procedure.⁴ As denatonium has the highest R_F value of the substances tested, it appeared possible that a simple column-chromatographic version of the thin-layer chromatographic procedure might be practicable with fraction collection and colorimetric determination of the picrate complex. A continuous high blank gave cause for doubt concerning the quantitative analysis of pure denatonium solution. Cetrimide was separated but when some perfumes spiked with standard solution were subjected to ion-exchange separation and column chromatography, serious interference was encountered. This interference was not removed by washing the carboxymethylcellulose column with ammoniacal ethanol, nor by extracting the acidic ethanol eluate with carbon tetrachloride before extracting denatonium into chloroform. It reacts with iodine vapour, picric acid and bromophenol blue but not with potassium bismuth iodide solution, and is derived from the perfume essential oil or from the ion-exchange materials used. A highly efficient column-chromatographic procedure offers potential advantages in both separation and quantitative analysis.

METHOD

STANDARD SOLUTIONS—

Denatonium benzoate (sold by Macfarlan Smith Ltd. under the trade name Bitrex), 10 μg ml⁻¹ solution in 95 per cent. ethanol; cetrimide, 0.1 per cent. solution in 95 per cent. ethanol; and solutions of other ingredients at appropriate concentrations in 95 per cent. ethanol were used. Solutions of denatonium benzoate, cetrimide and other quaternary ammonium compounds at 0.1 per cent. concentration in chloroform were used for direct application to thin-layer chromatographic plates for identification purposes.

ION-EXCHANGE SEPARATION—

Anion-exchange column—Convert a suitable resin, e.g., Permutit De-Acidite SRA 66, into the hydroxide form, wash it with water and prepare a slurry in 95 per cent. ethanol. Fill the column (Quickfit CR 12/10 is convenient) to a depth of at least 8 cm.

Carboxymethylcellulose column—Pre-cycle a suitable amount of Whatman CM11 or equivalent in accordance with the manufacturer's instructions. Prepare a slurry in 95 per cent. ethanol and fill the column to a depth of 8 cm with one batch of slurry. Place a glass-paper disc on top of the column.

Ammoniacal ethanol, approximately 0.5 N in ammonia—Dilute 5.5 ml of ammonia solution (sp. gr. 0.88) to 200 ml with 95 per cent. ethanol.

Acidic ethanol, approximately 1 N in acid—Dilute 17.8 ml of concentrated hydrochloric acid to 200 ml with 95 per cent. ethanol.

THIN-LAYER CHROMATOGRAPHY—

Plates—Prepare a slurry of 30 g of silica gel G with 60 ml of water containing 0.3 g of potassium bromide. Spread the slurry to a thickness of 0.25 mm on 200 × 200-mm plates and allow to dry. Complete the drying in an oven at 110 °C for 10 minutes. Store over dry silica gel.

Potassium bismuth iodide solution—Dissolve successively 1.0 g of potassium iodide and 0.1 g of potassium bismuth iodide in 20 ml of hot water, add glacial acetic acid dropwise until the solution is clear and dilute to 100 ml with 95 per cent. ethanol.

Potassium dichromate solution, 5.0 per cent., aqueous.

COLORIMETRY—

Picrate reagent—Dry a small amount of picric acid at 100 °C. Weigh 0.5 g and dissolve it in 22 ml of 0.1 N sodium hydroxide solution. Dissolve 0.53 g of sodium carbonate and 0.84 g of sodium hydrogen carbonate in water, mix the solution with the picrate solution and dilute to 500 ml.

Swinny syringe filter holder and cellulose filters—Gelman Instrument Company.
Glass syringe, of 2-ml capacity with needle and Luer fittings.

PROCEDURE (1): FOR ISOLATION OF QUATERNARY AMMONIUM COMPOUNDS—

Set up the ion-exchange column system with the carboxymethylcellulose column at the bottom, the resin column above it and a reservoir at the top. With a pipette transfer into the reservoir 25.0 ml of the ethanolic solution containing not more than 0.1 per cent. of total quaternary ammonium compounds. Allow the solution to percolate through the columns at the rate of about 1 ml min⁻¹. Wash through with 25 ml of 95 per cent. ethanol. Close the taps, disconnect the columns and place the reservoir on the carboxymethylcellulose column. Set the resin column aside for regeneration.

Wash the carboxymethylcellulose column with 25 ml of the ammoniacal ethanol and then with 25 ml of neutral ethanol. Elute the quaternary ammonium ions, as chlorides, with 20 ml of acidic ethanol and wash through with another 20 ml of 95 per cent. ethanol, collecting the eluate and washings in a 100-ml beaker. Transfer to a 250-ml separating funnel containing 60 ml of saturated salt solution and wash in with 60 ml of water. Extract the mixture with 20, 10 and 10-ml portions of carbon tetrachloride and reject the extracts. Repeat the extraction with three 20-ml portions of chloroform, combine the chloroform extracts and clarify and dry the solution with anhydrous sodium sulphate. Pour the chloroform solution into a clean beaker, evaporate it to 20 ml at a temperature not exceeding 60 °C, transfer the concentrated solution into a 25-ml calibrated flask and make the volume up to the mark with chloroform.

PROCEDURE (2): FOR THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION OF QUATERNARY AMMONIUM COMPOUNDS PRESENT IN MAJOR AMOUNTS (0.1 PER CENT. m/V)

Direct application of the chloroform solution of the isolated quaternary ammonium chlorides permits identification of any compounds present at about 0.1 per cent. concentration. Apply 5 μ l of the chloroform solution of the chlorides and 5 μ l of standard chloroform solutions of similar compounds of interest to the thin-layer chromatographic plates. Chromatograph the plate in a lined tank with a mixture of 9 volumes of chloroform and 1 volume of methanol. Allow the solvent to rise 15 cm from the origin, then remove and air dry the plate. Spray it lightly with potassium bismuth iodide solution and cover it with a glass plate. Denatonium gives a red spot at R_F 0.5. Relative R_F values for other quaternary ammonium compounds are given under Results.

PROCEDURE (3): FOR THIN-LAYER CHROMATOGRAPHIC DETERMINATION OF DENATONIUM BENZOATE—

Transfer the chloroform solution to a 50-ml beaker and evaporate off the chloroform at 60 °C, then add 0.20 ml of chloroform and spot 10 μ l of the resulting solution on to a thin-layer plate, together with a standard denatonium solution to give 1.5, 2.0 and 2.5- μ g spots. If other quaternary ammonium compounds are present in amounts that show when the chloroform solution is applied directly, removal of the bulk of these compounds present is then useful. Transfer the chloroform solution of the chlorides into a 50-ml beaker and evaporate off the chloroform at 60 °C. Dissolve the residue in 25 ml of water, add 1.0 ml of dichromate solution, heat to 70 °C, cover the beaker and allow the solution to cool slowly overnight. Filter it through a small filter-paper and wash the paper with 10 ml of water. Saturate the combined filtrate and washings with salt and extract the mixture with three 10-ml portions of chloroform. Evaporate the chloroform to dryness in a specimen tube, dissolve the residue in 0.20 ml of chloroform and spot 10 μ l of this solution on to a thin-layer plate. Also spot a range of standard solutions to give 1.5, 2.0 and 2.5 μ g of denatonium on the plate.

PROCEDURE (4): FOR THIN-LAYER CHROMATOGRAPHY AND COLORIMETRY—

Evaporate the 25 ml of the quaternary ammonium chloride solution from the ion exchange or from dichromate precipitation to dryness, take up the residue in a small volume of chloroform and apply the whole concentrate in a single spot on the chromatoplate, spotting not more than 3 μ l at a time. Develop the plate in chloroform - methanol mixture (9 + 1 V/V). Dry the plate in air and render the spot visible by treatment with iodine vapour. Scrape

off the denatonium spot, transfer it to the filter and extract it twice with 2 ml of chloroform-methanol mixture (1 + 1 v/v).

Evaporate the extract to dryness at 60 °C, dissolve the residue in 5 ml of water and transfer the solution to a 30-ml separating funnel, washing it in with another 5 ml of water. Add 1.0 ml of picrate reagent and 5.0 ml of chloroform and shake the mixture for 2 minutes. Allow the phases to separate and pour off the chloroform layer into a 25-ml beaker. Dry the chloroform solution over anhydrous sodium sulphate. Fill a 1-cm spectrophotometer cell with the solution and measure the optical density at 375 nm against a blank prepared in a similar way. The optical density obtained for 50 µg applied to the chromatoplate and extracted is about 0.210 and is about 70 per cent. of that obtained by direct colorimetric analysis of the same amount of standard denatonium solution.

RESULTS

The efficiency of the ion-exchange separation was measured by titration of 0.1 per cent. *m/V* ethanolic solution of quaternary ammonium compounds with sodium tetraphenylboron solution¹⁵ before and after the separation. The results, given in Table I, show losses of not more than 0.1 ml of titrant (0.01 M sodium tetraphenylboron) on titres of 5 to 7 ml, except for cetrimide sample A, with an apparent increase for benzalkonium chloride sample A of 0.2 ml.

TABLE I
YIELD FROM ION-EXCHANGE SEPARATION

Quaternary ammonium compound	Standard solution/ml	Direct titre/ml	Titre after separation/ml
Denatonium chloride	25	6.3	6.2
Cetrimide—			
Sample A	25	6.6	6.2
Sample B {	25	7.1	7.0
{	15	4.4	4.4
Benzalkonium chloride—			
Sample A	25	6.3	6.5
Sample B {	25	7.1	7.0
{	15	4.4	4.4
Benzethonium chloride	25	5.2	5.1
Cetylpyridinium bromide	25	5.0	4.9
Domiphen bromide	25	6.4	6.3

The degree of separation of other quaternary ammonium compounds from denatonium was tested on thin-layer chromatographic plates loaded with a variety of inorganic salts. Those with higher R_F values are given in Table II and show the quaternary ammonium-ion migration value (R_D) compared with that for denatonium. It can be seen that for most purposes silica gel *plus* bromide offers the best separation. The concentration for each salt is 0.10 mol per 100 g of dry silica gel.

TABLE II
EFFECT OF ANIONS ON R_D VALUES OF QUATERNARY AMMONIUM COMPOUNDS

	Bromide	Thiocyanate	Perchlorate	Picrate	Iodide
Benzalkonium	0.71	0.85	0.69	0.82	0.92
Benzethonium	0.58	0.73	0.63	0.74	0.92
Cetrimide	0.21	0.19	0.22	0.28	0.29
Cetylpyridinium	0.29	0.31	0.24	0.42	0.57
Domiphen	0.54	0.58	0.65	0.42	0.74
Denatonium (actual) R_F ..	0.56	0.50	0.33	0.55	0.47

Several experiments were carried out to evaluate the quantitative aspects of the thin-layer chromatographic results by visual assessment. One series of tests gave the results shown in Table III. Solutions of toiletry ingredients were spiked with denatonium benzoate at the specified 2 mg l⁻¹ level and at concentrations 10 and 20 per cent. below this level, and were subjected to procedures 1 and 3. The results illustrate the precision given by the procedure involving visual assessment. Statistical examination of the results is rendered difficult by the tendency to group around expected values.

TABLE III
THIN-LAYER CHROMATOGRAPHIC - VISUAL ESTIMATES OF ADDED DENATONIUM

Component of solution analysed	Amount present, per cent.	Denatonium added/mg l ⁻¹		
		1.6	1.8	2.0
		Denatonium found/mg l ⁻¹		
Perfume essential oil	3	} 1.8	1.9	1.9
and diethyl phthalate	1			
Polyvinylpyrrolidone	4	1.7	1.9	2.0
Polyvinylpyrrolidone acetate	4	1.6	2.0	1.9
National starch resin	4	0.8	1.6	1.8
Nonidet P40 [poly(ethylene glycol)]	4	1.6	Obscured	1.7
Tween 80	4	1.6	1.8	1.9
Lissapol (alkylsulphate)	4	1.9	2.0	1.9
Hexachlorophane	0.2	1.7	1.6	1.7
Cetrimide*	0.1	1.6	1.6	1.8

* The dichromate clean-up of procedure 3 was used for this substrate.

Procedure 4, the colorimetric analysis of material extracted from thin-layer spots, was carried out only on pure standards, with the results given in Table IV.

TABLE IV
COLORIMETRIC ANALYSIS OF THIN-LAYER SPOTS

<i>Optical densities obtained from 50-μg spots—</i>								
Plate 1	0.198	0.216	0.199	0.210	0.200	0.251		
Plate 2	0.187	0.234	0.199	0.221	0.213	0.185	0.275	
Over-all average	0.214							
Standard deviation	0.025							
<i>Optical density for 50 μg of denatonium benzoate (direct)—</i>								
	0.300							

The precision of the results for extracted thin-layer spots indicates that no advantage would be obtained from the application of colorimetry to separated sample spots. Procedures 1 and 3 are in use in this laboratory for the examination of routine samples of denatured ethanolic preparations. Commercial confidence precludes useful discussion of results in detail. The additional procedures described, *i.e.*, the qualitative identification of other quaternary ammonium compounds and the quantitative determination after ion-exchange separation, have been found useful with this type of sample. Table IV is included to show that while 98 per cent. recovery of steroids was obtained by this method,¹⁸ the recovery of denatonium salt was both low and uncertain and offered no advantage over visual assessment in this application.

This paper is published by permission of the Government Chemist, Dr. H. Egan.

REFERENCES

- Hay, J. E., British Patents 955309 and 955310, 1960.
- "Official Methods of Analysis," Eleventh Edition, The Association of Official Analytical Chemists, Washington, D.C., 1970, 20.076-20.080.
- Scott, G. V., *Analyt. Chem.*, 1968, **40**, 768.
- Sheiham, I., and Pinfold, T. A., *Analyst*, 1969, **94**, 387.
- Metcalfe, L. D., *J. Amer. Oil Chemists Soc.*, 1963, **40**, 25.
- Grossi, A., and Vece, R., *J. Gas Chromat.*, 1965, **3**, 170.
- Laycock, H. H., and Mullery, B. A., *J. Pharm. Pharmacol.*, 1966, **18**, Suppl. 95
- Vessman, J., and Stromberg, S., *Acta Pharm. Suec.*, 1969, **6**, 505.
- Boyd, G. E., and Larsen, Q. V., *J. Amer. Chem. Soc.*, 1967, **89**, 6038.
- Small, H., *Ibid.*, 1968, **90**, 2217.
- Metcalfe, L. D., *Analyt. Chem.*, 1960, **32**, 70.
- Bregoff, H. M., Roberts, E., and Delwiche, C. C., *J. Biol. Chem.*, 1953, **205**, 565.
- Garcia, J., and Couerbe, J., *Chemist Analyst*, 1956, **38**, 432.
- Holness, H., and Stone, W. R., *Analyst*, 1958, **83**, 71.
- Cross, J. T., *Ibid.*, 1965, **90**, 315.
- Covello, M., and Schettino, O., *Pharmaco Ediz. Practica*, 1966, **21**, 145.
- Gordon, J. E., *J. Chromat.*, 1965, **20**, 38.
- Masarachchia, R. A., and Gawienowski, A. M., *Steroids*, 1968, **11**, 718.

Received February 10th, 1972

Accepted May 19th, 1972

Automated Method for the Microdetermination of Sulphur

BY J. KEAY,* P. M. A. MENAGÉ AND G. A. DEAN†

(Division of Soils, CSIRO, W.A. Laboratories, Private Bag, P.O., Wembley, Western Australia)

Sulphate at concentrations of less than 30 mg l⁻¹ of sulphur is determined on the AutoAnalyzer by reduction to hydrogen sulphide, distillation of the latter and its subsequent colorimetric determination.

The method has been found to be suitable for aqueous solutions of sulphate, digests of leaf samples and animal faeces, and extracts of soils; it is applicable to a wide range of agricultural, biological and industrial materials.

METHODS for the determination of low concentrations of sulphate, below 30 mg l⁻¹ of sulphur, based on reduction of sulphate to hydrogen sulphide and the subsequent distillation of the latter, have several advantages. Firstly, sulphur is separated from any interfering substances in the sample and, secondly, sensitive methods are available for determining sulphide. The practical details of the sulphate reduction and distillation procedure were established by Johnson and Nishita¹ and further work by Gustafsson² defined more clearly the effects of interferences and variations of the procedures. These workers used the methylene blue colorimetric finish.

Although the molar extinction coefficient for methylene blue is at the upper limit of sensitivity, automation of the method presented practical difficulties. The simpler bismuth sulphide finish described by Dean³ was found to be sufficiently sensitive and much easier to adapt for automated analysis.

In the automated method for the determination of sulphate described in this paper the reduction and distillation procedure by Johnson and Nishita¹ has been combined with the colloidal bismuth sulphide finish by Dean.³

EXPERIMENTAL

REAGENTS—

Sodium hydroxide solution—Dissolve 40 g of analytical-reagent grade sodium hydroxide in distilled water and make the volume up to 1 litre.

Formic acid, 99 per cent.—Analytical-reagent grade.

Acetic acid, glacial—Analytical-reagent grade.

Reducing mixture—Transfer 500 ml of hydriodic acid (sp. gr. 1.7), 250 ml of formic acid (99 per cent.) and 125 ml of hypophosphorous acid (50 per cent. *m/m*) to a 2-litre boiling flask. Bubble nitrogen through the solution and boil the latter gently under reflux for about 15 minutes to remove sulphur present as an impurity in the reagents.

Bismuth reagent—Heat analytical-reagent grade bismuth subnitrate (2 g) or analytical-reagent grade bismuth nitrate pentahydrate (3.4 g) with 460 ml of glacial acetic acid until it has dissolved. Filter the solution through a No. 50 Whatman filter-paper, cool and add 500 ml of water containing 6 g of gelatine that has been previously dissolved by warming. Dilute the mixture to 2 litres.

Standard sulphur solutions—Dissolve 5.435 g of potassium sulphate (dried at 105 °C) in distilled water and dilute to 1 litre in a calibrated flask. This solution contains 1000 mg l⁻¹ of sulphur. Prepare suitable dilute standards to cover the required range.

INSTRUMENTATION—

The complete configuration of the manifold and a diagram of the flow sequence is shown in Fig. 1.

The sample solution containing up to 30 mg l⁻¹ of sulphur as sulphate is mixed with formic acid, segmented with air and pumped through a glass heating coil immersed in an oil-bath maintained at 120 °C. The design of the oil-bath has been described in a previous

* Present address: Kaymin Laboratories, Murray Road, Welshpool, Western Australia.

† Present address: Analogic, West Perth, Western Australia.

paper by two of the present authors.⁴ The reducing mixture is pumped through a similar coil in the oil-bath. The two pre-heated solutions are then allowed to mix, a stream of nitrogen is injected and the reaction is completed during passage of the mixture through the remaining length of glass coil in the bath.

The liquid and gas phases emerging from the heating coil separate in a condenser-separator and the liquid phase is pumped to waste.

