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Summaries of Papers in this Issue

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P. SANZ PEDRERO and J. M. LÓPEZ FONSECA

Department of Physical Chemistry, Facultad de Farmacia, Universidad de Santiago, Santiago, Spain.

Analyst, 1972, **97**, 81–86.

Nitrate Ion Selective Electrodes Based on Poly(vinyl chloride) Matrix Membranes

Details are given of the construction and performance of two nitrate-selective electrodes with sensor poly(vinyl chloride) membranes incorporating commercial Corning and Orion nitrate liquid ion exchangers.

J. E. W. DAVIES, G. J. MOODY and J. D. R. THOMAS

Chemistry Department, University of Wales Institute of Science and Technology, Cardiff, CF1 3NU, Wales.

Analyst, 1972, **97**, 87–94.

The Determination of Ammonia in Boiler Feed-water with an Ammonium-selective Glass Electrode

An investigation has been made into the accuracy of ammonium-selective (ammonium-responsive) glass electrodes for determining ammonia (10 to 1000 $\mu g\ l^{-1}$) in boiler feed-water and similar high-purity water samples from power stations. The electrode potential follows the Nernst equation in samples containing up to 10 000 $mg\ l^{-1}$ of ammonia, the pH of which is controlled between 8·0 and 8·4 by the addition of triethanolamine - hydrochloric acid buffer solution. However, interfering species in the buffer solution cause a detectable deviation from Nernstian response at low ammonia concentrations (less than 1000 $\mu g\ l^{-1}$). By use of a calomel - 0·1 N hydrochloric acid reference electrode, reproducible results have been obtained in static buffered solutions containing 10 to 1000 $\mu g\ l^{-1}$ of ammonia. Of the other impurities likely to be present in power-station waters only sodium caused a serious effect (100 $\mu g\ l^{-1}$ of sodium is equivalent to 25 $\mu g\ l^{-1}$ of ammonia). The within-batch standard deviations of analytical results were 2, 7, 17 and 33 $\mu g\ l^{-1}$ at concentrations of 10, 100, 500 and 1000 $\mu g\ l^{-1}$ of ammonia, respectively. Details of a recommended analytical procedure for discrete samples are given, and the application of the ammonium-selective electrode to continuous on-stream analysis is briefly discussed.

G. I. GOODFELLOW and H. M. WEBBER

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Analyst, 1972, **97**, 95–103.

The Use of Atomic-absorption Spectrophotometry for the Determination of Copper, Chromium and Arsenic in Preserved Wood

The application of atomic-absorption spectrophotometry to the determination of copper, chromium and arsenic in preservative-treated wood is described. The preserving compounds are rapidly leached from wood samples with a mixture of dilute sulphuric acid and hydrogen peroxide solution. Copper, chromium and arsenic in the leach solution are determined by atomic-absorption spectrophotometry and the results are compared with those obtained by colorimetric analysis.

With this method an analytical sensitivity for arsenic of 0·10 μg ml^{-1} has been achieved by use of an argon (entrained air) - hydrogen flame and a 10-cm propane burner.

A. I. WILLIAMS
Department of the Environment, Forest Products Research Laboratory, Princes Risborough, Aylesbury, Bucks.

Analyst, 1972, **97**, 104–110.

Spectrophotometric Determination of Phosphorus in Biological Samples after Dry Ashing without Fixatives

Simple dry ashing of samples without the use of fixatives can be used in the determination of phosphorus, in addition to metals, in biological materials. Two alternative spectrophotometric methods can be applied directly to a hydrochloric acid solution of the ash.

J. TUŠL
Research Institute for Animal Nutrition, Pohořelice, Czechoslovakia.

Analyst, 1972, **97**, 111–113.

Spectrophotometric Determination of Trace Amounts of Thorium in Lanthanum Oxide

A spectrophotometric method for the determination of trace amounts of thorium in lanthanum oxide or lanthanum metal has been developed.

The method is based on the determination of thorium with thoron after its separation from lanthanum by extraction with a mixture of ethyl acetate and acetone. The efficiency of the separation was tested with a lanthanum-140 tracer. Other elements present in lanthanum oxide as impurities do not interfere.

Thorium contents down to 10 p.p.m. can be determined in a 1-g sample with 1-cm cells used for absorbance measurement. Errors of determination are within the range ± 5 per cent. The relative standard deviations on samples containing 30 and 100 p.p.m. of thorium are 1·6 and 1 per cent., respectively.

D. RAJKOVIĆ
Analytical Department, Institute for Technology of Nuclear Raw Materials, Belgrade, Yugoslavia.

Analyst, 1972, **97**, 114-117.

The Determination of Molybdenum in Geological Materials by a Combined Solvent-extraction - Atomic-absorption Procedure

A method is described for the determination by atomic-absorption spectrophotometry of molybdenum, up to the 1000 p.p.m. level, in geochemical samples. After attack with acid, the molybdenum in dilute perchloric acid medium is complexed with benzoin α-oxime and extracted into chloroform. The extract is mixed with nitric and perchloric acids and evaporated to dryness and the residue is dissolved in ammonia solution. The ammonium molybdate formed is dissolved in a solution containing 1 per cent. v/v of perchloric acid *plus* 0·5 per cent. w/v of ammonium chloride and aspirated into a luminous air - acetylene flame.

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Analyst, 1972, **97**, 118–123.