By selecting a pump tube with a capacity equal to the volume of condensate, a liquid seal is formed at the base of the condenser-separator, thus preventing any loss of hydrogen sulphide. The gas phase containing the hydrogen sulphide is removed by the flow of nitrogen and is then passed over a slowly flowing stream of sodium hydroxide solution in which it is absorbed. The sodium hydroxide solution containing sodium sulphide is passed through a phase separator from which a sample is continuously withdrawn.

This sub-sample is allowed to react with a solution of bismuth nitrate so as to produce a brown colloidal suspension of bismuth sulphide, which is stabilised with gelatine. The optical density of the suspension of bismuth sulphide is measured in a colorimeter at 410 nm. Beer's law is obeyed for concentrations up to 25 mg l⁻¹ of sulphur.

Heating coil—As shown in Fig. 1, the unit consisted of two 30-cm long pre-heating coils joined to an approximately 100-cm long single coil. At a point 45 cm from the junction of the pre-heating coils, provision was made to inject a stream of nitrogen, following which a further 60-cm length of coil was provided to ensure that the reduction was taken to completion. The use of pre-heating coils has been found necessary to facilitate the rapid reduction of sulphate.

Several variations in coil design were tested for length and point of injection of nitrogen gas; the greatest sensitivity and recovery were obtained with the design described above. The heating coil was made of Pyrex glass (4.5 mm outside diameter) and was 17.5 × 3.15 cm overall.

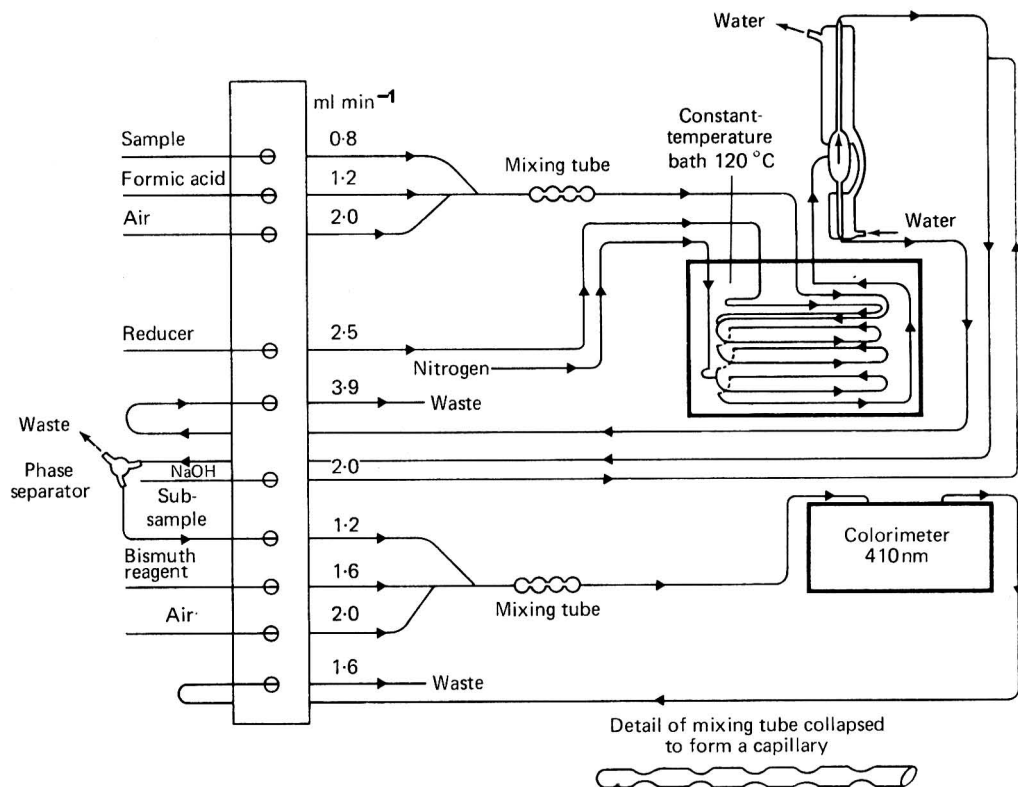


Fig. 1. Manifold and flow sequence for the automated determination of sulphur

Condenser - separator—The size of the condenser is critical as dead space must be kept to a minimum and at the same time provision must be made for complete condensation of acid vapours. The design illustrated in Fig. 2 was found to be the most suitable.

Nitrogen flow-rate—The flow-rate of nitrogen is also critical and, with the configuration given, a rate of 100 to 150 ml min⁻¹ was necessary to flush all the sulphide rapidly through the system without causing excessive back-pressure.

Air was found to be unsatisfactory as a carrier gas because iodine was formed in the condenser and was carried over into the sodium hydroxide solution.

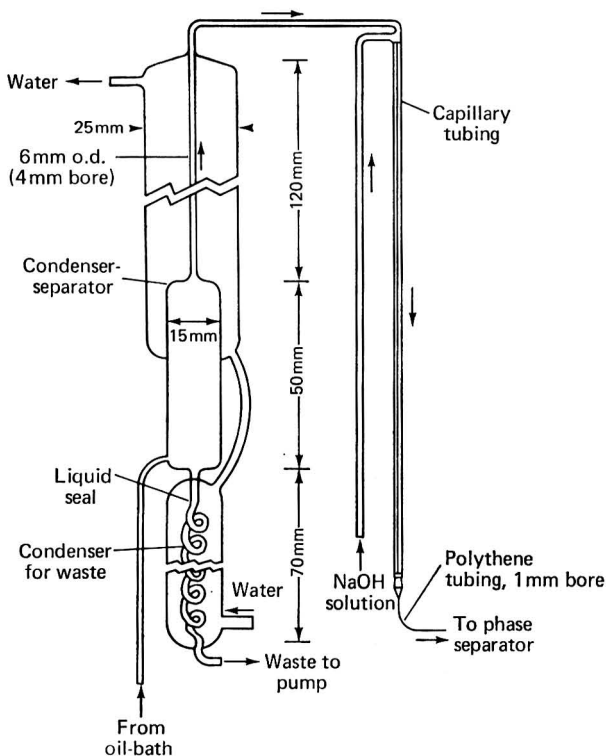


Fig. 2. Details of condenser - separator

CONDITIONING THE APPARATUS—

New glass tubing, heating coils and the condenser - separator were conditioned once with a high sulphur standard. In contrast with the manual method no further conditioning was required.

PRECAUTION—

To minimise any risk from poisonous phosphine that may be released into the laboratory, the waste from the still and the excess of solution from the phase separator were pumped through activated charcoal to a water pump.

RESULTS AND DISCUSSION

A recorder tracing showing the sensitivity and reproducibility of the method is shown in Fig. 3. After continuous determination of sulphur for 8 hours the reproducibility of the standards was still good and no base-line drift was noticed. The optical density was measured in an electronic colorimeter by using a 413-nm interference filter and a flow cell 15 mm in length. The sampling and wash times were set at 60 s, thus enabling thirty samples to be analysed per hour.

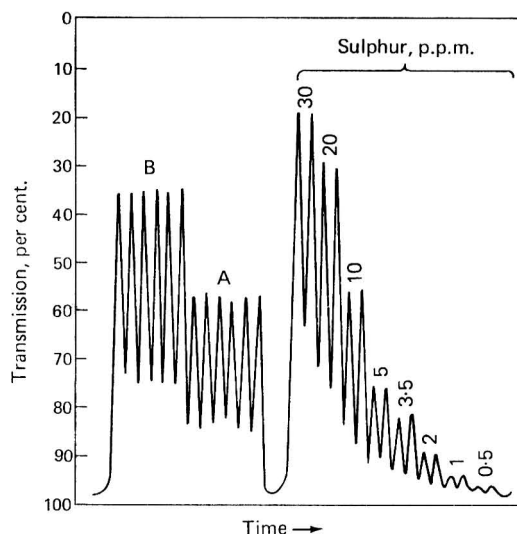


Fig. 3. Recorder tracing showing reproducibility of the automated method

Six samples consisting of poplar leaves (19064, two samples), pine needles (19056) cysteine and methionine (two samples) were treated by using the procedure described by Iismaa⁵ in which the sample is oxidised in a flask in the presence of oxygen and the sulphur dioxide formed absorbed in 0.4 M potassium hydroxide solution. The resulting digests were analysed to determine sulphur by two analysts, one of whom used the present automated method and the other a manual method. The manual method used was the reduction procedure of Johnson and Nishita,¹ followed by titration with mercury(II) chloride. The results are shown in Table I.

TABLE I
COMPARISON OF RESULTS BETWEEN AUTOMATED AND MANUAL METHODS

Sample	Amount/ mg	Final volume	Automated method					Mean, p.p.m.	Standard deviation, p.p.m.
			Sulphur in solution, p.p.m.						
Cysteine ..	100	1 litre	18.2	18.2	18.0	18.3	18.3	18.20	0.12
Methionine (a)	50		10.5	10.7	10.6	10.7	10.7	10.64	0.09
Methionine (b)	50		10.3	10.4	10.5	10.4	10.5	10.42	0.08
19064 (a) ..	250	100 ml	10.9	10.8	11.0	11.2	11.0	10.98	0.15
19064 (b) ..	250		10.2	10.2	10.4	10.4	10.2	10.28	0.11
19056 ..	250		4.4	4.5	4.5	4.6	4.5	4.50	0.07
Sample	Amount/ mg	Final volume	Manual method					Mean, p.p.m.	Standard deviation, p.p.m.
			Sulphur in solution, p.p.m.						
Cysteine ..	100	1 litre	18.5	18.8	18.8	18.8	17.8	18.54	0.43
Methionine (a)	50		11.2	9.9	11.1	11.0	11.9	11.02	0.72
Methionine (b)	50		11.1	11.9	11.9	11.9	10.2	11.40	0.75
19064 (a) ..	250	100 ml	11.5	11.7	11.7	11.7	11.7	11.66	0.09
19064 (b) ..	250		11.9	11.8	11.3	11.9	11.6	11.70	0.25
19056 ..	250		4.8	4.8	4.7	4.7	4.7	4.74	0.05

Analyses of variance were carried out on the results from the automated and manual methods shown in Table I. The error mean squares were 0.0081 (on 20 degrees of freedom) and 0.2252 (24 degrees of freedom), respectively, the automated method having a significantly lower error mean square ($p < 0.001$) than the manual method.

AUTOMATED METHOD—

Duplicate samples of methionine and of poplar leaves were analysed and the results were found to differ significantly ($p < 0.001$). This was probably caused by errors introduced during preparation of the two different digests. The sample means for cysteine and methionine were compared with the theoretical values (Table II).

Values for cysteine samples did not differ significantly from the theoretical value but there was a significant difference between the theoretical and determined values for the second methionine sample ($p < 0.01$), that for the automated method being lower than the theoretical value.

TABLE II
COMPARISON BETWEEN THEORETICAL AND DETERMINED VALUES FOR
AUTOMATED AND MANUAL METHODS

Sample	Mean sulphur content by automated method		Theoretical sulphur content, per cent.	Mean sulphur content by manual method	
	p.p.m.	Per cent.		p.p.m.	Per cent.
Cysteine	18.20	18.20	18.26	18.54	18.54
Methionine (a)	10.64	21.28	21.48	11.02	22.04
Methionine (b)	10.42	20.84	21.48	11.40	22.80
19064 (a)	10.98	0.44	*	11.66	0.46
19064 (b)	10.28	0.41	*	11.70	0.47
19056	4.50	0.18	*	4.74	0.19

* The poplar and pine samples were analysed to determine sulphur by two independent overseas laboratories. The analytical methods used were not disclosed. However, their results are shown below for comparison with those obtained here.

	Sample 19064	Sample 19056
Sulphur, per cent., by laboratory A	0.43	0.16
Sulphur, per cent., by laboratory B	0.40	0.14

MANUAL METHOD—

The results for the manual method are more variable than those for the automated method. However, a "t-test" of the results, by using Welch's approximation,⁶ showed that the manual method gives significantly higher values ($p < 0.01$) than the automated method.

EFFECTS OF VARIABLES—

Effect of water on the reduction of sulphate—This effect, which is caused by dilution of the reducing mixture, was tested by using two different mixtures, consisting of hydriodic and hypophosphorous acids plus acetic acid in one mixture and plus formic acid in the other. In both instances, the presence of water caused some loss in sensitivity but the loss was far greater when acetic acid was used in the reducing mixture. Both tests were carried out with acetic acid or formic acid, respectively, pumped continuously to the still with the sample, because it was observed that an excess of acid present in the heating coil prior to the introduction of the sample minimised the loss in sensitivity due to the presence of water.

A series of standard solutions, each containing 10 p.p.m. of sulphur, was prepared in formic acid at concentrations within the range 1 to 97 per cent. V/V . Automated analysis gave optical densities which varied with the concentration of acetic acid or formic acid.

The effect with formic acid is shown in Fig. 4.

The ratio of aqueous sample solution to reducing mixture is therefore critical because an increase in the ratio of sample to reducing mixture will result in a corresponding decrease in sensitivity.

Effect of acids and alkalis on the recovery of sulphur—Acidic or alkaline extracts are currently used in the determination of sulphate-sulphur. The effect of the different extractants on the recovery of sulphur was therefore tested. Ninety-nine per cent. formic acid was used in preference to the 90 per cent. acid (which is used in most manual procedures) because it could accommodate a wide range of acidic and alkaline extracts without changing the sensitivity of the method significantly.

Effect of temperature of heating bath on sulphur recovery—At 100 °C the sensitivity was low and reproducibility very poor. Increasing the temperature to 120 °C resulted in higher sensitivity and better reproducibility. No increase in optical density was observed at temperatures higher than 120 °C but the risk of phosphine formation is greater.

Fig. 5 illustrates the relationship between percentage transmission and temperature of distillation.

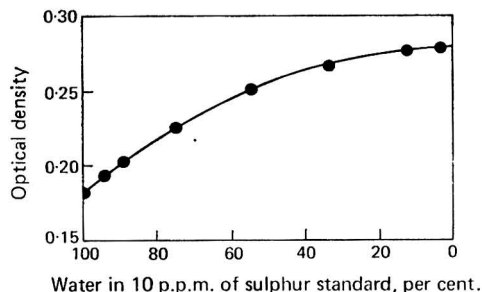


Fig. 4. Effect of the presence of water in sample on sulphur recovery

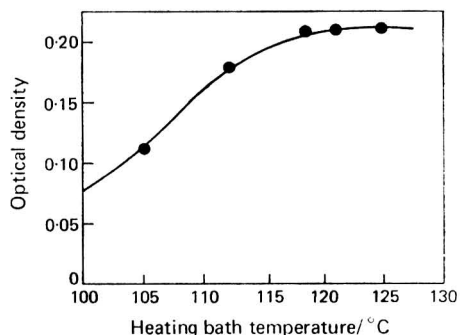


Fig. 5. Effect of temperature of distillation on percentage transmission

INTERFERENCES—

The effect of substances present in the samples, which could interfere at the reduction and distillation stages, has been investigated in detail by several authors, including Johnson and Nishita,¹ Gustafsson² and Freney.⁷ In this laboratory the extractants tested were 0.5 M sodium hydrogen carbonate, 1 M calcium chloride, 1 M sodium hydroxide and 1 M hydrochloric acid solutions. The extracts were analysed to determine sulphur without interference from the extractants. It is recommended, however, that standards should be made up in solutions the compositions of which are as nearly as possible the same as those used for extractants.

The distillation step prevents most substances present in samples from interfering with the formation of the colloidal suspension of bismuth sulphide. Solutions containing up to 1000 p.p.m. of sulphur as cysteine, cystine, methionine, thiourea and sulphanilic acid were tested for possible interference of organic sulphur compounds in the determination of sulphate. No interference was detected. However, as found by Freney,⁷ who used the manual method, all of the following inorganosulphur compounds (sodium salts) were reduced to some extent and would therefore interfere in the determination of sulphate: sulphide, thiosulphate, dithionite, dithionate, metabisulphite and persulphate.

Tests for nitrate interference showed that the recovery of sulphur was not affected by the presence of 50 p.p.m. of nitrate in solution.

We gratefully acknowledge the help of Dr. F. Hingston for his suggestions, Mr. J. A. Vallerini for the manual analysis of samples, and Mr. N. A. Campbell and Miss L. Foord for the statistical analysis of the results.

REFERENCES

1. Johnson, C. M., and Nishita, H., *Analyt. Chem.*, 1952, **24**, 736.
2. Gustafsson, L., *Talanta*, 1960, **4**, 226.
3. Dean, G. A., *Analyst*, 1966, **91**, 530.
4. Keay, J., and Menagé, P. M. A., *Ibid.*, 1969, **94**, 895.
5. Iismaa, O. J., *J. Aust. Inst. Agr. Sci.*, 1959, **25** (2), 136.
6. Brownlee, K. A., "Statistical Theory and Methodology in Science and Engineering," John Wiley and Sons, New York, 1960, p. 235.
7. Freney, J. R., *Soil Sci.*, 1958, **86** (5), 241.

Received March 20th, 1972
Accepted June 2nd, 1972

Sorption of Inorganic Phosphate by Laboratory Ware. Implications in Environmental Phosphorus Techniques

By J. C. RYDEN, J. K. SYERS AND R. F. HARRIS

(Department of Soil Science, University of Wisconsin, Madison, Wisconsin 53706, U.S.A.)

Acid-washed glass sorbed appreciable amounts of inorganic phosphate from distilled water systems and significant amounts (up to 20 per cent.) from lake waters within 1 to 6 hours of contact. Pre-treatment of polycarbonate with phosphate eliminated sorption of inorganic phosphate from distilled water and lake water and gave mean recovery values that had a low standard deviation. Lake waters should be filtered within 6 hours of collection. "Phosphated" polycarbonate is recommended for the storage of standard phosphate solutions containing small amounts of inorganic phosphate and for the sampling of lake waters and their storage in bulk subsequent to filtration. The use of acid-washed glassware should be avoided for these purposes.

THE recent increase in interest in the amounts, forms and mobility of inorganic phosphate in environmental samples containing phosphate levels of below $20 \mu\text{g l}^{-1}$ of phosphorus necessitates the rigorous examination of the potential problems involved in the accurate analysis of large numbers of samples that contain such low levels of phosphate. In particular, because samples are collected and stored in containers of some sort, it is essential to understand the characteristics of materials that are commonly used in laboratory ware for sorbing inorganic phosphate from solution. Specific aspects of environmental phosphate techniques to which sorption of phosphate by laboratory ware is particularly relevant include the collection and storage of water samples and the storage of sediment and soil extracts prior to analysis, the storage of dilute standard solutions of inorganic phosphate, the determination of the ability of sediments or soils to sorb or desorb inorganic phosphate, and the interpretation and calculation of phosphate turnover times and rates in aquatic systems.

Sorption of dissolved inorganic phosphate by glass^{1,2} and by polyethylene containers¹ has been reported. The significance of this effect, however, is ill defined, although it is apparently dependent upon the concentration of inorganic phosphate in solution.² Pre-treatment of glassware with acid is recommended³ for the removal of sorbed inorganic phosphate, but the effect of this pre-treatment on the subsequent sorption of inorganic phosphate from solution is not known.

This paper is concerned with the ability of glass, polypropylene and polycarbonate containers to sorb inorganic phosphate from distilled-water and lake-water systems as a function of pre-treatment of the containers, and a new procedure for eliminating the sorption of dissolved inorganic phosphate during storage is described. The implications of these findings in the techniques for obtaining satisfactory routine determinations of dissolved inorganic phosphate in environmental samples that contain low levels of inorganic phosphate (below $20 \mu\text{g l}^{-1}$ of phosphorus) are discussed.

EXPERIMENTAL

The ability of 50-ml glass, polypropylene and polycarbonate centrifuge tubes (Oak Ridge type, with screw caps) to sorb dissolved inorganic phosphate from distilled water was evaluated following three pre-treatments of each type of tube: (i) washing with distilled water; (ii) soaking in hot 1 + 1 hydrochloric acid followed by washing with distilled water³; and (iii) soaking in hot 1 + 1 hydrochloric acid, washing with tap water, soaking in 0.1 M potassium dihydrogen orthophosphate solution for 1 day, and washing thoroughly with tap water and then six times with distilled water. To evaluate the sorption of dissolved inorganic phosphate in a distilled-water system, 40-ml aliquots of solutions containing 0, 2, 5, 10, 20 and $60 \mu\text{g l}^{-1}$ of phosphorus (added as potassium dihydrogen orthophosphate) were placed

in the pre-treated tubes, which were shaken end-over-end for 2 days at 25 °C; a suitable aliquot was then taken for the determination of dissolved inorganic phosphate. The release of inorganic phosphate as a function of time from "phosphated" [pre-treatment (iii)] polycarbonate tubes to distilled water and to distilled water containing 166 mg l⁻¹ of hydrogen carbonate ion was evaluated in a similar manner. The effect of added hydrogen ions (1 mequiv) on the sorption of dissolved inorganic phosphate by acid-washed 250-ml calibrated flasks containing a dilute phosphate standard (10 µg l⁻¹ of phosphorus) was also determined.

The sorption of dissolved inorganic phosphate from lake waters collected in Lake Wingra (a hard-water lake bordering Madison, Wisconsin) and in Lake Mary (a soft-water lake in Vilas County, Wisconsin) by glass, polypropylene and polycarbonate following pre-treatment (ii), and by polycarbonate following pre-treatment (iii), was also determined. Water from Lake Wingra was collected in pre-treated tubes, centrifuged to remove suspended material and transferred to a second set of tubes that had been pre-treated in the same manner. Centrifugation was used instead of filtration through a Millipore filter so as to avoid contact with material other than that in which a particular sample was collected. The Lake Mary water had previously been stored at 4 °C and did not contain a detectable amount of dissolved inorganic phosphate. For this reason, 10 µg l⁻¹ of phosphorus was added to the filtrate following removal of the material with particle size above 0.45 µm by filtration through a Millipore filter (centrifugation was unsatisfactory for the removal of suspended material) prior to the transfer of the sample to the pre-treated tubes. Sets of tubes were analysed after storage for 1, 6 and 24 hours at 4 °C.

The effect of the method and time of storage on the concentration of dissolved inorganic phosphate in Lake Wingra water was also investigated. For this purpose, 2 litres of lake water were sampled in phosphated-polycarbonate bottles and returned to the laboratory. Approximately 1 litre of the water was filtered through a 0.45-µm Millipore filter and 25-ml aliquots of the filtrate were immediately transferred to 50-ml calibrated flasks that had been washed with distilled water and in which the colour was subsequently developed. The flasks were stored at 4 °C for various periods of time. The remainder of the lake water was not filtered and was stored in the collection container at 4 °C for various periods of time; immediately prior to analysis, samples were filtered through a 0.45-µm Millipore filter and aliquots taken for colour development.

Inorganic phosphate was determined by the method of Murphy and Riley⁴ by using a Unicam SP1800B spectrophotometer, scale expansion and a 4-cm path length. All reported values represent the mean of at least three determinations.

RESULTS

DISTILLED WATER—

With pre-treatment (i) (washing with distilled water), all of the dissolved inorganic phosphate was sorbed from the distilled-water system by glass, polypropylene and polycarbonate when 2 µg l⁻¹ of phosphorus was added, and by polycarbonate when 5 µg l⁻¹ of phosphorus was added (Table I). At each level of inorganic phosphate above 5 µg l⁻¹ of phosphorus, there was only a small variation in the recoveries for the three materials, the mean values having low standard deviations.

Pre-treatment with acid [pre-treatment (ii)] greatly increased the amount of added inorganic phosphate sorbed by glass, had little or no effect on that sorbed by polypropylene and decreased that sorbed by polycarbonate, compared with the sorptions after pre-treatment (i). Acid-washed glass sorbed all of the inorganic phosphate at the 5 µg l⁻¹ of phosphorus level, 96 per cent. of that added at the 10 µg l⁻¹ of phosphorus level and 82 per cent. of that added at the 20 µg l⁻¹ of phosphorus level; even at the highest level of added phosphate (60 µg l⁻¹ of phosphorus), 17 per cent. was sorbed. Similar results were obtained with new and well used glass tubes following washing with acid, which suggests that the sorption of dissolved inorganic phosphate is independent of the weathering of the glass. Sorption of dissolved inorganic phosphate by acid-washed glass was essentially complete after shaking with a solution containing 10 µg l⁻¹ of phosphorus for 1.5 hours (results not presented). Acid-washed polypropylene appears to be capable of decreasing the concentration of dissolved inorganic phosphate by a fairly constant amount (2.5 to 3.2 µg l⁻¹ of phosphorus). In contrast to the results for polypropylene, the acid-washing pre-treatment increased the standard deviation

of the mean recoveries of added phosphate obtained with glass and polycarbonate, compared with the results obtained after pre-treatment (*i*).

The recovery of dissolved inorganic phosphate by phosphated [pre-treatment (*iii*)] glass and polypropylene was highly variable at all levels of added phosphate, some mean values indicating sorption and others release, and high standard deviations were obtained for all values. In contrast, phosphated polycarbonate neither sorbed nor released a significant amount of inorganic phosphate at all levels of added phosphate. The standard deviations of the mean values were low and not significantly different from the expected error ($\pm 0.3 \mu\text{g l}^{-1}$ of phosphorus) in the colorimetric determination of inorganic phosphate, as used in this study. No inorganic phosphate was released into distilled water by phosphated polycarbonate tubes during 28 days.

In an experiment to determine the effect of added hydrogen ion on the sorption of dissolved inorganic phosphate by acid-washed 250-ml calibrated flasks containing a phosphate standard containing $10 \mu\text{g l}^{-1}$ of phosphorus, it was found that the extent of sorption after storage for 4 hours without shaking after an initial mixing was the same (30 per cent.) in the presence or absence of 1 mequiv of hydrogen ion.

LAKE WATERS—

The extent of sorption of dissolved inorganic phosphate by glass, polypropylene and polycarbonate, following washing with acid, from waters collected in Lake Mary and Lake Wingra was determined by comparing the values for inorganic phosphate remaining in solution with those obtained in phosphated polycarbonate. The validity of using the dissolved inorganic phosphate concentration obtained with phosphated polycarbonate as the reference concentration for Lake Wingra water is substantiated by the fact that there was no detectable release of inorganic phosphate from phosphated polycarbonate during 2 days of end-over-end shaking with distilled water containing the same concentration of hydrogen carbonate ion (166 mg l^{-1}) as Lake Wingra water. Also, there was no significant difference between the concentrations of dissolved inorganic phosphate remaining in solution in the lake water when stored in acid-washed or phosphated polycarbonate. The sorption of inorganic phosphate by acid-washed glass was appreciably less from the lake-water (Table II) than from the distilled-water system (Table I). By using the value of $10.1 \pm 0.2 \mu\text{g l}^{-1}$ of phosphorus, obtained with phosphated polycarbonate after 1 hour, as the true concentration of dissolved inorganic phosphate in Lake Wingra water (centrifuged sample), acid-washed glass sorbed from 10 to 20 per cent. of the inorganic phosphate over periods from 1 to 6 hours, respectively (Table II). Similar results were obtained for Lake Mary water. After 24 hours, however, there was an appreciable decrease in the concentration of dissolved inorganic phosphate remaining in Lake Wingra water with the three materials following washing with acid and even with phosphated polycarbonate. There was no corresponding decrease after 2 days in the distilled-water system with phosphated polycarbonate (Table I). Furthermore, the higher concentration of competing anions in Lake Wingra water, compared with distilled water, would tend to decrease rather than increase the sorption of dissolved inorganic phosphate. No significant decrease in the concentration of dissolved inorganic phosphate in Lake Mary water (filtered sample) was observed after storage for 24 hours in phosphated polycarbonate. There was essentially no sorption of dissolved inorganic phosphate by polypropylene and polycarbonate following washing with acid, relative to that obtained with phosphated polycarbonate, after 6 hours for Lake Wingra water and 24 hours for Lake Mary water, respectively (Table II). These findings contrast with those obtained in the distilled-water system (Table I).

After storage at 4°C for 1 day in phosphated polycarbonate, there was a significant decrease in the concentration of dissolved inorganic phosphate in Lake Wingra water that was not filtered immediately after collection (Table III). The decrease in the concentration of dissolved inorganic phosphate in the unfiltered water was 4.7 and $6.8 \mu\text{g l}^{-1}$ of phosphorus after storage for 1 and 7 days, respectively, relative to the value of $10.8 \mu\text{g l}^{-1}$ of phosphorus obtained for Lake Wingra water that was analysed as soon as possible (less than 1 hour) after collection. These results are consistent with those obtained on Lake Wingra water that was centrifuged after collection and stored at 4°C for 1 day (Table II), indicating that the component, either biological or chemical, that is responsible for the removal of dissolved inorganic phosphate from solution is not removed by the centrifugation procedure.

TABLE I

SORPTION OF DISSOLVED INORGANIC PHOSPHATE FROM A DISTILLED-WATER SYSTEM BY GLASS, POLYPROPYLENE AND POLYCARBONATE AFTER DIFFERENT PRE-TREATMENTS

Inorganic phosphate added/ $\mu\text{g l}^{-1}$ of phosphorus	Inorganic phosphate remaining in solution after 2 days/ $\mu\text{g l}^{-1}$ of phosphorus											
	Glass			Polypropylene			Polycarbonate			Polycarbonate		
	<i>i</i> *	<i>ii</i> †	<i>iii</i> ‡	<i>i</i> *	<i>ii</i> †	<i>iii</i> ‡	<i>i</i> *	<i>ii</i> †	<i>iii</i> ‡	<i>i</i> *	<i>ii</i> †	<i>iii</i> ‡
0	0.0	0.0	0.5 \pm 0.8	0.0	0.0	2.6 \pm 2.5	0.0	0.0	0.0	0.0	0.0	
2	0.0	0.0	3.8 \pm 3.4	0.0	0.0	2.5 \pm 1.8	0.0	0.0	0.0	0.0	0.0	
5	1.2 \pm 0.0	0.0	1.7 \pm 2.9	2.4 \pm 0.0	2.5 \pm 0.0	2.7 \pm 2.4	0.0	0.0	0.0	0.0	0.0	
10	5.6 \pm 0.7	0.4 \pm 0.7	10.2 \pm 2.5	7.6 \pm 0.7	7.4 \pm 0.0	8.5 \pm 0.9	4.8 \pm 0.0	0.0	0.0	0.0	0.0	
20	14.4 \pm 1.2	3.7 \pm 4.4	21.6 \pm 2.5	16.8 \pm 1.2	16.8 \pm 0.7	21.5 \pm 2.2	16.4 \pm 0.7	19.3 \pm 3.8	19.3 \pm 3.8	19.3 \pm 3.8	19.5 \pm 0.6	
60	53.3 \pm 0.7	49.8 \pm 3.2	68.5 \pm 5.6	52.1 \pm 0.7	57.4 \pm 0.6	57.6 \pm 2.8	55.7 \pm 0.7	58.1 \pm 1.4	58.1 \pm 1.4	58.1 \pm 1.4	60.5 \pm 0.5	

* Pre-treatment (*i*): washing with distilled water.

† Pre-treatment (*ii*): soaking in hot 1 + 1 hydrochloric acid followed by washing with distilled water.

‡ Pre-treatment (*iii*): soaking in hot 1 + 1 hydrochloric acid, washing with tap water, soaking in 0.1 M potassium dihydrogen orthophosphate solution, and washing thoroughly with tap and then distilled water.

TABLE II

SORPTION OF DISSOLVED INORGANIC PHOSPHATE FROM LAKE WATERS BY GLASS, POLYPROPYLENE AND POLYCARBONATE FOLLOWING WASHING WITH ACID, AND BY PHOSPHATED POLYCARBONATE AS A FUNCTION OF TIME OF CONTACT

Time of contact/hours	Inorganic phosphate remaining in solution/ $\mu\text{g l}^{-1}$ of phosphorus					
	Acid-washed glass	Acid-washed polypropylene	Acid-washed polycarbonate	Acid-washed glass	Acid-washed polypropylene	Acid-washed polycarbonate
<i>Lake Wingra</i> —						
1	8.9 \pm 1.2	9.6 \pm 1.7	9.0 \pm 1.2	9.0 \pm 1.2	10.1 \pm 0.2	10.1 \pm 0.2
6	7.9 \pm 0.2	9.2 \pm 0.2	10.1 \pm 0.2	10.1 \pm 0.2	10.3 \pm 0.6	10.3 \pm 0.6
24	6.1 \pm 0.5	5.3 \pm 0.4	6.3 \pm 2.0	6.3 \pm 2.0	7.1 \pm 1.6	7.1 \pm 1.6
<i>Lake Mary</i> —						
1	8.4 \pm 1.0	9.6 \pm 0.0	9.6 \pm 0.0	9.6 \pm 0.0	10.0 \pm 0.0	10.0 \pm 0.0
6	8.0 \pm 1.1	8.9 \pm 0.3	9.6 \pm 0.0	9.6 \pm 0.0	9.9 \pm 0.4	9.9 \pm 0.4
24	7.8 \pm 0.1	9.0 \pm 0.2	9.4 \pm 0.4	9.4 \pm 0.4	9.8 \pm 0.2	9.8 \pm 0.2

In contrast, there was no significant decrease in the concentration of dissolved inorganic phosphate in Lake Wingra water that was filtered immediately after collection and stored at 4 °C for periods of time up to 7 days in the calibrated glass flasks in which the colour was subsequently developed (Table III). Also, when a 25-ml aliquot was removed from 50 ml of filtered Lake Wingra water that had been stored for 7 days at 4 °C in a 50-ml calibrated glass flask, there was no difference in the concentration of dissolved inorganic phosphate in the sample removed (25 ml) and that remaining in the flask (25 ml), indicating that no detectable sorption of inorganic phosphate by the calibrated flasks that are used for routine phosphate determinations occurs.

TABLE III
EFFECT OF FILTRATION AND TIME OF STORAGE ON THE CONCENTRATION OF
DISSOLVED INORGANIC PHOSPHATE IN WATER FROM LAKE WINGRA

Method of storage	Inorganic phosphate ($\mu\text{g l}^{-1}$ of phosphorus) remaining in solution after				
	<1 hour*	2 hours	6 hours	1 day	7 days
Not filtered after collection, stored in phosphated polycarbonate	10.8 ± 0.3	10.6 ± 0.5	10.4 ± 0.3	6.1 ± 0.4	4.0 ± 0.2
Filtered after collection, stored in glass†	10.8 ± 0.3	10.8 ± 0.3	10.8 ± 0.3	10.6 ± 0.7	10.4 ± 0.3

* Time after collection.

† 50-ml calibrated flasks used for routine determinations of inorganic phosphate.

DISCUSSION

The results obtained for the sorption of dissolved inorganic phosphate from distilled-water systems by laboratory ware are not directly relevant to environmental phosphorus techniques. To evaluate the ability of laboratory ware to sorb dissolved inorganic phosphate from lake waters, however, it is essential to establish a basis for sorption of phosphate by using a distilled-water system because in this system the recovery of a known amount of added inorganic phosphate can be determined. In addition, the results obtained on a distilled-water system are important in determining the choice of laboratory ware to be used for the storage of dilute standard solutions of inorganic phosphate.

The washing with acid of glassware that is used for determinations of inorganic phosphate has been recommended³ for the removal of sorbed inorganic phosphate. The results obtained in this study for the sorption of dissolved inorganic phosphate indicate that washing with acid creates a greater problem than that which it aims to overcome because the sorption of inorganic phosphate by acid-washed glass was appreciably higher than the desorption of inorganic phosphate from phosphated glass. The fact that the sorption of inorganic phosphate by acid-washed glass was lower with lake waters is attributed to the higher concentration of anions in lake waters than in distilled water that compete with phosphate for sorbing sites on the glass. Even so, within 6 hours, approximately 20 per cent. of the inorganic phosphate was sorbed from two lake waters by acid-washed glass relative to the values obtained for lake waters stored in phosphated polycarbonate and acid-washed polypropylene and polycarbonate.

The significant sorption of dissolved inorganic phosphate by acid-washed glass from a distilled-water system dictates that solutions of low ionic strength and with low concentrations of dissolved inorganic phosphate should be in contact with glassware for the minimum period of time, when contact is unavoidable. In the analysis of solutions containing low concentrations of dissolved inorganic phosphate, it is preferable that essential glassware, such as calibrated flasks and pipettes, is washed immediately before and after use with distilled water. The addition of acid to dilute standard solutions of inorganic phosphate stored in acid-washed glass containers had no effect on the phosphate-sorption characteristics of the glass. The fact that there was no difference in the dissolved inorganic phosphate concentrations of dilute standard solutions of phosphate in the presence or absence of added hydrogen ion also suggests that the observed decrease in the concentration of dissolved inorganic phosphate is due to sorption and not to microbial assimilation. The washing of calibrated flasks with acid is not recommended during the preparation and temporary storage of dilute standard solutions of inorganic phosphate.

It is possible that the washing of glass with acid results in the protonation of Al-OH or Si-OH groups on the surface of the glass by a mechanism similar to that proposed by Parks⁵ and Hingston, Atkinson, Posner and Quirk⁶ for siliceous and aluminous surfaces. The specific sorption of inorganic phosphate by positively charged groups is well established (for example, for Al-OH₂⁺ groups⁷).

Because the error involved in the determination of inorganic phosphate is very low ($\pm 0.3 \mu\text{g l}^{-1}$ of phosphorus), high standard deviations for mean recoveries of inorganic phosphate indicate the wide variability in the sorption properties of the materials after a given pre-treatment. This effect is particularly apparent with acid-washed glass and both phosphated glass and polypropylene in a distilled-water system (Table I). The washing of polycarbonate with acid appears to produce sorption sites that have a fairly uniform and high binding energy for phosphate, which results in excellent recoveries and low standard deviations after the sites have been phosphated during pre-treatment (*iii*). The possibility of pre-treating polycarbonate with other anions, such as molybdate and hydrogen carbonate, is currently under investigation.