THE ANALYST

Analytical Application of the Cobalt(II) Catalytic Pre-wave in the Presence of Thiamine in its Disulphide Form

BY P. SANZ PEDRERO AND J. M. LÓPEZ FONSECA

(Department of Physical Chemistry, Facultad de Farmacia, Universidad de Santiago, Santiago, Spain)

The cobalt catalytic pre-wave in the presence of thiamine in its disulphide form, and in a borate medium, was studied as a function of the thiamine (disulphide form) and cobalt(II) concentrations and of the pH of the solution. The effects of temperature, the addition of neutral salts and the presence of surface-active agents on the pre-wave are described. The effect of thiamine (disulphide form) is shown on the electrocapillary curves. The electrode process involves the formation of complexes between cobalt(II) and thiamine, in its thiolic form, adsorbed on the dropping-mercury electrode, and the electrochemical decomposition of these complexes with liberation of the catalytic ligand. The analytical application of this pre-wave was studied.

IN a general study of the polarographic behaviour of thiamine in the presence of cobalt(II), a well defined cobalt pre-wave was obtained in a borate medium after converting the thiamine into its disulphide form by the action of an alkaline solution of iodine.[1] The pre-wave mechanism involves the formation of complexes between cobalt(II) and the thiolic form of thiamine, which is produced by reduction of the disulphide form of thiamine in the dropping-mercury electrode. These complexes are reduced to the potentials of the pre-wave, thus regenerating the ligand, which is then able to participate again in this cycle of reactions. Similar mechanisms have been proposed for the cobalt(II),[2–5] nickel(II)[6–12] and indium(III)[13,14] catalytic pre-waves.

In this paper, an extensive study of the properties of this pre-wave is described with the purpose of completing the interpretation of its mechanism and indicating the optimum conditions for its application to the analytical determination of thiamine.

EXPERIMENTAL

APPARATUS—

The dropping-mercury electrode used in these experiments at a height of 45 cm of mercury, with no applied potential, had the following characteristics in 0·06 M borax solution: m 2·13 mg s^{-1} and t 4·24 s. A saturated calomel electrode (S.C.E.) was used as the reference electrode, and its electrical contact with the cell was made by means of an agar - potassium chloride bridge. Unless otherwise stated, the experiments were carried out at 25 °C. Recording of current - potential curves was made with a Radiometer PO4 Polarograph with the damping switch set at position 5. The pH values were measured with a Radiometer pH meter, Model 26.

REAGENTS—

Thiamine hydrochloride—A Fluka product.

Gelatin—A Sigma product.

Polyvinylpyrrolidone—Supplied by Koch-Light Laboratories Ltd.

Other chemicals used were of Merck reagent grade.

Two standard thiamine (disulphide form) solutions were used, as follows.

Standard thiamine solution, 10^{-3} M—Dissolve an appropriate amount of thiamine hydrochloride in about 40 ml of water, add 5 ml of 0·1 M sodium hydroxide solution and, after the yellow colour has disappeared, 1 ml of 0·05 M iodine solution. Make the volume up to 50 ml with water.

Standard thiamine solution, 10^{-4} M—To about 40 ml of a solution containing the appropriate amount of thiamine hydrochloride add 0·1 M sodium hydroxide solution until the

pH of the solution reaches 11, leave for about 10 minutes to ensure that the thiamine is converted into its thiolic form, then add 0·15 ml of 0·05 M iodine solution. Dilute the solution to 50 ml with water.

Both of the standard thiamine solutions give reproducible pre-waves 30 minutes to 5 hours after preparation.

PROCEDURE—

The procedure used is identical with that given in a previous paper.[1]

The catalytic current values, i_{cat}, measured are average peak currents corrected for the residual current. The electrocapillary curves were recorded by measuring the drop time of 10 drops at a given potential.

RESULTS AND DISCUSSION

STUDY OF THE PRE-WAVE—

Polarograms A in Fig. 1 show well defined cobalt pre-waves in the presence of thiamine (disulphide form) in a 0·4 mM cobalt(II) chloride - 0·06 M borax system (pH 9·5). This medium also contains iodine and sodium hydroxide at concentrations of 10^{-5} and 10^{-4} M, respectively, which originate from the standard thiamine solution. The presence of these two substances does not modify the pre-wave current values, although the iodine (or iodide ion) slightly increases the cobalt(II) background wave. Thus it can be seen that the pre-waves show a rounded maximum with a potential peak of −1·04 V *versus* S.C.E.

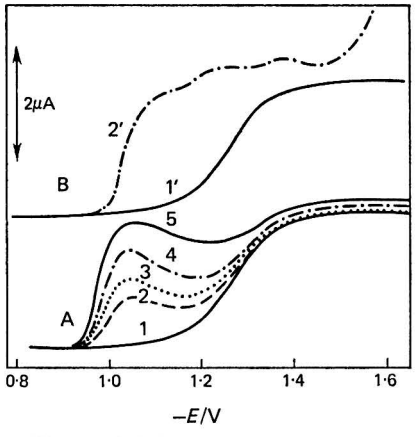


Fig. 1. Cobalt pre-wave in the presence of thiamine (disulphide form). Polarograms A: 0·06 M borax - 0·4 mM Co(II), pH 9·5; thiamine concentration × 10^{-5} M: 1, 0; 2, 0·5; 3, 1; 4, 2; and 5, 8. Polarograms B: 0·06 M borax - 0·45 M boric acid - 0·4 mM Co(II), pH 7·7; thiamine concentration: 1', 0; and 2', 6 × 10^{-5} M

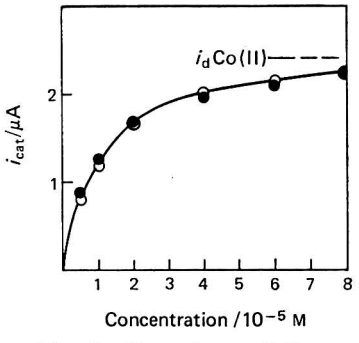


Fig. 2. Dependence of the pre-wave catalytic current (i_{cat}) on the thiamine (disulphide form) concentration in two buffer systems with a 0·4 mM Co(II) concentration: ●, pH 9·5; and ○, pH 8·2

The decrease in the pH from 9·5 to 7·7 caused by the addition of boric acid to the 0·06 M borax medium does not change the pre-wave limiting current. Nevertheless, as shown in polarograms B in Fig. 1, in buffers of pH up to 8·3 the pre-waves obtained at moderately high thiamine concentrations are poorly defined because of the presence of two small maxima. The potentials at which these maxima appear coincide with those of the observed currents when thiamine that has not been converted into its disulphide form is used. These currents were considered to be catalytic hydrogen waves.[1] In these polarograms at more negative potentials the beginning of a wave can be seen, which, when it appears also in the absence of cobalt(II), is included in the so-called "pre-sodium currents."[15]

In Fig. 2, the pre-wave peak currents are represented as a function of the thiamine (disulphide form) concentration. The results were obtained in two buffers of different pH,

both solutions being 0·4 mM with respect to cobalt(II). The catalytic current does not vary linearly with the thiamine concentration but it tends slightly towards the same limiting value as that of the cobalt(II) diffusion current.

The effect of the cobalt(II) concentration on the pre-wave catalytic currents was studied in two supporting electrolytes. The cobalt(II) concentration was adjusted to between 0·1 and 0·4 mM in the 0·06 M borax medium. As the solubility of cobalt in this medium is limited to a concentration of 0·4 mM,[16] a 0·06 M borax - 0·3 M boric acid buffer was used in order to study the influence of higher cobalt(II) concentrations. Variation in the peak current for a 1×10^{-5} M thiamine (disulphide form) concentration *versus* the cobalt(II) concentration is shown in Fig. 3.

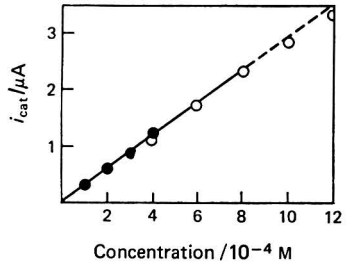


Fig. 3. Dependence of the pre-wave catalytic current on the Co(II) concentration. Thiamine (disulphide form) concentration 1×10^{-5} M: ●, 0·06 M borax; and ○, 0·06 M borax - 0·3 M boric acid

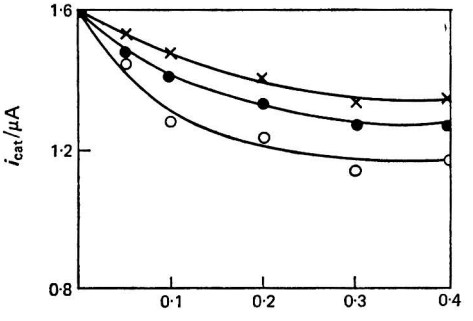


Fig. 4. Influence of the addition of neutral salts on the pre-wave catalytic current; 0·06 M borax - 0·4 mM Co(II) - 2×10^{-5} M thiamine (disulphide form) system: ×, LiCl; ●, NaCl; and ○, KCl

The kinetic nature of the pre-wave was made evident by the fact that its peak current, when less than 25 per cent. of the cobalt(II) diffusion current, was not influenced by the height of the electrode mercury.[1] This conclusion is confirmed by the high pre-wave temperature coefficient. In a 0·06 M borax - 0·4 mM cobalt(II) - $2{\cdot}5 \times 10^{-6}$ M thiamine (disulphide form) system the average temperature coefficient in the 20 to 30 °C range is 3·9 per cent. $°C^{-1}$. On increasing the thiamine concentration in this medium, the pre-wave temperature coefficient gradually diminishes.

The effect of neutral salts on the pre-wave was studied by adding amounts of different alkali-metal chlorides to a 0·06 M borax - 0·4 mM cobalt(II) - 2×10^{-5} M thiamine (disulphide form) system. An increase in the ionic strength caused by the addition of a salt is accompanied by a decrease in peak currents (Fig. 4), by a sharper decrease in the pre-wave currents following the peak and by a slight displacement of the peak potentials to more positive values. In Fig. 4, the effect of the particular cation on catalytic currents can be seen; the i_{cat} values diminish in the order $Li^+ > Na^+ > K^+$.

Surface-active agents exercise a strong influence on pre-wave catalytic currents. In the 0·06 M borax - 0·4 mM cobalt(II) - 2×10^{-5} M thiamine (disulphide form) system, polyvinylpyrrolidone causes a decrease in the catalytic current, with almost total elimination of the pre-wave but without affecting the cobalt(II) background wave. The results of the effect of this surface-active agent on the catalytic current are summarised in Table I. Gelatin behaves in a similar way to polyvinylpyrrolidone in an identical system, although the pre-waves are not well defined because of the deformation of the cobalt(II) background wave brought about by this surface-active agent. With a 0·008 per cent. gelatin concentration the pre-wave is eliminated completely.