Although errors due to the sorption of dissolved inorganic phosphate by acid-washed glass from lake water will be significant only at low levels of inorganic phosphate, the use of acid-washed glass should be avoided when other materials, particularly polycarbonate, are available. The common practice of washing collection containers with the lake water that is to be collected, immediately prior to sampling, probably has little effect in minimising the sorption of dissolved inorganic phosphate by the container, because sorption is not rapid. Phosphated polycarbonate appears to be the most suitable material for the storage of dilute standard solutions of inorganic phosphate and for the collection of lake waters and their storage after filtration. The selection of phosphated polycarbonate is based on the insignificant sorption and the low standard deviation of the mean recovery of inorganic phosphate. When polycarbonate is unavailable, acid-washed polypropylene could be used.

It is safe to assume that the decrease in the concentration of dissolved inorganic phosphate in Lake Wingra water that was stored in an unfiltered condition in phosphated polycarbonate is not due to sorption by the storage container. The observed decrease in the concentration of dissolved inorganic phosphate could be due to various factors, including microbial assimilation and sorption of phosphate by finely divided particulate material that is either present in the natural lake water or is formed as a precipitate during storage. If water samples are filtered within 6 hours of collection, they can be stored for at least 7 days at 4 °C in calibrated glass flasks, washed with distilled water, which are used for the routine determination of dissolved inorganic phosphate. Storage of the bulk sample at 4 °C, after filtration, in phosphated polycarbonate would also appear to be satisfactory.

This research was supported in part by the College of Agricultural and Life Sciences, University of Wisconsin, by the Office of Water Resources Research Project No. WRC 71-10 (OWRR A-038-WIS), and by the Eastern Deciduous Forest Biome Project, International Biological Program, National Science Foundation subcontract 3351, under Interagency Agreement AG-199, 40-193-69, with the Atomic Energy Commission, Oak Ridge National Laboratory.

REFERENCES

1. Hassenteufel, W. R., Jagitsch, R., and Koczy, F. F., *Limnol. Oceanogr.*, 1963, **8**, 152.
2. Lee, G. F., "Eutrophication: Causes, Consequences, Correctives," National Academy of Sciences, Washington, D.C., 1969, p. 646.
3. "Methods of Chemical Analysis of Water and Wastes," Environmental Protection Agency, Cincinnati, Ohio, 1971, p. 235.
4. Murphy, J., and Riley, J. P., *Analytica Chim. Acta*, 1962, **27**, 31.
5. Parks, G. A., *Adv. Chem. Ser.*, No. 67, 1967, 121.
6. Hingston, F. J., Atkinson, R. J., Posner, A. M., and Quirk, J. P., *Nature, Lond.*, 1967, **215**, 1459.
7. Muljadi, D., Posner, A. M., and Quirk, J. P., *J. Soil. Sci.*, 1966, **17**, 230.

Received March 13th, 1972

Accepted June 2nd, 1972

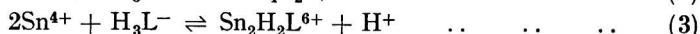
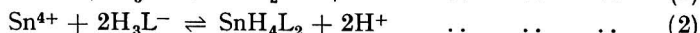
The Complexation of Tin(IV) with Catechol Violet

By B. W. BUDESINSKY

(Department of Chemistry, University of Arizona, Tucson, Arizona 85721, U.S.A.)

The various factors involved in the formation of the complex of tin(IV) with catechol violet have been studied and their significance is discussed.

THE formation of a complex between tin(IV) and catechol violet (3,3',4,4'-tetrahydroxy-sulphonphthalein) has recently been described by Wakley and Varga,¹ who assumed that the following equilibria occurred—



and calculated the corresponding equilibrium constants (they called them over-all formation or stability constants, β , which could be misleading because they do not conform to the standard definition of these terms); L is the completely deprotonised ligand of catechol violet. However, there was no indication of how many hydrogen atoms are actually in the complex as the experiments were carried out only at pH 3.0 and in 1 M sodium chloride solution. These authors also determined the stability constants of hydrogen complexes but did not realise that they are of no use for metal complexes if the latter are investigated at only one acidity. For a complex $\text{M}_m\text{H}_j\text{L}_n$, the value of j must be determined by means of the following equation (for its use, see reference 2)—

$$\log K_{mn}\alpha_{M(Y)}^m\alpha_{L(H)}^n = \log \beta - j \text{ pH} \quad \dots \quad (4)$$

where K_{mn} is the effective (conditional) stability constant, β is the over-all stability constant and $\alpha_{M(Y)}$ and $\alpha_{L(H)}$ are the coefficients of side reactions of the metal M with a ligand of Y (from buffer, masking reagent, etc.) and ligand L with hydrogen ions, respectively.

Consequently, the data provided by the paper can be presented rigorously only in terms of effective stability constants. The concept of the effective stability constant was introduced by Schwarzenbach and Flaschka³ just to describe equilibria where the side reactions cannot be taken into account. The constant, K_{mn} , is given by the equation—

$$K_{mn} = c_{mn}c_M^{-m}c_L^{-n} \quad \dots \quad (5)$$

where c_{mn} is the actual concentration of the complex (with the molar ratio of metal to ligand $m:n$) and c_M and c_L are the total concentrations of the metal and ligand, respectively, that are not complexed with each other. The logarithmic effective stability constants can be obtained from the values reported by Wakley and Varga¹ by addition of the value of $a \times 3$ (equal to $a\text{pH}$), where a is the number of dissociated hydrogen ions in equations 1 to 3. In this way we obtained the values 10.80, 20.90 and 15.92 for complexes of composition 1:1, 1:2 and 2:1, respectively.

An investigation of catechol violet - hydrogen complexes was made first by Ryba, Cifka, Malat and Suk.⁴ These authors pointed out that their values correspond to the dissociation of the first, second and third phenolic hydrogen atoms. Recently, Heys, Kinns and Perrin⁵ found pH values of 7.921 and 10.230 (at 20 °C and an ionic strength of 0.01 M) for the dissociation of the second and third phenolic hydrogen atoms, in agreement with the values reported by Ryba, Cifka, Malat and Suk⁴ (7.82 and 9.80), but in disagreement with those of Wakley and Varga¹ (7.51 and 8.33). There are a number of other papers dealing with xylenol orange, semixylenol orange, methylthymol blue, chrome azurol S and many other sulphonphthaleins, and all report the dissociation of the group $-\text{SO}_3\text{H}$ in a strongly acidic medium (usually concentrated sulphuric acid) due to the strong field effect of the positively charged central carbon atom. A relatively small chromogenic effect is involved. Therefore, the value of 0.26 reported by Wakley and Varga¹ for the pK value of the group $-\text{SO}_3\text{H}$ is in disagreement with all other findings and again lacks supporting evidence. The difficulties in explaining the formation of a tin(IV) complex arise from this disagreement.

The connection between the structure and absorption properties of various deprotonised species of the complexing agent is the key to understanding the chromogenic effect of metal complex formation. The first enumeration was given by Schwarzenbach, Brandenberger, Ott and Hagger⁶ in 1937. They stated that the occurrence of various bathochromic species of the reagent is determined by the extent of π -electron delocalisation in the system $ZO-C_6H_4-\overset{+}{C}-C_6H_4-OZ$. This delocalisation can occur either by dissociation (when Z is equal to zero, the only instance taken into account by the authors¹) or association (when Z represents H) of both the phenolic hydrogen atoms. In metal complex formation, metal - oxygen bonds can replace hydrogen - oxygen bonds completely (complexes with the ratio of metal to ligand of 2:1) or partially (complexes of ratio 1:1 and 1:2). It is true that in the formation of both hydrogen and metal complexes the formation of chelates with other functional groups on the benzene rings is usually involved. Such behaviour has been described in the system thorium - xylenol orange.⁷

The computer-assisted method of Nagano and Metzler,⁸ used by Wakley and Varga,¹ is, in principle, a good method for the investigation of ionic equilibria in solution. A drawback with this method may be hidden in the definition of the statistical weighing factor, ω_k , equalising the measurement of various absorbances at different wavelengths. In fact, Nagano and Metzler did not present any proper definition of the weighing factor. By use of this factor all absorbances and wavelengths become equivalent and the conclusion was drawn¹ that the best results can be obtained in the widest range of the spectrum. Such a conclusion is misleading. In addition to the effect of various absorbance values, the occurrence of isosbestic points should also be taken into account. It is well known that the most reliable values are obtained for wavelengths with the most significant differences in the molar absorptivities of individual absorbing species.

REFERENCES

1. Wakley, W. D., and Varga, L. P., *Analyt. Chem.*, 1972, **44**, 169.
2. Budesinsky, B. W., and Svec, J., *Analytica Chim. Acta*, 1971, **55**, 115.
3. Schwarzenbach, G., and Flaschka, H., "Complexometric Titrations," Methuen, London, 1969.
4. Ryba, O., Cifka, J., Malat, M., and Suk, V., *Colln Czech. Chem. Commun.*, 1956, **21**, 349.
5. Heys, G., Kinns, H., and Perrin, D. D., *Analyst*, 1972, **97**, 52.
6. Schwarzenbach, G., Brandenberger, M., Ott, G. H., and Hagger, O., *Helv. Chim. Acta*, 1937, **20**, 490.
7. Budesinsky, B. W., and Svec, J., *Analytica Chim. Acta*, in the press.
8. Nagano, K., and Metzler, D. E., *J. Amer. Chem. Soc.*, 1967, **89**, 2891.

Received May 2nd, 1972
Accepted June 22nd, 1972

Enthalpimetric Determination of Nitrogen-containing Bases

BY L. S. BARK AND J. K. GRIME

(Department of Chemistry and Applied Chemistry, University of Salford, Salford, M5 4WT)

Milligram amounts of bases of various classes, *viz.*, compounds that contain primary, secondary or tertiary amino groups, quaternary ammonium compounds and simple ammonium salts, have been determined by utilising their reaction with a known and excess amount of sodium tetraphenylboron.

The excess of the boron compound has been determined by direct injection enthalpimetry by using potassium chloride. The results were all within 1.5 per cent. of the theoretical value. The standard deviation of the method applied to the determination of aniline (38 mg) was 0.27 mg.

ENTHALPIMETRIC and thermometric methods for the determination of basic substances have generally involved the use of either the heat of neutralisation in aqueous¹⁻⁴ or non-aqueous media,⁵⁻⁸ the heat of dilution of the titrant⁹ or the catalimetric use of the solvent.¹⁰⁻¹³ Other more selective methods include the oxidation of sulphonamides with hypochlorite solution¹⁴ and the determination of primary amines by using a diazotisation reaction.¹⁵⁻¹⁷ Acetylation has also been used.¹⁸ The heats of ionisation of the bases have been reported⁹ to be endothermic and, as the heat of reaction between the protons from the base and the hydroxyl ions from the titrant is exothermic, the over-all enthalpy change of the combined neutralisation reactions is small. The enthalpy changes of reactions are, in general, enhanced by the entropy changes, and reactions that involve precipitation have relatively large entropy changes and consequently often have large enthalpy changes. Hence, in theory, a suitable method for the determination of bases could involve a precipitation reaction and could be made sensitive with a reagent that is known to give highly insoluble materials.

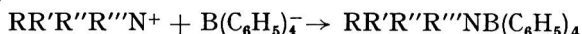
It was decided to use sodium tetraphenylboron, which has been applied in the gravimetric determination of ammonium and quaternary ammonium compounds and also of some salts of primary, secondary and tertiary bases.¹⁹⁻²⁴

Not all bases react at the same rate with this reagent²⁵ and preliminary investigations indicated that the use of continuous thermometric methods did not give the precision that is required in an analytical method. This effect was attributed to the non-completion of some of the reactions in the period of time taken for a continuous titration, and also to the need to have an excess of the sodium tetraphenylboron present at all times. Accordingly, the use of direct injection enthalpimetry²⁶ was considered. The theory of and the experimental conditions that are necessary for this method have been reviewed elsewhere.²⁷

The relatively low solubility of sodium tetraphenylboron in common solvents (a saturated solution in water is less than 0.90 M) restricts its use as a direct titrant. The need to have standard conditions for the determination of organic functional groups by precipitation reactions has been discussed for the determination of aldehydes as their phenylhydrazones^{28,29} and it has been shown that an indirect or back-titration method is superior to direct methods. The method proposed therefore involves the precipitation of the base with a known and excess amount of sodium tetraphenylboron, and the subsequent determination of the excess of sodium tetraphenylboron as potassium tetraphenylboron by using a concentrated standard solution of potassium chloride.

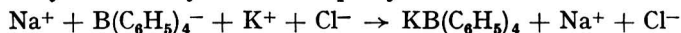
The over-all reactions are as follows.

Precipitation of the base—



where R, R', R'' and R''' can be hydrogen, alkyl or aryl, or the compound can be a heterocyclic base.

The nature of the base pre-determines the state of the reaction after a given time; however, the presence of a suitable excess of the precipitant can ensure that the reaction is completed in a standard time.

Determination of the excess of sodium tetraphenylboron—

The "heat pulse" given by the addition of a fixed volume of potassium chloride solution is a measure of the excess of sodium tetraphenylboron present, and thence the amount of base originally present can be calculated.

EXPERIMENTAL

APPARATUS—

The circuit for the basic electrical bridge system has already been reported.²⁸ The titration vessel was a thick-walled polythene bottle of nominal capacity 30 cm³, surrounded by a casing of expanded polystyrene to minimise thermal losses during the period of the titration.

The injection pipette, shown in Fig. 1, was specially designed to be submersible and to aid in the efficient mixing of the solutions. The injection apparatus was a 2-cm³ glass syringe. The mixtures were stirred with a rotating multi-paddle stirrer.

MATERIALS—

Stock (0.1 M aqueous) solutions of sodium tetraphenylboron were standardised against purified potassium chloride by direct thermometric titration. The stock solutions were stored in opaque bottles at room temperature (solutions were prepared weekly, no significant decomposition occurring during 1 week).

Potassium chloride solution (2 M) was prepared from recrystallised analytical-reagent grade salt.

The bases listed below were dissolved in 0.1 M hydrochloric acid.

(a) *Compounds containing a primary amino group*—Aniline, benzylamine, 3-aminopyridine, 4-aminopyridine, 4-aminoacetophenone and 2-aminobenzoic acid.

(b) *Compounds containing a secondary amino group*—Phenylhydrazine hydrochloride, *N*-methylaniline, diethylamine, piperidine and morpholine.

(c) *Compounds containing a tertiary amino group*—*NN*-Dimethylaniline, triethylamine, pyridine, hexamethylenetetramine, acridine and 8-nitroquinoline.

(d) *Quaternary ammonium salts and ammonium salts*—Tetraethylammonium bromide and ammonium chloride.

PROCEDURE—

A known volume of the base in 0.1 M hydrochloric acid was treated in the reaction vessel with a known and excess amount of an aqueous solution of sodium tetraphenylboron (10 cm³ of 0.1 M solution). The volume was adjusted to 25 cm³ with 0.1 M hydrochloric acid. The pipette containing the potassium chloride solution was submerged in the mixture, which was then stirred vigorously for 2 to 3 minutes. (During this period, the solution in the pipette attained thermal equilibrium with the mixture in the reaction vessel, which also attained practical equilibrium with its immediate surroundings, as shown by the trace on the record chart.)

The potassium chloride solution was injected into the reaction mixture by means of a syringe driven with a synchronous motor, so that the time taken for the injection of the fixed volume was constant (2 s).

The "temperature pulse" was recorded and measured. A calibration graph was prepared by using known amounts of the particular base in procedures similar to those described.

From the calibration graph and the observed "temperature pulse," the amount of the base originally present was calculated.

RESULTS

All the compounds considered were determined over the range 0.03×10^{-3} to 0.7×10^{-3} mol; each compound gave results within 1.5 per cent. of the theoretical value.

The standard deviation of the method was determined for aniline. Ten samples of nominal mass 38 mg were analysed by using the procedure outlined. The standard deviation calculated from these results was 0.27 mg.

Typical results obtained with each class of base are shown in Table I.

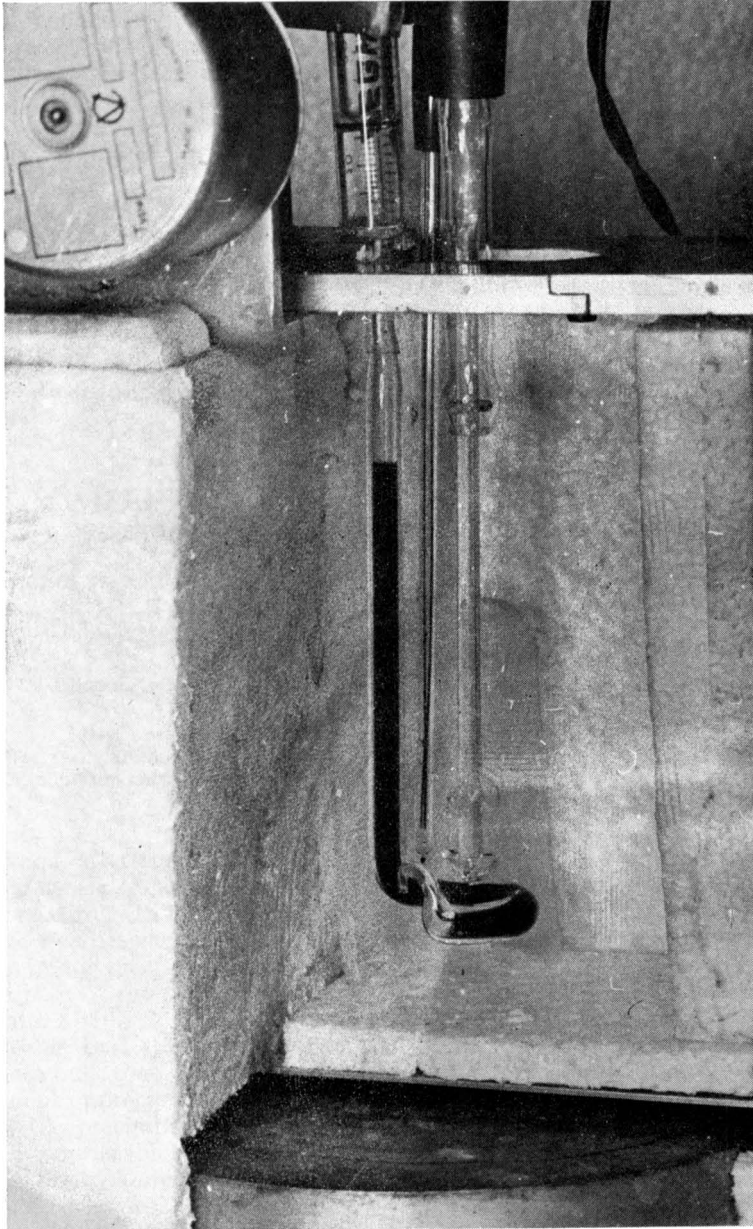
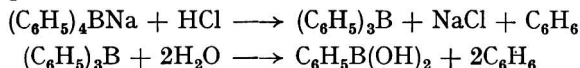


Fig. 1. Injection pipette

DISCUSSION

FACTORS THAT AFFECT THE METHOD—

The endothermic heat of dilution of the concentrated solution of potassium chloride reduces the size of the "temperature pulse" given by the exothermic precipitation of potassium tetraphenylboron and, consequently, the calibration graph does not necessarily pass through the origin. However, this effect can be compensated for and minimised by using the exothermic heat of mixing of the hydrochloric acid present in the solution. The acidity of the solution must, however, be controlled, because in concentrated acidic solutions sodium tetraphenylboron decomposes as follows—



Hence, by careful control of the amount of acid present, it is possible to avoid decomposition of the sodium tetraphenylboron, to provide a sufficient number of protons to protonate the base and also to liberate enough heat during the dilution of the base to offset the endothermic heat of dilution of the potassium chloride, resulting in zero heat of mixing. It has been found that 0.1 M hydrochloric acid provides such a solution. The decomposition of sodium tetraphenylboron is negligible during the period of time taken for the titration in acid of this particular concentration and the mixture has zero heat of mixing.