TABLE I

EFFECT OF POLYVINYLPYRROLIDONE ON THE PRE-WAVE PEAK CURRENT

System: 0·06 M borax - 0·4 mM cobalt(II) - 2×10^{-5} M thiamine (disulphide form)

Polyvinylpyrrolidone concentration, per cent. $\times 10^{-3}$	0	1	2	4	6	8	11
$i_{cat}/\mu A$	1·68	1·56	1·46	1·12	0·68	0·28	0·0

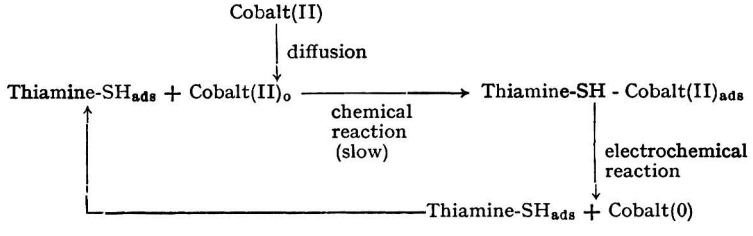
ELECTROCAPILLARY CURVES—

The dropping-mercury electrode electrocapillary curves were obtained in 0·06 M borax medium in the absence and presence of 10^{-4} M thiamine (disulphide form). Curve 1 in Fig. 5 corresponds to the borate system that was also 10^{-4} M with respect to iodine and 10^{-3} M with respect to sodium hydroxide, that is, the concentrations of these reagents originating from the standard solution of thiamine used. These substances are also present in the same concentrations in the solution with which curve 2 is obtained. As can be seen, the presence of thiamine (disulphide form) in the solution, although its concentration is of the same order as the concentration used in polarography, causes a decrease in the interfacial tension of the dropping-mercury electrode. This effect is maintained up to a potential of −1·0 V, which is close to the pre-wave peak potential, and indicates that an organic species is adsorbed on the surface of the dropping-mercury electrode.

MECHANISM OF THE ELECTRODE PROCESS—

The thiolic form of thiamine produced by reduction of the disulphide form in the dropping-mercury electrode gives complexes with cobalt(II) in which the cobalt is reduced with a much smaller overpotential than it is in the aquo-cobalt ion. The fact that the pre-wave peak current is independent of the mercury height and also the high temperature coefficient of i_{cat} shows that the pre-wave is kinetic in nature and that it is governed by the re-combination rate of the thiolic form, liberated by the decomposition of the complexes, with the cobalt(II) diffusing to the electrode.[1]

The decrease in the pre-wave limiting current after forming a peak indicates the effect of the potential on the adsorption of a species taking part in the electrode process.[17,18] It is therefore reasonable to suggest that this process involves the participation of the thiolic form of thiamine (catalytic ligand) adsorbed on the electrode surface. This can be expressed by the following scheme.



The electrocapillary curves shown in Fig. 5 are in agreement with the processes shown in this scheme and give evidence of the presence of a species adsorbed up to potentials at which the pre-wave begins, this species being desorbed progressively as the cathodic potential increases.

The effect of neutral salts on pre-wave peak currents is consistent with the adsorption of the catalytic ligand. As the ionic strength or the specific adsorption of the salt cation increases, the absolute value of the negative potential, ψ_1 (the potential on the external surface of the Helmholtz region), decreases. As in the borate medium the cobalt ion passes through the diffuse layer with a positive charge,[16] a decrease in the negative value of ψ_1 should lead to a decrease in the chemical reaction rate of formation of the thiamine-SH - cobalt(II) complexes if this reaction takes place in the immediate proximity of the electrode,[19–22] as occurs when the adsorbed ligand participates. On the other hand, an increase in the ionic strength or in the adsorption of the cation causes a decrease in the adsorptivity of the catalytic ligand, as expressed by the Frumkin equation.[17] Both effects lead to a decrease in the pre-wave peak currents shown in Fig. 4.

Finally, the almost complete elimination of the pre-wave by the addition of polyvinyl-pyrrolidone, or gelatin, indicates that the surface-active agent causes a decrease both in ligand adsorption, on displacing it from the electrode surface, and in the chemical and electrochemical reaction rates.

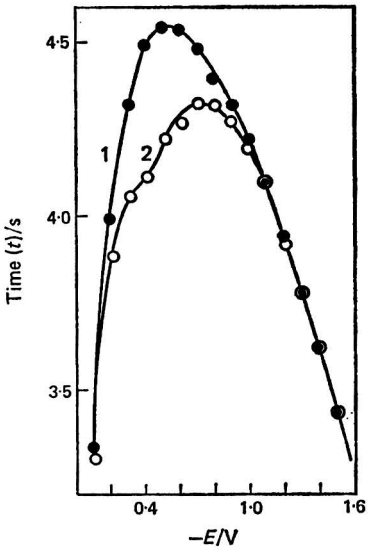


Fig. 5. Electrocapillary curves: 1, 0·06 M borax containing 10^{-4} M I_2 - 10^{-3} M NaOH; and 2, 0·06 M borax with 1×10^{-4} M thiamine (disulphide form)

ANALYTICAL APPLICATION—

In the analytical determination of thiamine, greater sensitivity is obtained by increasing the cobalt(II) concentration in the medium. To effect this increase it is necessary to lower the pH of the supporting electrolyte.[16] The system used was 1 mM cobalt(II) - 0·06 M borax - 0·4 M boric acid (pH 7·85). Greater accuracy is obtained with thiamine concentrations up to 1×10^{-5} M, as the slope of the graph of catalytic current *versus* concentration decreases with increasing concentration of thiamine (disulphide form) (Fig. 2).

The results for the thiamine determination in the range of concentrations 1×10^{-6} to 10×10^{-6} M are shown in Table II. The determinations of concentration were performed in four successive series, by using 10^{-4} M thiamine (disulphide form) solutions obtained from the same thiamine solution. A simple calibration graph was used as a reference. In accordance with these results, the analytical evaluation performed with this method can be made with satisfactory precision and accuracy.

TABLE II

ANALYTICAL DETERMINATION OF THIAMINE

Thiamine concentration			
Taken/10^{-6}M	Found/10^{-6}M (average of four determinations)	Relative error, per cent.	Range
1·00	1·01	+1·0	1·00 to 1·05
2·00	1·99	−0·5	1·95 to 2·05
4·00	3·98	−0·5	3·95 to 4·05
7·00	7·08	+1·1	7·00 to 7·15
10·00	10·04	+0·4	9·80 to 10·15

We thank Miss T. Bujeiro, Mr. J. C. Tutor and Mr. M. Miguez for their collaboration and the Ministry of Education and Science for the scholarship granted to enable this work to be carried out.

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Nitrate Ion Selective Electrodes Based on Poly(vinyl chloride) Matrix Membranes

BY J. E. W. DAVIES, G. J. MOODY AND J. D. R. THOMAS

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Details are given of the construction and performance of two nitrate-selective electrodes with sensor poly(vinyl chloride) membranes incorporating commercial Corning and Orion nitrate liquid ion exchangers.

THREE nitrate-selective electrodes are now commercially available.[1] Although their over-all performances are relatively similar (Tables I and II), only the Orion 92–07 model seems to have been widely used. Applications include the determination of nitrate in soils,[2–7] waters[7–12] and plant extracts,[7,13–16] and for following the rate of nitrate reduction by sheep rumen bacilli.[17] The electrode has also been used for potentiometric titrations[18] and lanthanum(III) complex studies,[19] and for determining oxides of nitrogen in flowing gas streams[20] and nitrate in explosives.[21] Flow-through models have also been described.[12,22]

The Orion 92–07 electrode incorporates a tris(substituted *o*-phenanthroline)nickel(II) ion exchanger dissolved in an organic solvent to give an ion-exchanging liquid that is insoluble in water.[23–25] No information is available on the ion exchanger material used for the Beckman or Corning* nitrate electrodes. Coetzee and Frieser[26,27] have described a useful nitrate-selective electrode with a 10 per cent. v/v solution of methyltrioctanoylammonium nitrate exchanger in decanol in an Orion 92-series electrode body. Bakelite membranes prepared by polymerising various phenol - formaldehyde - nickel nitrate - ammonia mixtures showed a linear 60 mV per decade response between 10^{-1} and 10^{-5} M nitrate activity. Although sulphate, phosphate and ferricyanide do not interfere, other anions such as bromide, chloride, perchlorate and thiocyanate constitute very serious interferents.[28]

The Orion 92-series of liquid membrane electrodes requires frequent re-charging of the organic ion exchanger. This inconvenience, with the Orion 92–20 calcium electrode, has been solved by incorporating calcium liquid ion exchanger in a membrane based on a poly(vinyl chloride) matrix.[29] This PVC - calcium electrode performs as well as any commercial calcium electrode and, moreover, is still functional after 2 years' frequent use in stirred solutions.[29] (G. J. Moody, R. B. Oke and J. D. R. Thomas, unpublished work.)

For the Orion 92–07 nitrate electrode, the manufacturer gives varying "lifetimes" from 30 days up to several months, whereas Potterton and Shults[30] have found it necessary, and troublesome, to re-charge the Orion nitrate electrode every 3 weeks when in constant use. Greater interference from chloride is reported for an Orion nitrate electrode in which the internal solutions had not been changed for 5 months.[15]

This paper describes the fabrication and general performance of two nitrate-selective electrodes based on nitrate liquid ion exchangers incorporated in a poly(vinyl chloride) matrix.

EXPERIMENTAL

PREPARATION OF THE PVC - NITRATE ION EXCHANGER MEMBRANES—

The Corning 477316 or Orion 92–07–02 nitrate liquid ion exchanger (0·42 g) was added to a solution of 0·177 g of poly(vinyl chloride) [Breon 113 (now known as Breon S 110/10), B.P. Chemicals (U.K.) Ltd.] in 6 ml of tetrahydrofuran. This solution was poured into a glass ring (33 mm i.d.) resting on a glass plate, and the system was allowed to evaporate over a period of 2 days. The glass ring with adhering membrane was then inverted and left for 1 day to allow the solvent to evaporate from the under-surface. No attempt was made to establish the optimum proportion of ion exchanger in the PVC matrix, although for the analogous PVC - calcium electrode[29] a PVC content of at least 20 per cent. is required for a functional membrane.[31]

*This Corning exchanger is tridodecylhexadecylammonium nitrate in n-octyl *o*-nitrophenyl ether.

TABLE I

SPECIFICATIONS OF VARIOUS NITRATE-SELECTIVE ELECTRODES

	Electrode					
	Beckman No. 39618	Corning No. 476134	Orion No. 92–07	PVC (Corning) exchanger (this work)	PVC (Orion) exchanger (this work)	Coetzee-Frieser[a]
Effective concentration range/M	—	10^{0} to 10^{-6}	10^{-1} to 10^{-5}	10^{-1} to 10^{-4}	10^{-1} to 2×10^{-4}	10^{-1} to 10^{-3}
Effective pH range	2 to 12 (at $> 10^{-1}$ M NO_3^-)	2·5 to 10 (in 10^{-2} M KNO_3)	2 to 12 (in 10^{-1} M $NaNO_3$)	2·5 to 8 (in 10^{-2} M $NaNO_3$)	2·5 to 8 (in 10^{-2} M $NaNO_3$)	—
Potential response at 25 °C/mV per decade	—	—	56·6	~57	~57	57
Resistance/MΩ	100	—	<30	—	—	—
Response in pure nitrate[b]	A few seconds	<60 s	A few seconds	A few minutes	A few minutes	~ 1 minute
Operational lifetime	> 100 hours	10 to 15 days	30 days Several months 3 weeks[c]	> 11 weeks	2 to 3 weeks	1 month
Length/cm	12·8	12·8	14·9	11	11	14·9
Diameter/cm	1·25	1·5	1·75	0·7	0·7	1·75
Principal interferents (X) (where $K_{NO_3X} > 1$)	I^-; ClO_3^-; ClO_4^-	I^-; ClO_4^-	I^-; ClO_3^-; ClO_4^-	I^-; ClO_3^-; ClO_4^-	I^-; ClO_3^-; ClO_4^-	—

Manufacturer's data unless otherwise stated.