TABLE I
TITRATION OF TYPICAL BASES FROM EACH CLASS

Class	Compound	Amount added/g	Amount found/g
(a)	Aniline	0.029 90	0.030 00
		0.038 87	0.038 25, 0.038 25, 0.038 45, 0.038 50
(b)	<i>N</i> -Methylaniline	0.018 50	0.018 52
		0.012 34	0.012 38, 0.012 34
(c)	<i>NN</i> -Dimethylaniline	0.012 04	0.012 04
		0.024 08	0.023 59
		0.030 10	0.030 10
(d)	Tetraethylammonium bromide Ammonium chloride	0.018 47	0.018 65
		0.007 92	0.007 90
		0.031 68	0.031 65

The suspension, after the precipitation of the base as its tetraphenylboron salt, is very bulky and spurious results can be obtained if the stirring is inefficient and does not produce a homogeneous mixture. In order to facilitate the production of homogeneity in the suspension, the pipette was designed so that a multi-paddle stirrer could be accommodated and stir at all levels in the reaction vessel. The pipette is in the form of a loop that fits peripherally in the base of the reaction cell and does not interfere with the rapid circulation of the precipitate.

It is noticeable that the precipitate of the organic-substituted tetraphenylboron has considerably more bulk than that of potassium tetraphenylboron, and as there is a physical limit to the amount of precipitate that can be stirred effectively, a practical limit is imposed, by the bulk of the precipitate formed, on the determination of a particular base. This restriction is more noticeable in molecules of relatively large mass and is observed when the series aniline, *N*-methylaniline, *NN*-dimethylaniline is considered; the upper limit of the determination decreases as the relative molecular mass increases from aniline to *NN*-dimethylaniline.

Analytical methods that involve visual indication of the end-point may fail if emulsions or precipitates are present in the reaction mixture or if the sample itself is coloured, but methods that involve thermometric indication of the end-point are not affected by these factors and, consequently, coloured solutions of impure bases can be titrated successfully by using the above technique. The technique has been applied successfully to the determination of the amine content of several highly coloured industrial samples, which could not be analysed by using visual indication of the end-point.

REFERENCES

1. Parsons, J. S., *Abstr. Pap. 132nd Meet. Amer. Chem. Soc.*, 1957, 7B.
2. Popper, E., Roman, L., and Marcu, P., *Talanta*, 1965, **12**, 245.
3. Drăgulescu, C., and Policec, S., *Studii Cerc. Baza Științe Timișoara, Științe Chim.*, 1962, **9**, 33.
4. Ogawa, M., Yasuyo, N., Tetsu, K., and Fujie, T., *Kyoritsu Yakka Daigaku Kenkyu Nempo*, 1970, **15**, 21.
5. Forman, E. J., and Hume, D. N., *J. Phys. Chem.*, 1959, **63**, 1947.
6. —, —, *Talanta*, 1964, **11**, 129.
7. Meade, T. E., *J. Phys. Chem.*, 1962, **66**, 2149.
8. Keily, H. J., and Hume, D. N., *Analyt. Chem.*, 1956, **28**, 1294.
9. Vaughan, G. A., and Swithenbank, J. J., *Analyst*, 1967, **92**, 364.
10. Vajgand, V. J., and Gaál, F. F., *Glasn. Hem. Društ., Beogr.*, 1966, **31**, No. 2, 103.
11. —, —, *Talanta*, 1967, **14**, 345.
12. Vajgand, V. J., Kiss, T. A., Gaál, F. F., and Zsigrai, I. J., *Talanta*, 1968, **15**, 699.
13. Vajgand, V. J., Gaál, F. F., and Brusin, S. S., *Ibid.*, 1970, **17**, 415.
14. Schafer, H., and Wilde, E., *Z. analyt. Chem.*, 1949, **130**, 396.
15. Daftary, R. D., and Halder, B. C., *Analytica Chim. Acta*, 1961, **25**, 538.
16. Yamazaki, M., and Takeuchi, T., *Kogyo Kagaku Zasshi*, 1969, **72**, 1263.
17. —, —, *Ibid.*, 1969, **72**, 1500.
18. Somiya, T., *J. Soc. Chem. Ind.*, 1932, **51**, 135.
19. Kohler, M., *Z. analyt. Chem.*, 1953, **138**, 9.
20. Gloss, G. H., *Chemist Analyst*, 1953, **42**, 50.
21. Rüdorff, W., and Zannier, H., *Z. analyt. Chem.*, 1953, **140**, 241.
22. Hahn, F. L., *Ciencia, Méx.*, 1954, **14**, 249.
23. Newman, E. J., and Peter, G., "Sodium Tetrphenylboron," Monograph No. 38, Hopkin and Williams, Chadwell Heath, 1959.
24. Carr, P. W., *Analyt. Chem.*, 1971, **43**, 756.
25. Crane, F. E., *Ibid.*, 1956, **28**, 1794.
26. Wasilewski, J. C., Pei, P. T.-S., and Jordan, J., *Ibid.*, 1964, **36**, 2131.
27. Bark, L. S., and Bark, S. M., "Thermometric Titrimetry," Pergamon Press, Oxford, 1969.
28. Bark, L. S., and Bate, P., *Proc. 3rd Natn. Conf. Analyt. Chem., Brasov, Romania*, 1971, **4**, 335.
29. —, —, *Analyst*, 1971, **96**, 881.

Received March 17th, 1972

Accepted June 26th, 1972

The Determination of Steam-volatile *N*-Nitrosamines in Foodstuffs by Formation of Electron-capturing Derivatives from Electrochemically Derived Amines*

BY T. G. ALLISTON, G. B. COX AND R. S. KIRK

(Department of Trade and Industry, Laboratory of the Government Chemist,
Cornwall House, Stamford Street, London, SE1 9NQ)

A method for the determination of six steam-volatile *N*-nitrosamines in a range of foodstuffs is described. *N*-Nitrosamines are isolated by steam distillation and are subjected to controlled-potential electrochemical reduction. The amines thus produced are converted into polyfluorinated amides, which are analysed by gas chromatography by means of electron-capture detection. Sensitivities of 1 μg per kilogram of original foodstuff are attained for all the *N*-nitrosamines studied. Low levels of some *N*-nitrosamines have been detected in several foodstuffs, some of which had not been treated with nitrate - nitrite preservatives.

THE carcinogenic properties of many of the *N*-nitrosamines are well documented.^{1,2} Because of the widespread use of sodium nitrate and nitrite as preservatives, it has for some time been realised that there exists the possibility of the formation of *N*-nitrosamines in certain foodstuffs.³ In recent years, much work has been directed towards the detection and determination of volatile *N*-nitrosamines with special regard to food analysis.

Among the methods developed for *N*-nitrosamine determination are thin-layer chromatography,⁴ polarography,⁵ spectrophotometry⁶ and gas-liquid chromatography.⁷ Many of these methods, including gas chromatography with non-specific detectors, have been hampered by their lack of sensitivity or specificity. Recently, gas-chromatographic methods that involve the use of specific detectors have been reported. These include the combination of detectors specific for nitrogen with the confirmation of results by use of a mass spectrometer.^{8,9} Two reports of methods that involve the use of an electron-capture detector have also appeared. In one of these,¹⁰ *N*-nitrosodimethylamine is oxidised to the electron-capturing nitramine, while in the other, due to Eisenbrand, G., and Preussmann, R. (private communication), the cleavage of *N*-nitrosamines with hydrogen bromide in acetic acid with subsequent reaction of the resultant amines with heptafluorobutanoyl chloride to yield volatile amides is used.

N-Nitrosamines are reduced electrochemically in alkaline solution to give high yields of the secondary amines.^{11,12} This report describes the utilisation of such a reduction process in a method of determination that results ultimately in the preparation and gas-chromatographic determination of heptafluorobutanoyl derivatives of the electrolysis products of six *N*-nitrosamines: *N*-nitrosodimethylamine (DM), *N*-nitrosodiethylamine (DE), *N*-nitrosodi-*n*-propylamine (DP), *N*-nitrosodi-*n*-butylamine (DB), *N*-nitrosopiperidine (PIP) and *N*-nitrosopyrrolidine (PYR).

EXPERIMENTAL

APPARATUS—

Gas chromatographs—Pye series 104 gas chromatographs with 10 mCi nickel-63 electron-capture detectors were used. The detectors were operated at 500 μs pulse space and at a temperature of 250 °C. The columns were a 2.75 m \times 2 mm i.d. stainless-steel column, packed with 15 per cent. *m/m* of FFAP (Carbowax 20M terminated with 2-nitroterephthalic acid) (Phase Separations Ltd.) on 80 to 100-mesh Chromosorb W, operated at a temperature of 60 °C; and a 5.8 m \times 2 mm i.d. stainless-steel column, packed with 20 per cent. *m/m* of FFAP on 80 to 100-mesh Chromosorb W and operated at 110 °C. Nitrogen at a flow-rate of 50 ml min⁻¹ was used as the carrier gas. Samples of 5- μl volume were injected.

Electrolysis cell—The cell (see Fig. 1) was a flat-bottomed vessel constructed from a 75 mm i.d. flat-flange joint. A tap was fitted in the centre of the base. Through the five

* Presented at Euroanalysis I, Heidelberg, August 28th to September 1st, 1972.

© SAC; Crown Copyright Reserved.

sockets in the lid passed a stirrer, which reached the mercury cathode - electrolyte interface, the cathode connection, a gas bubbler, a saturated calomel electrode and an anode compartment. The anode compartment consisted of a tube closed at one end by a porous ceramic disc, 0.5 cm thick (Activion Ltd., Kinglassie, Fife). The anolyte was 0.1 M sodium hydroxide solution, and the anode was a platinum sheet (22 gauge, 5 × 5 cm).

A potentiostat [J. A. Radley (Laboratories) Ltd.] was used to control the cathode potential relative to that of the saturated calomel electrode.

MATERIALS—

Reagents were of analytical grade unless otherwise specified.

Cyclohexane, Spectrosol grade.

Heptafluorobutanoyl chloride (PCR Inc.), redistilled.

Sodium chloride.

Sodium carbonate.

Sodium hydroxide.

Sodium sulphate (anhydrous).

Sulphuric acid, 3 M.

Hydrochloric acid, 2 M.

N-Heptafluorobutanoyl derivatives of amines—Derivatives of diethylamine, di-n-propylamine, di-n-butylamine, piperidine and pyrrolidine were prepared from the amine and heptafluorobutanoic acid anhydride.¹³

NN-Dimethylheptafluorobutanamide, boiling-point 44 °C at 18 mm of mercury pressure—This compound was prepared by passing dry dimethylamine through a solution of heptafluorobutanoyl chloride in cyclohexane.

PROCEDURE—

Mince the sample and weigh 200 g into a 1-litre distillation flask together with 100 g of sodium chloride. Pass steam into the initially dry mixture until 250 ml of distillate have been collected. Add 30 g of sodium hydroxide to this distillate and distil the resulting solution until 150 ml have been collected. Then add 25 g of anhydrous sodium sulphate and 3 ml of 3 M sulphuric acid to the latter distillate, redistil, and divide the 100 ml of distillate into two equal portions.

Make one portion of the distillate alkaline with 5 ml of 0.01 M sodium hydroxide solution. De-gas the solution in the electrolysis cell for 1 minute by the passage of a stream of nitrogen and stirring. Electrolyse for 1 hour with stirring, at a cathode potential of -1.8 V versus a saturated calomel electrode. Acidify the reduced solution with 1 ml of 2 M hydrochloric acid and evaporate the mixture to dryness.

To the remainder of the distillate add 5 ml of 0.01 M sodium hydroxide solution and 1 ml of 2 M hydrochloric acid, and evaporate the solution to dryness.

Dissolve each dry residue in 2 ml of water and add 0.5 ml of 1 M sodium hydrogen carbonate solution and 1 ml of a 0.5 per cent. solution of heptafluorobutanoyl chloride in cyclohexane to each of the solutions. Shake the mixtures for 15 minutes, separate the cyclohexane layers and analyse them by gas chromatography.

Compare the differences in peak heights on the recorder traces given by the derivatives under consideration in the reduced and unreduced samples (diluted when necessary) directly with the peak heights from a $1 \mu\text{g ml}^{-1}$ standard solution of the six heptafluorobutanamides in cyclohexane.

RESULTS AND DISCUSSION

The response of the electron-capture detector was linear only with amounts of sample derivative below 7.5 ng. Above this level, the response varied in a manner peculiar to each detector, and the derivative solutions were diluted when necessary in order to maintain concentrations within the linear range. The lower limits of detection of the derivatives were measured and are reported in Table I.

NN-Di-n-butylheptafluorobutanamide had a retention time close to that of an impurity in the reagent at a column temperature of 60 °C and could be determined only at 110 °C, at which temperature the peaks due to *NN*-dimethyl- and *NN*-diethylheptafluorobutanamides were not resolved. Accordingly, gas-chromatographic analyses were carried out at both temperatures.

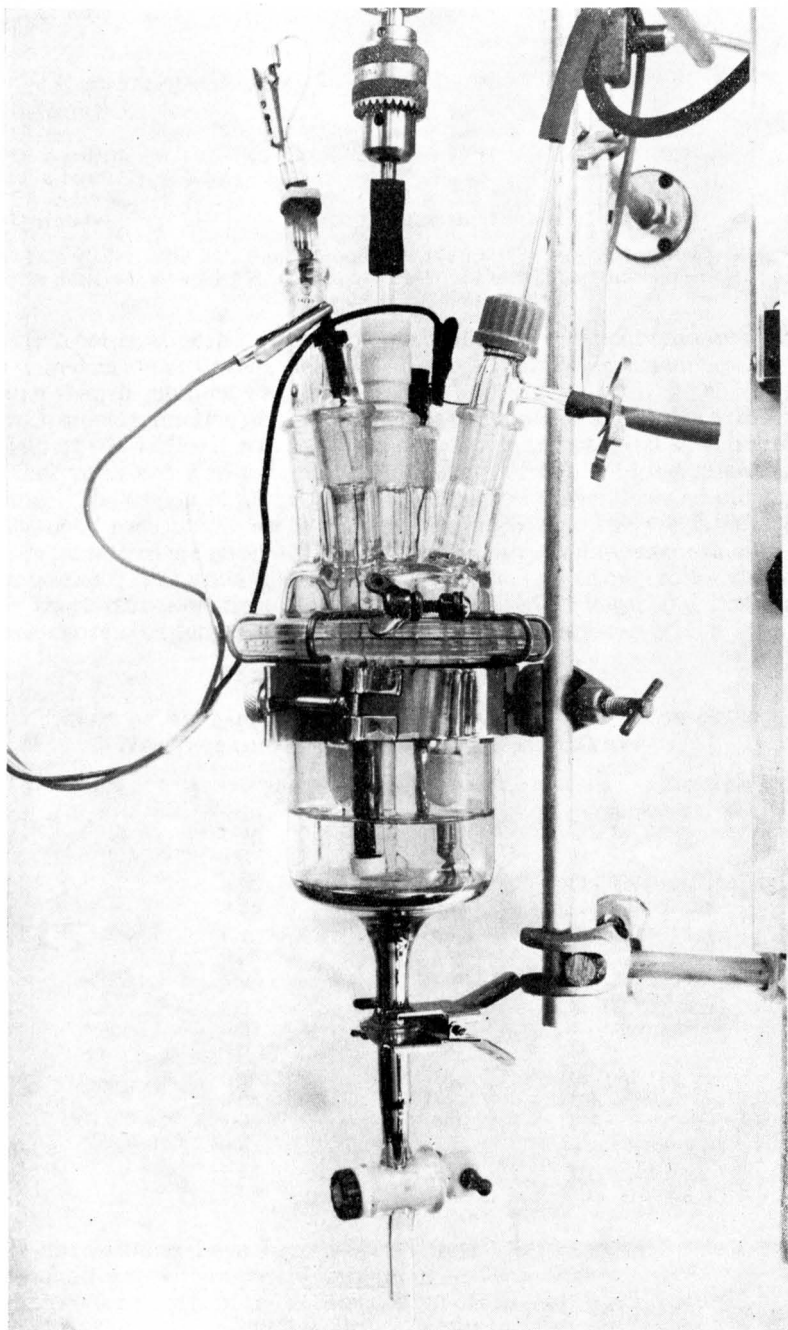


Fig. 1. Electrolysis cell

TABLE I
LOWER LIMITS OF DETECTION OF HEPTAFLUOROBUTANAMIDES

Heptafluorobutanamide	Lower limits of detection/g, on—	
	Column 1*	Column 2†
Dimethyl	5.3×10^{-11}	—
Diethyl	8.0×10^{-11}	—
Di- <i>n</i> -propyl	1.0×10^{-10}	3.5×10^{-11}
Di- <i>n</i> -butyl.. .. .	—	5.0×10^{-11}
Piperidyl	5.4×10^{-10}	7.8×10^{-10}
Pyrrolidyl	6.3×10^{-10}	1.0×10^{-10}

* Column of 15 per cent. *m/m* FFAP on Chromosorb W, 9 feet in length, temperature 60 °C.

† Column of 20 per cent. *m/m* FFAP on Chromosorb W, 19 feet in length, temperature 110° C.

The derivative preparation, a modification of the method of Eisenbrand and Preussmann (see above), resulted in quantitative yields of all six heptafluorobutanamides for periods of agitation greater than 5 minutes.

The electrochemical reduction of *N*-nitrosamines to amines at a mercury cathode proceeds with a high yield at pH 12.¹¹ It was found to be possible to use 0.001 M sodium hydroxide solution as catholyte in order to minimise the amounts of solid residue resulting from the evaporation stage. Reduction of 15 and 60 minutes duration were carried out on solutions containing 0.25 μ g of each of the six *N*-nitrosamines studied. The yields (after reduction and derivative preparation) are given in Table II. Reduction of solutions containing 1 and 5 μ g of each *N*-nitrosamine gave results that fell well within the ranges quoted for the more dilute solutions. The short reduction time of 15 minutes resulted in lower and less reproducible yields; in consequence, the longer period was used in the analyses.

TABLE II
YIELDS OF HEPTAFLUOROBUTANAMIDES AFTER REDUCTION OF
N-NITROSAMINES AND DERIVATIVE FORMATION

<i>N</i> -Nitrosamine	Amount/ μ g	Reduction time/minutes	Yield, per cent.
Dimethyl	0.25	60	103, 97, 95, 99, 99
	0.25	15	103, 98, 87, 97
	1.0	60	93
Diethyl	0.25	60	115, 106, 108, 114, 118
	0.25	15	105, 110, 88, 98
Dipropyl	0.25	60	82, 84, 79, 77, 82
	0.25	15	76, 71, 59, 56
	1.0	60	80
Dibutyl	0.25	60	72, 79, 72, 73, 77
	0.25	15	50, 49, 49, 48
	1.0	60	71
Piperidyl	0.25	60	96, 104, 100, 104
	0.25	15	104, 88, 81
	1.0	60	103
Pyrrolidyl	0.25	60	105, 105, 108, 115
	0.25	15	97, 92, 95
	1.0	60	106

Volatile *N*-nitrosamines have been isolated from food samples by steam distillation under various conditions with reported recoveries of 70 to 100 per cent.^{5,8,14} The use of alkaline conditions often results in the decomposition of the sample, with attendant difficulties in distillation, while the use of acidic conditions might catalyse nitrosation reactions caused by the presence of sodium nitrite in the sample. A neutral steam distillation was therefore used to remove the volatile *N*-nitrosamines from the sample, and two further distillations, one from alkali and one from acid, were used to remove acidic and basic materials that co-distilled with the *N*-nitrosamines. Neutral, volatile materials were lost in the evaporation stage following the reduction. Adventitious amines (mostly dimethylamine and diethylamine from the air or glassware: the acidic distillation step was shown to be effective in retaining

TABLE III
YIELDS OF HEPTAFLUOROBUTANAMIDES PREPARED FROM AQUEOUS SOLUTIONS OF
N-NITROSAMINES AND SPIKED FOODS

Amount of <i>N</i> -Nitrosamine/ μg	Sample type	Yield of heptafluorobutanamides, per cent.					
		Dimethyl	Diethyl	Dipropyl	Dibutyl	Piperidyl	Pyrrolidyl
0.25	Aqueous	93	113	78	53	90	55
		104	100	76	40	52	52
		70	84	52	44	70	52
		88	84	64	26	83	40
		90	92	70	—	88	66
		76	100	64	40	85	68
		92	108	76	52	90	64
		80	96	72	48	80	64
		76	76	64	40	80	*
		76	80	76	56	82	*
	Cheshire cheese	82	*	86	39	77	*
10.0	Aqueous	71	91	79	65	78	66
		71	83	78	55	86	69
0.1	Cod	*	*	69	35	89	*

* Unspiked sample contained the particular *N*-nitrosamine.

amines emanating from the sample) were monitored by analysis of the unreduced portion of the sample; the differences in peak heights obtained with the reduced and unreduced samples were taken as a measure of the *N*-nitrosamine content.

A number of *N*-nitrosamine solutions were analysed in order to determine the over-all yields and reproducibility of the method. These yields are reported in Table III, which also

TABLE IV
N-NITROSAMINES IN FOODSTUFFS

Sample	<i>N</i> -Nitrosamine content/ $\mu\text{g kg}^{-1}$								
	DM	DE	DP	DB	PIP	PYR			
Cheddar cheese (1)	T	T	ND	ND	ND	ND
Cheddar cheese (2)	ND	ND	ND	ND	ND	ND
Cheshire cheese	T	1.5	ND	ND	ND	1.0
Norwegian goat's milk cheese	ND	ND	ND	ND	ND	ND
Corned beef	ND	ND	ND	ND	ND	2.0
Pork luncheon meat (1)	ND	ND	ND	T	ND	1.5
Pork luncheon meat (2)	ND	ND	ND	ND	ND	ND
Back bacon (uncooked)	ND	*	ND	ND	ND	1.5
Back bacon (fried)	ND	1.5	ND	ND	ND	3.0
Streaky bacon (uncooked)	ND	ND	ND	ND	ND	ND
Streaky bacon (fried)	T	ND	ND	ND	ND	3.5
Pig's liver (uncooked)	ND	1.5	ND	ND	ND	1.5
Pig's liver (fried)	ND	ND	ND	ND	ND	11.0
Haddock (uncooked)	ND	ND	ND	ND	ND	ND
Haddock (fried)	ND	ND	ND	ND	ND	ND
Fresh cod (uncooked) (1)	ND	ND	ND	ND	ND	2.1
Fresh cod (uncooked) (2)	T	ND	ND	ND	ND	1.3
Fresh cod (uncooked) (3)	ND	ND	ND	ND	ND	1.6
Fresh cod (fried)	ND	ND	ND	ND	ND	1.0
Stale cod (uncooked)	1.0	1.5	ND	ND	ND	6.0
Stale cod (fried)	1.0	1.5	ND	ND	ND	6.0
Smoked cod (1)	ND	ND	ND	ND	T	4.0
Smoked cod (2)	ND	ND	ND	ND	ND	ND
Tinned herrings	ND	ND	ND	ND	ND	2.5
Cooking fat (well used for frying bacon)	ND	ND	ND	ND	T	5.0

T = Trace amounts. ND = Not detected.

* Contamination by adventitious diethylamine (as shown in the chromatograms of the non-reduced sample) precluded measurement.

DM = *N*-nitrosodimethylamine; DE = *N*-nitrosodiethylamine; DP = *N*-nitrosodipropylamine; DB = *N*-nitrosodibutylamine; PIP = *N*-nitrosopiperidine; PYR = *N*-nitrosopyrrolidine.

gives yields of heptafluorobutanamides from meat, cheese and fish samples to which had been added a mixture of the six *N*-nitrosamines. In these last results no figures are given when prior analysis of the unspiked sample demonstrated the presence of a particular *N*-nitrosamine. From the values of the over-all yields, together with the limits of detection of the derivatives, it is calculated that an original concentration of $1 \mu\text{g kg}^{-1}$ of the *N*-nitrosamines in 200 g of sample can readily be determined by the proposed procedure.

Several food samples have been analysed in order to demonstrate the scope of the method, and the *N*-nitrosamine contents of these samples are summarised in Table IV. "Trace amounts" referred to in this table represent positive findings of less than $1 \mu\text{g kg}^{-1}$, but greater than $0.5 \mu\text{g kg}^{-1}$. Values below this lower limit are not reported. In only one sample, the fried pig's liver, was any one of the *N*-nitrosamines under investigation present to a level greater than $10 \mu\text{g kg}^{-1}$. The other sample that contained levels of *N*-nitrosopyrrolidine greater than $5 \mu\text{g kg}^{-1}$ was a particularly strongly smelling sample of cod (although fresh samples contained significantly lower amounts of *N*-nitrosamines). It is of interest to note that neither of these samples had been cured with sodium nitrite. A few of the samples analysed have also been analysed by another method, involving gas chromatography and mass spectrometry, in this laboratory.¹⁵ Preliminary results indicate reasonable agreement between results from the two methods.

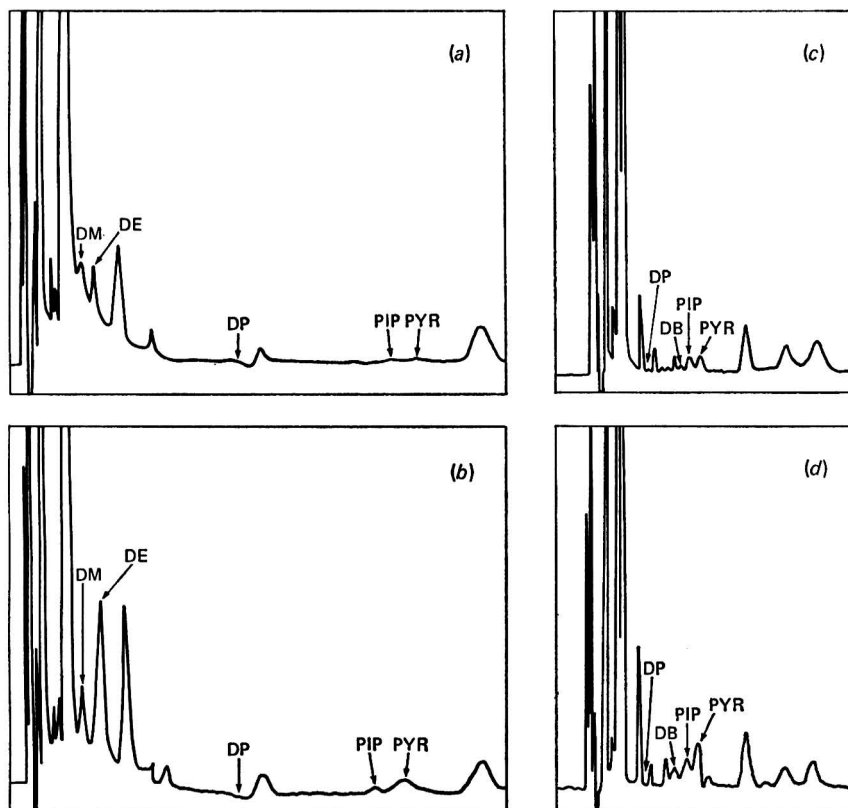


Fig. 2. Chromatograms of heptafluorobutanamides from analysis of Cheshire cheese: (a), unreduced sample; (b), reduced sample (60°C , 15 per cent. FFAP on Chromosorb W, 9-foot column); (c) unreduced sample; (d), reduced sample (110°C , 20 per cent. FFAP on Chromosorb W, 19-foot column). For abbreviations, see text

None of the foodstuffs analysed gave rise to interfering peaks in the chromatograms, although with some samples a number of components that had retention times longer than those of the amine derivatives were seen. A typical pair of chromatograms (reduced and unreduced portions) from the analysis of Cheshire cheese are shown in Fig. 2.

The present method can be extended to the determination of other steam-volatile *N*-nitrosamines. In Table V relative retention times for a number of heptafluorobutanamides, including the six under detailed investigation, are listed, although during the analyses reported in Table IV no evidence for the presence of the *N*-nitrosamine precursors of the other heptafluorobutanamides was found. The extension of this method to the analysis of the less volatile and non-volatile *N*-nitrosamines is currently being investigated.

TABLE V
RELATIVE RETENTION TIMES FOR HEPTAFLUOROBUTANAMIDES

Heptafluorobutanamide	Relative retention time	
	Column 1*	Column 2†
Dimethyl	0.33	—
Methylethyl	0.40	—
Diethyl	0.41	—
Methylisopropyl	0.45	—
Methyl- <i>n</i> -propyl	0.56	—
Ethyl- <i>n</i> -propyl	0.64	—
Di- <i>n</i> -propyl	1.00	1.00
Diallyl	1.14	—
Methyl- <i>n</i> -butyl	1.17	—
Ethyl- <i>n</i> -butyl	1.31	—
Methylisopentyl	1.56	—
Piperidyl	2.51	2.45
Pyrrolidyl	2.58	2.62
Di- <i>n</i> -butyl	3.32	2.19

* Column of 15 per cent. *m/m* FFAP on Chromosorb W, 9 feet in length, temperature 60 °C.

† Column of 20 per cent. *m/m* FFAP on Chromosorb W, 19 feet in length, temperature 110 °C.

We thank Dr. J. B. Headridge for initial discussion of this work, Mr. D. C. Street for carrying out some of the experimental work, and the Government Chemist for permission to publish this paper.

REFERENCES

1. Druckrey, H., Preussmann, R., Ivankovic, S., and Schmahl, D., *Z. Krebsforsch.*, 1967, **69**, 103.
2. Magee, P. N., and Swan, P. F., *Br. Med. Bull.*, 1969, **25**, 240.
3. *B.I.B.R.A. Inf. Bull.*, 1965, **4**, 47.
4. Preussman, R., Neurath, G., Wulf-Lorentzen, G., Daiber, D., and Hengy, H., *Z. analyt. Chem.*, 1964, **202**, 187.
5. Walters, C. L., Johnson, E. M., and Ray, N., *Analyst*, 1970, **95**, 485.
6. Daiber, D., and Preussmann, R., *Z. analyt. Chem.*, 1964, **206**, 344.
7. Foreman, J. K., Palframan, J. F., and Walker, E. A., *Nature, Lond.*, 1970, **225**, 554.
8. Crosby, N. T., Foreman, J. K., Palframan, J. F., and Sawyer, R., Paper presented at I.A.R.C. Meeting on Analysis and Formation of Nitrosamines, Heidelberg, October, 1971.
9. Howard, J. H., Fazio, T., and Watts, J. O., *J. Ass. Off. Analyt. Chem.*, 1970, **53**, 269.
10. Sen, N. P., *J. Chromat.*, 1970, **51**, 301.
11. Lund, H., *Acta Chem. Scand.*, 1957, **11**, 990.
12. Whitnack, G. C., Weaver, R. D., and Kruse, H. W., *Rep. U.S. Dept. Commerce Office Tech. Serv.*, 1963, AD 413029.
13. Clarke, D. D., Wilk, S., and Gitlow, S. E., *J. Gas Chromat.*, 1966, **4**, 310.
14. Eisenbrand, G., Hodenberg, A. V., and Preussmann, R., *Z. analyt. Chem.*, 1970, **251**, 22.
15. Crosby, N. T., Foreman, J. K., Palframan, J. F., and Sawyer, R., *Nature, Lond.*, 1972, **238**, 342.

Received May 17th, 1972

Accepted June 26th, 1972

Potentiometric Determination of Monuron in Herbicide Formulations

BY S. H. YUEN AND J. M. C. PALMER

(Imperial Chemical Industries Limited, Agricultural Division, Jealott's Hill Research Station, Bracknell, Berkshire)

Monuron is extracted from a dispersion of the sample in hydrochloric acid into chloroform and the residue after evaporation of the chloroform is hydrolysed with boiling 24 N sulphuric acid. The liberated dimethylamine is distilled into 2 per cent. boric acid and titrated potentiometrically with standard hydrochloric acid. The method has been applied to technical monuron and aqueous suspension formulations with an error of 0.9 per cent.

MONURON, *N'*-(4-chlorophenyl)-*NN*-dimethylurea, is structurally related to diuron. For the macrodetermination of diuron and monuron, Lowen and co-workers^{1,2} described three titrimetric methods; these methods are time consuming mainly because of the high stability of the herbicides. A rapid method for assaying diuron³ depends on the liberation of the dimethylamine in boiling 24 N sulphuric acid followed by its distillation and titration with standard hydrochloric acid, with methylene blue - methyl red as indicator. The experimental conditions of this method were found to be equally applicable in the determination of monuron, but the results were over-estimated owing to the obscure end-point. This difficulty was caused by the buffering effect of 4-chloroaniline, which is distilled together with the dimethylamine and is soluble in dilute boric acid solution. The inflection point occurred at pH 5.9 to 6.3, instead of pH 5.1 when dimethylamine alone was titrated. The methylene blue - methyl red indicator (pH 5.0 to 5.3) was therefore unsuitable and is used only for determining the reagent blank. Several other indicators were tried without success. However, the end-point was satisfactorily located potentiometrically and was evaluated by calculating the first and second derivatives of the potential, $d\text{mV}$ and $d^2\text{mV}$, as a function of volume,⁴ with the equivalence point occurring at the maximum of the $d\text{mV}$ graph.

METHOD

APPARATUS—

Steam-distillation apparatus.

pH meter—A direct-reading pH meter, equipped with a glass and a saturated calomel electrode and a magnetic stirrer, was used.

REAGENTS—

Hydrochloric acid, 4 N.

Sulphuric acid, 24 N.

Sodium hydroxide solution, 10 N.

Boric acid solution, 2 per cent.

Mixed indicator—Mix equal volumes of aqueous solutions containing 0.4 per cent. of water-soluble methyl red and 0.2 per cent. of methylene blue.

Standard hydrochloric acid, 0.05 N.

PROCEDURE—

Weigh accurately a sample containing 0.3 to 0.4 g of monuron into a 250-ml separating funnel, add 100 ml of 4 N hydrochloric acid, extract the mixture successively with three 50-ml portions of chloroform, by shaking the funnel for 1 minute each time, and collect the extracts in a 250-ml boiling flask. Evaporate off the solvent at a temperature of 40 to 50 °C under reduced pressure with a rotary evaporator, add 30 ml of 24 N sulphuric acid and boil the solution under reflux for 30 minutes. Add cautiously 100 ml of water to the cooled solution, transfer the contents to a 500-ml Kjeldahl flask, cool again, then add 85 ml of

10 N sodium hydroxide solution. Distil the solution into a 500-ml conical flask containing 50 ml of 2 per cent. boric acid solution until 150 ml of the distillate have been collected. Transfer the contents of the flask into a 600-ml beaker and titrate them potentiometrically with 0.05 N hydrochloric acid.

Carry out concurrently a determination on the reagents used, detecting the end-point visually with the mixed indicator.

One millilitre of 0.05 N hydrochloric acid is equivalent to 9.935 mg of monuron (relative molecular mass 198.7).

$$\text{Monuron content of sample, per cent. } m/m = \frac{0.9935 V}{\text{Amount of sample in grams}}$$

where V ml is the volume of 0.05 N hydrochloric acid used (corrected for the blank).

RESULTS AND DISCUSSION

Monuron Col is an aqueous suspension containing 50 per cent. of active ingredient together with surfactants. To establish the accuracy of the method, analyses were carried out on 0.3-g portions of pure monuron alone and in the presence of an equal amount of blank Col formulation, which contained all of the components except the active ingredient.

The recovery of monuron, based on fourteen determinations, averaged 99.5 per cent. with a coefficient of variation of 0.9 per cent., which is considered to be the error of the method. The precision of the method applied to various aqueous formulations was found to be acceptable, with agreement between duplicates within 0.3 per cent. for samples containing 35 to 60 per cent. of monuron.

The method is not affected by the presence of ammonium ions and free amines, but any volatile bases liberated from other components of the formulation interfere. Both diuron and fenuron are determined as monuron, but diuron can be distinguished by the presence of insoluble 3,4-dichloroaniline in the distillate, while with monuron the distillate is clear. Linuron and monolinuron are hydrolysed to yield methoxymethylamine, which is partially distilled into the boric acid solution. Other herbicides, such as bipyridylum compounds, triazines, halogenated pyridines and phenoxyacetic acids, have no effect.