[a] Reference 26.
[b] Depends on concentration and on whether solutions are stirred or unstirred.
[c] Reference 30.

TABLE II

SELECTIVITY COEFFICIENTS, K_{NO_3X}, OF NITRATE LIQUID ION-EXCHANGE MEMBRANE ELECTRODES

	Nitrate electrode						
						PVC Models[a]	
Interfering anion (X)	Beckman No. 39618	Corning No. 476134[b]	Orion 92–07	Orion 92–07[c]	Coetzee - Frieser Model[d,e]	Corning exchanger	Orion exchanger
F^-	$6{\cdot}6 \times 10^{-3}$	—	6×10^{-6} 9×10^{-4}	5×10^{-4}	—	$8{\cdot}7 \times 10^{-4}$ (5×10^{-2} M)	7×10^{-4} (5×10^{-2} M)
Cl^-	0·02	4×10^{-3}	6×10^{-3} 4×10^{-3}	8×10^{-3} 0·02 to 0·05[f]	0·23	5×10^{-3} (5×10^{-1} M)	4×10^{-3} (5×10^{-1} M)
Br^-	0·28	0·011	0·13; 0·9 0·09 to 0·24[g]	—	—	—	—
I^-	5·6	25	20 2·7 to 22·2[g]	—	—	17 (5×10^{-5} M)	16 (5×10^{-5} M)
NO_2^-	0·066	—	0·04 0·06	0·09	~0·5	0·066 (5×10^{-2} M)	0·06 (5×10^{-2} M)
SO_4^{2-}	10^{-5}	$<10^{-3}$	6×10^{-4} 3×10^{-5}	—	$2{\cdot}5 \times 10^{-3}$	$<10^{-5}$ (5×10^{-1} M)	3×10^{-4} (5×10^{-1} M)
CO_3^{2-}	$1{\cdot}9 \times 10^{-4}$	—	2×10^{-4} 6×10^{-3}	—	—	—	—
HCO_3^-	—	$<10^{-3}$	0·02 9×10^{-3}	—	—	[h]	[h]
$CH_3CO_2^-$	5×10^{-3}	$<10^{-3}$	6×10^{-3} 4×10^{-4}	—	—	—	—
ClO_3^-	1·1	—	2 1·14 to 1·45[g]	0·89	—	1·66 (5×10^{-4} M)	1·66 (5×10^{-4} M)
ClO_4^-	95·5	$>10^3$	10^3	—	—	800 (5×10^{-5} M)	550 (5×10^{-5} M)
CN^-	0·02	—	0·02 0·01	—	—	—	—
PO_4^{3-}	$7{\cdot}4 \times 10^{-3}$	—	3×10^{-4} 10^{-4}	—	—	[h]	[h]
$H_2PO_4^-$	—	—	3×10^{-4} 5×10^{-5}	—	—	—	—
HPO_4^{2-}	—	—	8×10^{-5} 3×10^{-5}	—	—	—	—

[a] Mixed solution method. Values in parentheses are interferent concentrations in each instance.
[b] Presented as the reciprocals of manufacturer's apparent selectivity constants.
[c] Reference 30.
[d] Based on 10 per cent. v/v methyltrioctanoylammonium nitrate organic exchangers in decanol.[26,27]
[e] Originally expressed as log selectivities.[26] [f] Reference 11. [g] Reference 32. [h] See text.

CONSTRUCTION OF THE PVC - NITRATE ELECTRODES—

The constructional details of each PVC - nitrate electrode are as already described[29] for the PVC - calcium electrode, with membrane discs (6 mm in diameter and 0·35 mm thick) cut out with a cork borer. The master membrane (33 mm in diameter) provides sufficient material for about ten electrodes. Each of the several nitrate electrodes fabricated from different master membranes gave essentially identical activity ranges, slopes and selectivities.

APPARATUS FOR EVALUATING THE PERFORMANCES OF THE PVC - NITRATE ELECTRODES—

Ag; AgCl(s)	NO_3^- (5×10^{-2} M) Cl^- (5×10^{-2} M)	Corning or Orion exchanger in PVC	Test sample	Reference electrode

← PVC - nitrate electrode →

Measurements were taken with the above electrochemical cell, by using a Beckman Research Model pH meter. The E.I.L. calomel electrode, Type RJ23, with remote micro-junction constituted a satisfactory reference electrode. The phenylmercury acetate preservative normally used in Orion 92–07 electrodes was not included in the internal nitrate - chloride reference solutions.

Before use each PVC - nitrate electrode was soaked in 0·1 M sodium nitrate solution for 1 day; the electrodes were also stored in this solution. All solutions were stirred in a thermostatically controlled 50-ml beaker (25 ± 0·1 °C). The water used for the preparation of the solutions was obtained by distilling de-ionised water from alkaline permanganate solution.