REFERENCES

1. Lowen, W. K., and Baker, H. M., *Analyt. Chem.*, 1952, **24**, 1475.
2. Lowen, W. K., Bleidner, W. E., Kirkland, J. J., and Pease, H. L., in Zweig, G., *Editor*, "Analytical Methods for Pesticides, Plant Growth Regulators and Food Additives. Volume IV. Herbicides," Academic Press, New York, 1964, p. 157.
3. Yuen, S. H., and Milosevic B., *Analyst*, 1969, **94**, 820.
4. Berl, W. G., *Editor*, "Physical Methods in Chemical Analysis," Volume II, Academic Press, New York, 1951, p. 131.

Received December 7th, 1970
Amended November 19th, 1971
Accepted June 21st, 1972

Use of Vitreous Carbon as a Working Electrode for a Coulometric Redox Titration

By V. J. JENNINGS, A. DODSON AND A. M. ATKINSON

(*Department of Chemistry and Metallurgy, Lanchester Polytechnic, Priory Street, Coventry*)

Vitreous or glassy carbon has been examined as to its suitability for use as a working electrode in the coulometric generation of bromine. It has been found to compare well with platinum for this purpose, although an appreciable increase in the electrode potential is required to maintain a given current density. The theoretical current efficiency for the generation of bromine has been found to be better than 99.9 per cent. The theoretical value has been confirmed by the titration of 100 μ equiv of arsenic(III) with an accuracy and precision each of 0.1 μ equiv.

VITREOUS carbon, sometimes called glassy carbon, has been the subject of a number of published investigations concerning its suitability for use as an electrode in electroanalytical chemistry^{1,2} since its introduction in 1962.³ It is an attractive material because of its good electrical conductivity, resistance to chemical attack and its impermeability to liquids and gases. Its main limitation reported so far has been the higher background currents found at this electrode compared with the more conventional electrodes of platinum and mercury. Such currents were found to limit the precision of results obtained when this material was used as a working electrode in controlled potential coulometry.⁴

High background currents are not desirable in amperostatic or coulometric titrations although, as the potential of the working electrode and the current flow are both constant, their effect should be less significant than in controlled potential coulometry, in which the current decreases with time. In this paper the successful use of a vitreous carbon electrode for a coulometric titration involving bromine is described. The current - potential curves for the generation of bromine from a bromide - sulphuric acid solution have been examined in order to determine the theoretical current efficiency following the method of Lingane Langford and Anson,⁵ which was applied to the coulometric generation of iodine from iodide.⁶ A comparison is made of the effect of current density on the working electrode potential when the electrode is either platinum or vitreous carbon.

The potentiometric titration of small amounts (100 μ equiv or 2000 μ g) of arsenic(III), in the form of dissolved arsenic(III) oxide, with electrogenerated bromine at a vitreous carbon electrode has been examined. The precision found for the titration is compared with that found when using a platinum working electrode. Previous workers³ have shown that 1000 μ g of arsenic(III) can be determined with a precision of about 0.1 per cent. when a platinum working electrode was used.^{7,8}

EXPERIMENTAL

APPARATUS—

A Thorn coulometric titrator, TE 110, was used to provide the constant electrolysis current, which for these titrations was 5 mA. The true current value obtained was 4.998 mA and was determined by measuring the potential drop across a 50- Ω (± 0.05 per cent.) resistor to within 0.1 mV with a Beckman Research pH meter used in its potentiometer mode.

The time intervals used for bromine generation were measured by using the digital timer of the coulometer to 0.1 s, the digital timer being checked against two calibrated stop-watches.

The vitreous carbon electrode used was a 3 mm diameter rod sealed into a Pyrex tube with an epoxy resin. The rod was supplied by Le Carbone (Great Britain) Ltd., and was of grade V 25. The surface area exposed to the electrolyte solution was 2.6 cm².

The titration cell consisted of a 250-ml beaker containing 150 ml of electrolyte solution. The counter electrode of platinum foil was contained in a 1 M solution of potassium sulphate, which was separated from the main cell by a sintered disc of porosity 4. When required, the potential of the vitreous carbon electrode was measured against a closely situated saturated calomel electrode.

For potentiometric titrations, the redox potential of the solution within the cell was measured with a bright platinum electrode. The blank value was found to be equivalent to about 1 μ equiv of arsenic(III) and was subtracted from the total titre found. The titration end-points were taken at a platinum indicator electrode potential of +0.600 V (*versus* S.C.E.) from graphs of the potential *versus* the time for which current was passed.

REAGENTS—

AnalaR grade reagents were used.

Potassium bromide solution, 1 M.

Sulphuric acid, 3 M.

Appropriate volumes of the above solutions together with distilled water were added to the cell so as to give the required concentrations, which for arsenic(III) titrations were 0.1 M of potassium bromide and 0.5 M of sulphuric acid.

Arsenic(III) solution, 0.001 M—This solution was prepared with either mass standardised 99.99 per cent. pure arsenic(III) oxide or the AnalaR grade reagent by dissolving 0.4 g of the oxide (weighed to the nearest 0.01 mg) in 40 ml of warm 2.5 M sodium hydroxide solution, acidifying with 5 M sulphuric acid and making the volume up to 2 litres with water in a Grade A calibrated flask. The pH of the final solution was about 1.8. Titrations of from 25 to 5 ml of the solution [100 to 20 μ equiv of arsenic(III)] were carried out at room temperature, which varied from 18 to 22 °C.

RESULTS

Fig. 1 shows the effect of current density on the working electrode potential when the electrode was made of either platinum or vitreous carbon. Fig. 2 shows the effect of variation of bromide concentration on the potential of a vitreous carbon working electrode at different current densities. The result obtained at 10^{-5} M bromide concentration was very similar to that found for sulphuric acid alone, *i.e.*, with no added potassium bromide. At 10^{-5} M bromide concentration the potential at each current value was unsteady, but slowly reached a more cathodic value with time.

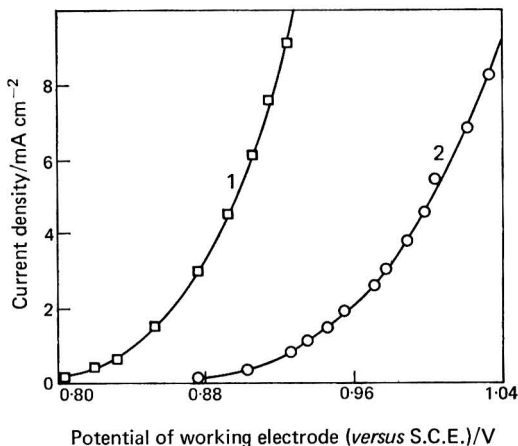


Fig. 1. Effect of current density on the potential of vitreous carbon and platinum electrodes: 1, platinum; and 2, vitreous carbon. $[H_2SO_4] = 0.5$ M and $[Br^-] = 0.1$ M

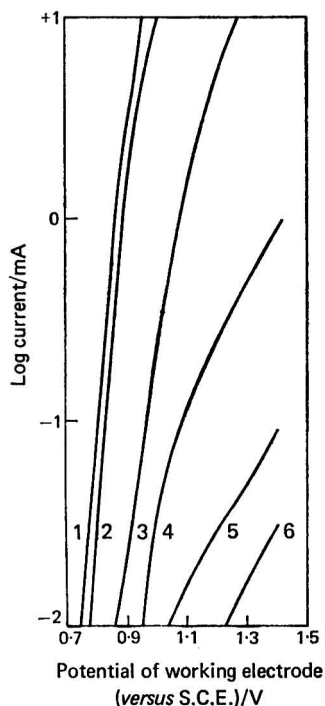


Fig. 2. Effect of bromide concentration in 0.5M sulphuric acid. $[\text{Br}^-]$: 1, 0.2 M; 2, 0.07 M; 3, 10^{-2} M; 4, 10^{-3} M; 5, 10^{-4} M; and 6, 10^{-5} M

Table I shows a comparison of the results obtained for the potentiometric titration of 100 μequiv of arsenic(III) when either a platinum or a vitreous carbon electrode was used. Table II shows results for the determination of 100 μequiv of arsenic(III) taken by pipette from two separate solutions prepared with 99.99 per cent. pure arsenic(III) oxide. Table III shows the effect of sample size when the latter is reduced in three stages to 20 μequiv . The titration errors given are based on the assumption of zero error for the titration of 100 μequiv .

TABLE I

COMPARISON OF VITREOUS CARBON AND PLATINUM WORKING ELECTRODES FOR THE COULOMETRIC BROMINE TITRATION OF 100 μequiv OF ARSENIC(III)

Electrode	Arsenic(III) found/ μequiv							Mean	Relative standard deviation
	99.85	99.82	99.77	99.81	99.84	99.84	99.95		
Platinum	99.85	99.82	99.77	99.81	99.84	99.84	99.95	99.84	0.06
Vitreous carbon . .	99.77	99.86	99.86	99.74	99.84	99.92	99.79	99.83	0.06

DISCUSSION AND CONCLUSIONS

Fig. 1 shows that an additional overpotential of about 0.1 V is required to oxidise bromide at a vitreous carbon electrode compared with a platinum electrode. The overpotential required for the oxidation of bromide at a smooth platinum electrode is known to be small,^{9,10} being less than 0.05 V for a current density of 100 mA cm^{-2} , while the current density used in the present work was below 5 mA cm^{-2} .

In Fig. 2 it can be seen that between the concentrations 0.2 and 0.07 M of bromide ions the theoretical current efficiency for the generation of bromine is greater than 99.9 per cent.

TABLE II

ACCURACY OF COULOMETRIC BROMINE TITRATION OF ARSENIC(III) BY USING
A VITREOUS CARBON WORKING ELECTRODE

Arsenic(III) taken/ μ equiv	100.65	99.35
Arsenic(III) found (mean value)/ μ equiv	100.74	99.54
Number of results	6	7
Relative standard deviation	0.09	0.06
Titration error, per cent.	+0.09	+0.19

It was not possible to obtain complete current - potential curves because the maximum current available from the coulometer was about 10 mA.

The mean values and standard deviations found for the coulometric titration of 100 μ equiv of arsenic(III) by using either a vitreous carbon or a platinum electrode are very similar. The 0.1 per cent. relative standard deviation found is to be expected when use is made of Grade A calibrated glassware.

A small positive error of 0.1 to 0.2 per cent. was found for the accuracy of the determination of arsenic(III), which again might be expected having regard to the magnitude of the blank value of about 1 per cent.

It is accordingly concluded that vitreous carbon makes a satisfactory working electrode for the generation of bromine and that it compares well with platinum for the titration of arsenic(III).

TABLE III

EFFECT OF SAMPLE SIZE ON THE COULOMETRIC BROMINE TITRATION OF
ARSENIC(III) BY USING A VITREOUS CARBON WORKING ELECTRODE

Arsenic(III) taken/ μ equiv	79.93	39.94	19.99
Arsenic(III) found (mean value)/ μ equiv	80.00	40.09	20.10
Number of results	5	5	5
Relative standard deviation	0.07	0.12	0.15
Titration error, per cent.	+0.1	+0.4	+0.6

REFERENCES

- Jennings, V. J., Forster, T. E., and Williams, J., *Analyst*, 1970, **95**, 718.
- Zittel, H. E., and Miller, F. J., *Analyt. Chem.*, 1965, **37**, 200.
- Yamada, S., and Sato, H., *Nature, Lond.*, 1962, **193**, 261.
- Plock, C. E., and Vasquez, J., *Talanta*, 1969, **16**, 1490.
- Lingane, J. J., Langford, C. H., and Anson, F. C., *Analytica Chim. Acta*, 1957, **16**, 165.
- Marinenko, G., and Taylor, J. K., *Analyt. Chem.*, 1967, **39**, 1568.
- Meyers, R. J., and Swift, E. H., *J. Amer. Chem. Soc.*, 1948, **70**, 1047.
- Lee, J. K., and Adams, R. N., *Analyt. Chem.*, 1958, **30**, 240.
- Knobel, M., *Editor*, "International Critical Tables," Volume VI, McGraw-Hill, New York, 1928, p. 340.
- Latimer, W. M., "Oxidation States," Second Edition, Prentice Hall Inc., New York, 1952, p. 60.

Received September 20th, 1971

Accepted July 4th, 1972

Book Reviews

RECENT ADVANCES IN THE ANALYTICAL CHEMISTRY OF THE NOBLE METALS. By F. E. BEAMISH and J. C. VAN LOON. *International Series of Monographs in Analytical Chemistry, Volume 48*. Pp. xvi + 511. Oxford, New York, Toronto, Sydney and Braunschweig: Pergamon Press. 1972. Price £14.

The appearance in 1966 of Professor Beamish's book, "The Analytical Chemistry of the Noble Metals," led us into the *sanctum sanctorum* of seven noble characters whose known behaviour can best be described as unusual, peculiar and unpredictable.

There may, on occasions, be good commercial reasons for not disclosing information on the analytical chemistry of these metals, but a change in attitude to the withholding of information of this nature has been brought about by a recognition that advances in analytical chemistry are best achieved by a free flow of information. This is particularly so in the field of modern instrumentation, although by no means in that area alone.

In this respect, the potential value of this latest, equally commendable book is evident, *e.g.*, in the application of electrometric, atomic-absorption, radioactivation, X-ray spectrometric and spectrochemical procedures for determining major and minor amounts of gold, palladium, rhodium, iridium, ruthenium and osmium in a multifarious range of raw materials and finished products.

It would be misleading to give an impression that the present authors have disregarded advances in other analytical fields, *e.g.*, those of solvent extraction, ion exchangers and chromatography. These areas are also covered in depth, the authors recognising that not all analytical laboratories are equipped with the necessary, often very expensive, instrumentation; even where they are so equipped, preliminary separations can be used to advantage, *e.g.*, for concentration purposes or to circumvent instrumental interferences.

The essential differences between this book and its inseparable companion, "The Analytical Chemistry of the Noble Metals," are the comprehensive coverage given to more recent innovations and the inclusion of a detailed, critical and up-to-date survey of information to supplement the earlier volume. However, this book is not simply a collection and co-ordination of published information with over 1600 supporting references. Its chapter headings, with the number of pages given in parentheses, are: *Methods of Separation* (74); *Atomic Absorption* (29, including 1 on flame photometry); *Neutron Activation* (85); *Spectrochemical* (51); *X-ray Fluorescence* (14); *Electrometric* (52), including sub-sections on polarographic, coulometric, amperometric, potentiometric and electrogravimetric methods; *Spectrophotometric* (120); *Gravimetric* (30); and *Titrimetric* (13).

With its predecessor, this complementary book provides an authoritative account of current practice in a branch of analytical chemistry where the analyst is frequently confronted with difficult and awkward problems.

W. T. ELWELL

ANALYTICAL SPECTROMETRY. By L. DE GALAN. Pp. viii + 279. London: Adam Hilger Ltd. 1971. Price £6.

The late appearance of this review does not indicate that this book is of little or no interest. It is a student textbook written originally in 1969 to support courses in analytical spectrometry at technical college level in The Netherlands. The English translation, which first appeared in 1971, contains some minor corrections and modifications to the original version but has the same basic contents with chapters on ultraviolet - visible, infrared and flame spectrometry, emission spectrometry by non-flame sources, X-ray spectrometry, nuclear magnetic resonance spectrometry, mass spectrometry and activation analysis. Theory is kept to a minimum but there are very useful chapters on monochromators and on a comparison of the various techniques and their application, which includes one often neglected factor, the time required to train an analyst in any specific procedure. The depth of treatment of any particular technique will be insufficient to satisfy a teacher with specialist leanings, but the approach and general level are appropriate to the many L.R.I.C. courses in analytical chemistry organised at Technical Colleges and Colleges of Technology in this country.

The author is to be congratulated on resisting the temptation to include historical perspective in concentrating his attention on the basic principles and current developments in each field. The lack of detailed theory will be insufficient for University undergraduate courses but this is, of course, intentional and the book will provide an excellent introduction to current spectrometric practice at the L.R.I.C. level. The only valid criticism is of the price. For a student textbook of moderate proportions, £6 seems excessive and one can only hope that a paperback version is either available now or will be available shortly, as this book should be read by as many students of analytical chemistry as possible.

J. M. OTTAWAY

ANNUAL REPORTS ON NMR SPECTROSCOPY. Edited by E. F. MOONEY. Volume 4. Pp. xvi + 548. London and New York: Academic Press. 1971. Price £10; \$29.

Review series of the type to which this volume belongs have two functions. Firstly, they present periodic bibliographies of published work in defined areas, following a pattern now well established in chemistry whereby the reviewer links together the formidable list of references with a text intended to serve as a rough guide and aid to digestion. Secondly, they provide a vehicle for specialist review articles less constrained by the objective of covering a fixed period, and offering the author more scope for including background information, critical review and a personal selection of topics.

Two of the articles in this latest volume are regular annual progress reports, on high-resolution proton magnetic resonance by G. R. Bedford, and on fluorine-19 nuclear magnetic resonance by K. Jones and E. F. Mooney, and cover work published in 1969. In both articles the authors have tackled these ever more onerous tasks with thoroughness, providing 426 references on proton nuclear magnetic resonance and 237 on fluorine-19.

The other four articles belong to the second of the above categories, and include references to work published up to about the middle of 1970. One in particular, I. O. Sutherland's 165-page contribution on the study of kinetics of conformational changes by nuclear magnetic resonance, contains enough introductory theory and critical review of experimental methods, as well as providing the most thorough survey of applications currently available, to be regarded as a valuable working handbook and reference source. The review by R. Haque and D. R. Buhler on nuclear magnetic resonance applications in pesticide chemistry will be a welcome item for industrial and agricultural chemists working in this area, and there must be many who would like to see more such contributions to the literature from specialists in areas of applied chemistry. C. W. Haigh's article on the use of iterative computer programmes in analysing nuclear magnetic resonance spectra is not so much a review as a useful and entirely practical guide to the software that the experienced Swansea group has found most effective, and I recommend it to anyone involved in or embarking on the analysis of strongly coupled spectra. M. E. A. Cudby and H. A. Willis present the current situation in structural analysis of polymers by high-resolution proton magnetic resonance, including experimental details such as the choice of solvents, selective deuteration and double resonance; applications to various vinyl homopolymers and to copolymers are reviewed.

The book contains good author and subject indices, an essential feature in a work of this nature. The total number of literature references is almost 1400. The production follows the high standard of earlier volumes. Regrettably, the continually rising prices of books, combined with the fact that only a proportion of the subject matter is likely to interest a particular reader, will make ownership of these periodic review volumes unattractive to many potential users; I confidently predict that the authors of the articles will be bombarded with reprint requests.

J. K. BECCONSALL

APPOINTMENTS VACANT

"Academic Appointments. Regular details of available lecturing and research positions, fellowships etc. Send for details and order form to Academic Appointment Listings, 12/161 Holden Rd., London N12."