RESULTS AND DISCUSSION

CALIBRATION OF THE PVC - NITRATE ELECTRODES IN PURE NITRATE SOLUTIONS—

Potentials were recorded on standard solutions of analytical-reagent grade sodium nitrate of nitrate concentration decreasing from 10^{-1} to 10^{-5} M. The electrode was then immediately exposed to standard nitrate solutions of increasing nitrate concentration up to the initial 10^{-1} M. The electrode was quickly blotted with tissue paper between measurements. Concentrations were converted into activities as previously described,[29] by using the equation—

$$-\log f = 0{\cdot}511z^2\sqrt{I}/(1+\sqrt{I}) \quad \ldots \quad (1)$$

where f and z are the activity coefficient and ionic charge of the nitrate ion, respectively, and I is the ionic strength of the solution. The potential response of the PVC (Corning exchanger) - nitrate electrode (shown in Fig. 1) is linear over the range 10^{-1} to 10^{-4} M nitrate, and so is the response of the PVC (Orion exchanger) electrode. Such response patterns compare favourably with the commercial nitrate electrodes (Table I), although manufacturers' claims usually include the plateau region beyond the lower linear sections of calibration graphs, but it is more practical to give the linear detection limit as in Fig. 1.

The "constant" term in equation (2) was initially −4 mV for the PVC (Corning exchanger) electrode but became more positive by about 10 mV when the electrode was operated over a period of 11 weeks.

$$E = \text{Constant} - 0{\cdot}059 \log a_{NO_3^-} \quad \ldots \quad (2)$$

The potential in any one nitrate-containing solution varied by only about ±2 mV during 1 day's use.

For the corresponding PVC (Orion exchanger) electrode the potential in any one nitrate-containing solution varied by about ±4 mV during 1 day while the constant term was about −12 mV with a positive drift of about 5 mV over a period of 2 weeks. Compared with this finding, Mahendrappa[5] reported much greater variation in the constant term with "constants" of −10 mV for freshly assembled Orion 92–07 electrodes, reaching −80 to −85 mV within 1 day, and −100 to −110 mV during a 4-week period. However, it was found[5] that the calibration slope did not change with time, although different electrodes gave different calibration slopes ranging from 64 to 50 mV per activity decade, the wide spread being attributed to defective assembly of the electrodes.[5] Potterton and Shults[30] have also found slope deviations for the Orion 92–07 electrode, while the potentials were reproducible to ±0·8 and ±2·9 mV over periods of 3 hours and 5 days, respectively.

The initial calibration slope (56 to 57 mV per activity decade) of the PVC (Orion exchanger) - nitrate electrode remained reasonably constant for 2 to 3 weeks but the response

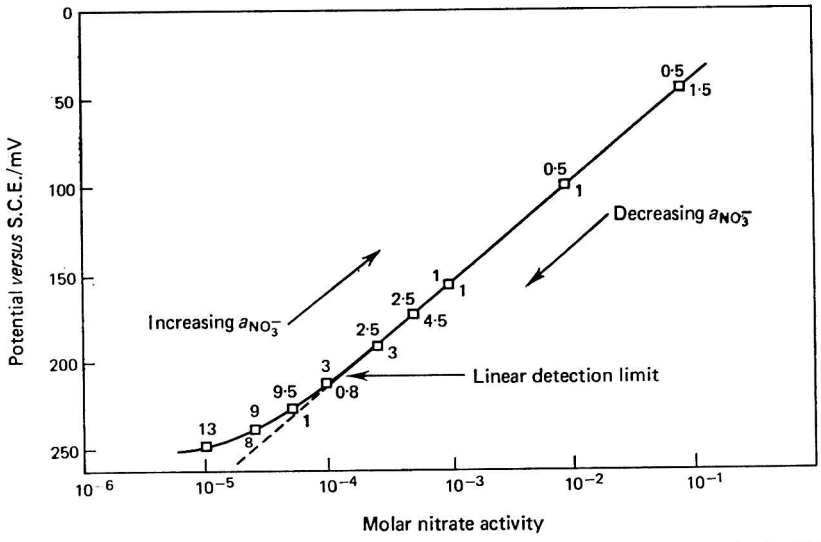

Fig. 1. Calibration of the PVC (Corning ion exchanger) - nitrate electrode at pH 6 and 25 °C. Slope = 58 mV per decade. Figures adjacent to the symbols refer to the time in minutes required for steady potentials to be attained as the nitrate activity is increased or decreased according to the directions of the arrows

then became noisy and sluggish. The deterioration of this electrode is attributed to the solubility in water of the liquid ion exchanger. For the PVC (Corning exchanger) - nitrate electrode the calibration slope of 57 mV per activity decade fell to about 54 to 55 mV at the end of an 11-week study period but without any loss of response times or any serious noise developments.

INTERFERENCES—

Cationic interference—As a check on possible cationic interference, both of the PVC - nitrate electrodes were also calibrated in pure solutions of ammonium, copper(II) and lanthanum nitrates at 25 °C (Fig. 2). There is essentially no interference for the cations studied. Myers and Paul[3] have obtained similar results for the Orion 92–07 nitrate electrode with ammonium, calcium and copper(II) - silver mixtures. This is of some importance in any electrode soil analysis that involves a preliminary copper(II) sulphate extraction stage.

Anionic interference—The electrode will respond variously to foreign anions (X) as well as nitrate according to the equation—

$$E = \text{Constant} - 0{\cdot}059 \log [a_{NO_3} + K_{NO_3X}(a_X)^{1/n}] \quad .. \quad .. \quad (3)$$

where n is the charge on the foreign anion, and K_{NO_3X} is the so-called "selectivity coefficient." Several methods are available for determining this selectivity term,[1,32,33] but because of the unrealistic features of separate solutions compared with situations that involve actual measurements,[1,29,32,33] only the mixed solution approach has been followed in this study. This involved potential measurements of a series of solutions, each containing a fixed interferent concentration but variable nitrate concentrations. A typical potential response of the PVC (Corning exchanger) electrode at different nitrite interferent levels is shown in Fig. 3. Unlike the normal pattern of interference, the selectivity values calculated from the a_{NO_3}/a_{NO_2} ratio are essentially independent of concentration.