Analyst

Quality Assurance

The Technical Services Division of Johnson & Johnson Ltd. require a forward thinking young man aged 25 years or under who has the ability to cope with general analytical work over a wide range of products manufactured in the U.K.

Applicants must have obtained a degree or equivalent in Chemistry and should preferably have had some experience of analytical work but this is not essential.

The modern laboratories are located in a pleasant area on the South Coast and generous assistance will be given towards the cost of rehousing where applicable. Salary will be negotiable.

Applicants should write stating age, qualifications and career to:

The Employee Relations Manager,
Johnson & Johnson Ltd.,
Southampton Road, Cosham
Portsmouth, PO6 4RL, Hants.

BUREAU OF ANALYSED SAMPLES LTD.

announce the issue of the following

NEW SAMPLES

Chemical Standards

- No. 318 0.01% Oxygen in Steel
- No. 380 Aluminium Alloy (B.S. 3L.51)
- No. 385 Leaded Brass (B.S. 218 & 249)

Spectroscopic Standards

- Nos. 601-606 Plain Carbon Cast Steels
- Nos. 656-660 Low Phosphorus Engineering Irons

For further details please write to:—

NEWHAM HALL, NEWBY,
MIDDLESBROUGH, TEESIDE, ENGLAND.
TS8 9EA

or Telephone Middlesbrough 37216 (STD 0642)

QUALITY CONTROL

Vacancies exist for analytical chemists in the Quality Control Department of the Company at Havant, Hants. Candidates should be qualified at graduate or HNC levels, preferably with some experience in the analysis of foods, pharmaceuticals or related products. The work involves the development of new analytical methods, the improvement of existing methods and routine analysis. Knowledge and understanding of modern instrumental techniques will be an advantage.

A non-contributory pension and life assurance scheme is in operation.

Please apply with full details to:—

Personnel Manager,
John Wyeth & Brother Limited,
Huntercombe Lane South,
Taplow, Maidenhead,
Berks, SL6 0PH.



Science & Technology Subscription Agency

**3 Dyers Buildings, Holborn,
London EC1**

Dr. I. Sutherland Dr. P. R. Masek M.I.Inf.Sc.

Expert subscription service on all foreign and domestic scientific and technical periodicals, continuations and serials for all libraries.

For a fast, reliable and continuing subscription service, contact:

**SCIENCE & TECHNOLOGY
SUBSCRIPTION AGENCY**

**3 Dyers Buildings
Holborn, London EC1, England**

The Determination of Polycyclic Aromatic Hydrocarbons in Mineral Oils by Thin-layer Chromatography and Mass Spectrometry

A procedure involving column chromatography and preparative thin-layer chromatography, with high-resolution mass spectrometry, has been developed for the determination of polycyclic aromatic hydrocarbons in mineral oils. The approximate content of these hydrocarbons can be determined by preparative thin-layer chromatography and a more accurate result obtained by analysing certain fractions from this separation by mass spectrometry. The mono- and dicyclic aromatic hydrocarbon contents can also be determined from the analysis of these fractions and considerable additional information is obtained about the types of polycyclic aromatic hydrocarbons present, as designated by *Z* number in the hydrocarbon formula C_nH_{2n+Z} .

Limitations of the procedure result from the presence of sulphur compounds in the thin-layer chromatographic fractions, although assumptions can be made that permit certain of these compounds to be determined in the mass-spectrometric analysis. Also, the latter method is not applicable to oils containing appreciable amounts of compounds with relative molecular masses higher than 500.

C. A. GILCHRIST, A. LYNES, G. STEEL and B. T. WHITHAM

Shell Research Ltd., Thornton Research Centre, P.O. Box 1, Chester, CH1 3SH.

Analyst, 1972, **97**, 880-888.

Specific Detection of Aromatic Compounds Containing Sulphur and Thiazole Compounds on Thin-layer Chromatographic Plates

Thin-layer chromatographic procedures involving the use of silica gel G plates have been developed for the specific detection of aromatic sulphur-containing and thiazole compounds in amounts of 4 μ g. A mixture of chloroform - methanol (9 + 1) was used as the developing system. Thiazole compounds with a free *ortho* position with respect to sulphur and aromatic sulphur-containing compounds (benzene derivatives) have been detected by using ninhydrin, and all aromatic sulphur-containing compounds can be detected by using copper(II) acetate containing hydroxylammonium chloride as the spray reagent. A method is described for distinguishing between the benzene-derived and heterocyclic sulphur-containing compounds.

M. S. BHATIA, K. L. BAJAJ, SIMRAN SINGH and I. S. BHATIA

Department of Chemistry and Biochemistry, Punjab Agricultural University, Ludhiana, India.

Analyst, 1972, **97**, 889-890.

Separation and Thin-layer Chromatographic Determination of Denatonium Benzoate and Other Quaternary Ammonium Denaturants in Spirituous Preparations

Denatonium benzoate is an effective partial denaturant at 2 mg l⁻¹ concentration in alcoholic preparations such as toiletries. While its presence is easily detected by tasting the preparation, an analytical procedure for its identification and determination is desirable. Denatonium salts are separated by ion exchange on carboxymethylcellulose and are determined by using thin-layer chromatography, which also separates other quaternary ammonium compounds. Some other methods that have been applied to solve this problem are briefly described.

M. J. GLOVER and A. J. BLAKE

Department of Trade and Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, SE1 9NQ.

Analyst, 1972, **97**, 891-896.

MONOGRAPHS FOR TEACHERS

Modern Analytical Methods

by D. BETTERIDGE
and H. E. HALLAM

Modern Analytical Methods is one of The Chemical Society's series of paperback monographs which present concise and authoritative accounts of selected well defined topics in chemistry for those who teach the subject at 'A' level and above and for students of further and higher education.

It discusses the principles underlying the most important methods of quantitative and qualitative analysis used today. Samples for analysis may arise from diverse sources and contain a variety of molecules or elements at various levels of concentration. Thus separation methods, organic reagents, nuclear, electrochemical, spectroscopic and titrimetric methods are amongst those dealt with in some detail. Within the bounds of elementary algebra, equations are developed which show how the optimum conditions for the application of a method may be deduced and conditional constants are used throughout. The numerous illustrations support the text by clarifying principles or by exemplifying important methods which are dealt with briefly because they do not involve new principles.

234pp 75 diagrams £2.00
(CS Members £1.50)
ISBN 0 85186 759 6

Orders enclosing the appropriate remittance, should be sent to:
The Publication Sales Officer, The Chemical Society, Blackhorse Road, Letchworth, Herts SG6 1HN.
For information on other titles in the series write to: The Marketing Officer, The Chemical Society, Burlington House, London W1V 0BN.



**THE CHEMICAL
SOCIETY**

SCIENCE & TECHNOLOGY AGENCY

3 DYERS BUILDINGS, HOLBORN, LONDON E.C.1.

Journals available from
Science and Technology Agency:

**Atomic Absorption and Flame
Emission Spectroscopy Abstracts**
Bimonthly £24

**X-Ray Fluorescence Spectroscopy
Abstracts**
Quarterly £24

Thin-Layer Chromatography Abstracts
Bimonthly £24

**Gas Chromatography—Mass
Spectrometry Abstracts**
Quarterly £37

**Nuclear Magnetic Resonance
Spectrometry Abstracts**
Bimonthly £28

Laser—Raman Spectroscopy Abstracts
Quarterly £30

X-Ray Diffraction Abstracts
Quarterly £30

Neutron Activation Analysis Abstracts
Quarterly £30

Electron Microscopy Abstracts
Quarterly £30

Mössbauer Spectrometry Abstracts
Quarterly £30

Sample copies on request from:
Science and Technology Agency,
3 Dyers Buildings, London E.C.1,
England. 01-405 9322

Automated Method for the Microdetermination of Sulphur

Sulphate at concentrations of less than 30 mg l⁻¹ of sulphur is determined on the AutoAnalyzer by reduction to hydrogen sulphide, distillation of the latter and its subsequent colorimetric determination.

The method has been found to be suitable for aqueous solutions of sulphate, digests of leaf samples and animal faeces, and extracts of soils; it is applicable to a wide range of agricultural, biological and industrial materials.

J. KEAY, P. M. A. MENAGÉ and G. A. DEAN

Division of Soils, CSIRO, W. A. Laboratories, Private Bag, P.O., Wembley, Western Australia.

Analyst, 1972, **97**, 897-902.

Sorption of Inorganic Phosphate by Laboratory Ware. Implications in Environmental Phosphorus Techniques

Acid-washed glass sorbed appreciable amounts of inorganic phosphate from distilled water systems and significant amounts (up to 20 per cent.) from lake waters within 1 to 6 hours of contact. Pre-treatment of polycarbonate with phosphate eliminated sorption of inorganic phosphate from distilled water and lake water and gave mean recovery values that had a low standard deviation. Lake waters should be filtered within 6 hours of collection. "Phosphated" polycarbonate is recommended for the storage of standard phosphate solutions containing small amounts of inorganic phosphate and for the sampling of lake waters and their storage in bulk subsequent to filtration. The use of acid-washed glassware should be avoided for these purposes.

J. C. RYDEN, J. K. SYERS and R. F. HARRIS

Department of Soil Science, University of Wisconsin, Madison, Wisconsin 53706, U.S.A.

Analyst, 1972, **97**, 903-908.

The Complexation of Tin(IV) with Catechol Violet

The various factors involved in the formation of the complex of tin(IV) with catechol violet have been studied and their significance is discussed.

B. W. BUDESINSKY

Department of Chemistry, University of Arizona, Tucson, Arizona 85721, U.S.A.

Analyst, 1972, **97**, 909-910.

Enthalpimetric Determination of Nitrogen-containing Bases

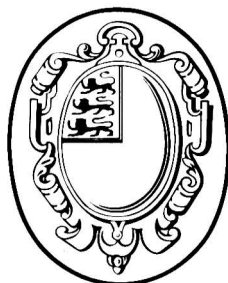
Milligram amounts of bases of various classes, *viz.*, compounds that contain primary, secondary or tertiary amino groups, quaternary ammonium compounds and simple ammonium salts, have been determined by utilising their reaction with a known and excess amount of sodium tetraphenylboron.

The excess of the boron compound has been determined by direct injection enthalpimetry by using potassium chloride. The results were all within 1.5 per cent. of the theoretical value. The standard deviation of the method applied to the determination of aniline (38 mg) was 0.27 mg.

L. S. BARK and J. K. GRIME

Department of Chemistry and Applied Chemistry, University of Salford, Salford, M5 4WT.

Analyst, 1972, **97**, 911-914.



National Physical Laboratory

Pesticide Analytical Standards

A range of 40 pesticides, of certified high purity, is available for use as reference and calibration standards in pesticide analysis. Use of these samples is recommended in the official handbook of the Collaborative International Pesticides Analytical Council. Typical examples from the present list, which is being rapidly extended, are:

γ -BHC	99.89%	α -endosulfan	99.8%
2,4-D (acid)	99.66%	β -endosulfan	99.5%
pp'-DDT	99.61%	monuron	99.92%
op'-DDT	99.3%	2,4,5-T	99.8%

Further details and prices are available from:

**Mr R R Collerson,
Division of Chemical Standards
National Physical Laboratory
Teddington
Middlesex TW11 0LW**

Telephone: 01-977 3222 ext. 3184

The Determination of Steam-volatile *N*-Nitrosamines in Foodstuffs by Formation of Electron-capturing Derivatives from Electrochemically Derived Amines

A method for the determination of six steam-volatile *N*-nitrosamines in a range of foodstuffs is described. *N*-Nitrosamines are isolated by steam distillation and are subjected to controlled-potential electrochemical reduction. The amines thus produced are converted into polyfluorinated amides, which are analysed by gas chromatography by means of electron-capture detection. Sensitivities of 1 μg per kilogram of original foodstuff are attained for all the *N*-nitrosamines studied. Low levels of some *N*-nitrosamines have been detected in several foodstuffs, some of which had not been treated with nitrate - nitrite preservatives.

T. G. ALLISTON, G. B. COX and R. S. KIRK

Department of Trade and Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, SE1 9NQ.

Analyst, 1972, **97**, 915-920.

Potentiometric Determination of Monuron in Herbicide Formulations

Monuron is extracted from a dispersion of the sample in hydrochloric acid into chloroform and the residue after evaporation of the chloroform is hydrolysed with boiling 24 *N* sulphuric acid. The liberated dimethylamine is distilled into 2 per cent. boric acid and titrated potentiometrically with standard hydrochloric acid. The method has been applied to technical monuron and aqueous suspension formulations with an error of 0.9 per cent.

S. H. YUEN and J. M. C. PALMER

Imperial Chemical Industries Limited, Agricultural Division, Jealott's Hill Research Station, Bracknell, Berkshire.

Analyst, 1972, **97**, 921-922.

Use of Vitreous Carbon as a Working Electrode for a Coulometric Redox Titration

Vitreous or glassy carbon has been examined as to its suitability for use as a working electrode in the coulometric generation of bromine. It has been found to compare well with platinum for this purpose, although an appreciable increase in the electrode potential is required to maintain a given current density. The theoretical current efficiency for the generation of bromine has been found to be better than 99.9 per cent. The theoretical value has been confirmed by the titration of 100 μequiv of arsenic(III) with an accuracy and precision each of 0.1 μequiv .

V. J. JENNINGS, A. DODSON and A. M. ATKINSON

Department of Chemistry and Metallurgy, Lanchester Polytechnic, Priory Street, Coventry.

Analyst, 1972, **97**, 923-926.

Spectrographers the world over know all about Specpure[®] chemicals & metals



but there are other products in our range

Specpure[®] Calibration Kit contains 73 materials, selected from the Specpure[®] range of ultra-pure metals and chemicals, together with a detailed analytical report on each material. With this kit in your laboratory you can select standard reference materials for immediate use in analysing a wide variety of samples.

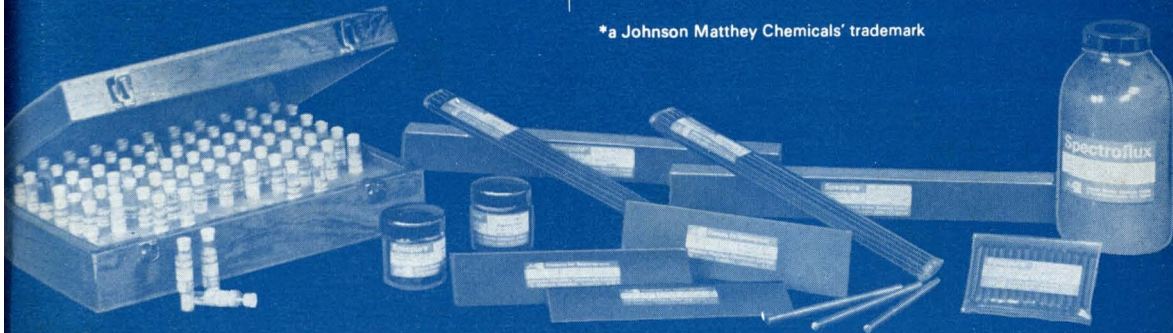
Specpure[®] Carbon and Graphite is available as rods, powders and preformed electrodes in grades suitable for use in all types of spectrographic analysis, whether in routine production control or in the most critical research work.

Johnson Matthey Chemicals' Silver Electrodes for Vacuum Spectrometry have exactly the degree of purity and the physical characteristics needed for this application and are relied on by major companies in their routine quality control analyses of metallurgical samples.

Spectroflux* materials form a range of prefluxed borate fluxes that are specially formulated to assist the analyst in the rapid and accurate analysis of ores, refractories and slags. Whether you are using instrumental or chemical methods, you can select from the Spectroflux* range the flux that is best suited to your needs.

Ask for publications on these products today!

*a Johnson Matthey Chemicals' trademark



Johnson Matthey Chemicals Limited

74 Hatton Garden, London EC1P 1AE, England Tel: 01-405 6989



THE QUEEN'S AWARD TO INDUSTRY
1968 1970

Sole distributor in USA: Jarrell Ash Division, Fisher Scientific Co., 590 Lincoln St., at Route 128 Waltham, Massachusetts 02154
Sole distributor in Japan: Tanaka Matthey KK, 14, 2-Chome, Kayabacho, Nihonbashi, Chuo-Ku, Tokyo

THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

CONTENTS

	Page
ORIGINAL PAPERS	
Analysis of Aluminosilicate Materials by X-ray Fluorescence Spectrometry— D. G. Ashley and K. W. Andrews	841
The Quantitative Determination of Uncombined Calcium Hydroxide and Oxide in Clinker and Cement by X-ray Diffraction— M. Bartl and D. Pekárek ..	848
Determination of Water of Crystallisation in Gypsum by Infrared Spectroscopy in the Presence of Clay Minerals— M. Bartl	854
Determination of Boron in Plants by Emission Spectroscopy with the Nitrous Oxide - Hydrogen Flame— J. C.-M. Pau, E. E. Pickett and S. R. Koirtyohann ..	860
The Assay of Non-fat Milk Solids by the Determination of Orotic Acid in Milk Chocolate and in the Milk— R. J. Motz	866
Analysis of Fluoroborate Alloy Plating Solutions to Determine Iron, Cobalt and Nickel— T. Keily and R. C. Woodford	872
A Colorimetric Method for the Determination of Chloral Hydrate— S. S. Kamat, V. P. Barve and H. S. Mahal	877
The Determination of Polycyclic Aromatic Hydrocarbons in Mineral Oils by Thin-layer Chromatography and Mass Spectrometry— C. A. Gilchrist, A. Lynes, G. Steel and B. T. Whitham	880
Specific Detection of Aromatic Compounds Containing Sulphur and Thiazole Compounds on Thin-layer Chromatographic Plates— M. S. Bhatia, K. L. Bajaj, Simran Singh and I. S. Bhatia	889
Separation and Thin-layer Chromatographic Determination of Denatonium Benzoate and Other Quaternary Ammonium Denaturants in Spirituous Preparations— M. J. Glover and A. J. Blake	891
Automated Method for the Microdetermination of Sulphur— J. Keay, P. M. A. Menagé and G. A. Dean	897
Sorption of Inorganic Phosphate by Laboratory Ware. Implications in Environ- mental Phosphorus Techniques— J. C. Ryden, J. K. Syers and R. F. Harris ..	903
The Complexation of Tin(IV) with Catechol Violet— B. W. Budesinsky	909
Enthalpimetric Determination of Nitrogen-containing Bases— L. S. Bark and J. K. Grime	911
The Determination of Steam-volatile N-Nitrosamines in Foodstuffs by Formation of Electron-capturing Derivatives from Electrochemically Derived Amines— T. G. Alliston, G. B. Cox and R. S. Kirk	915
Potentiometric Determination of Monuron in Herbicide Formulations— S. H. Yuen and J. M. C. Palmer	921
Use of Vitreous Carbon as a Working Electrode for a Coulometric Redox Titra- tion— V. J. Jennings, A. Dodson and A. M. Atkinson	923
Book Reviews	927
Summaries of Papers in this Issue	vi, vii, x, xii, xiv