The selectivity coefficients obtained for seven anions for each electrode (Table II) are in reasonable agreement with those for the commercial electrodes. However, it must be stressed that the methods by which some of the listed selectivity values for commercial electrodes are obtained, and interferent concentration levels, are not available. In common with the commercial electrodes, iodide, chlorate and particularly perchlorate constitute the most serious interferents for both PVC electrodes (Table II).

Selectivities given by manufacturers for hydrogen carbonate, carbonate or phosphate, for example, are unrealistic because these ions hydrolyse in aqueous solution, and the

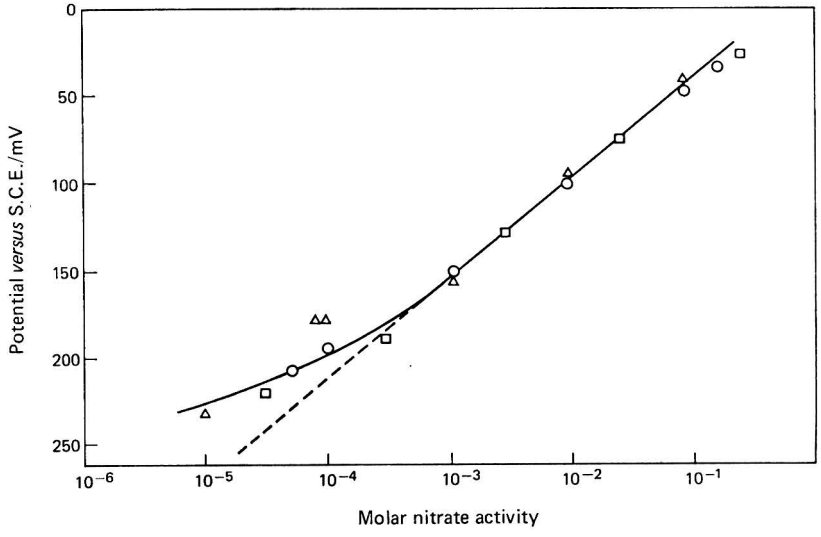

Fig. 2. Calibration of the PVC (Orion ion exchanger) - nitrate electrode at pH 6 and 25 °C by using different metal nitrates: □, lanthanum(III) nitrate; ○, copper(II) nitrate; and △, ammonium nitrate. Slope = 57 mV per decade

resulting hydroxyl anions could also contribute to the measured selectivity. Thus, the PVC (Orion exchanger) electrode in nitrate solutions containing 5×10^{-3} M sodium hydrogen carbonate and 5×10^{-3} M sodium phosphate gave calibration slopes of 45 and 31 mV per decade, respectively, and are to be contrasted with the reported[12] calibration slope of 50 mV per decade for the Orion 92–07 nitrate electrode in nitrate solutions containing 8×10^{-3} M sodium hydrogen carbonate.

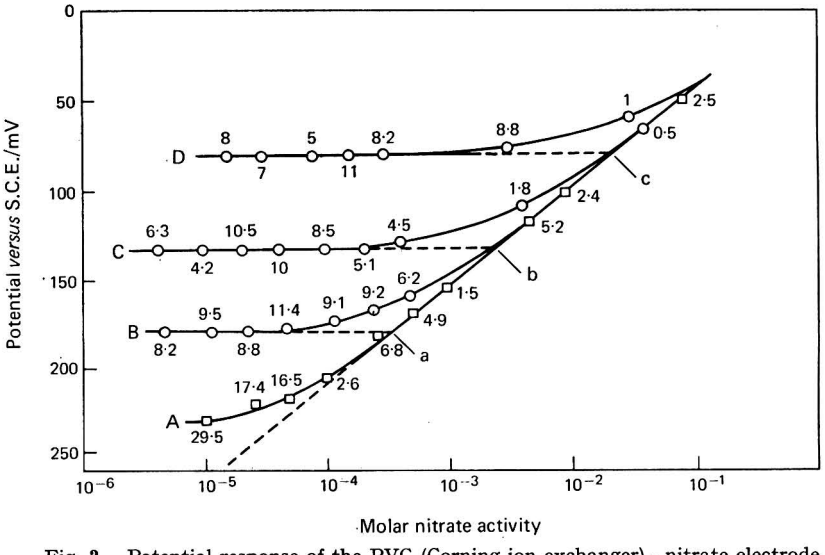

Fig. 3. Potential response of the PVC (Corning ion exchanger) - nitrate electrode in three mixed nitrate - nitrite solutions at 25° C: A, calibration graph, slope = 55·3 mV per decade; B, $[NO_2^-] = 5 \times 10^{-3}$ M; C, $[NO_2^-] = 5 \times 10^{-2}$ M; and D, $[NO_2^-] = 5 \times 10^{-1}$ M. Selectivity coefficients, $K_{NO_3NO_2}$: a, 0·06; b, 0·066; and c, 0·066. Figures adjacent to the symbols refer to the time in minutes required for steady potentials to be attained

EFFECT OF pH—

Hydrogen - hydroxyl ion interference is commonly expressed as a pH range rather than as a K_{NO_3H} factor.[1,29] The operative range for commercial nitrate electrodes covers the pH range 2 to 12 (Table I), but this is dependent on nitrate concentration and becomes considerably restricted at low nitrate levels. The pH interference for the PVC electrodes has been made by following both the potential and pH responses in analytical-reagent grade nitric acid (10^{-2} and 10^{-3} M) after successive small additions of sodium hydroxide (Fig. 4). These results indicate a similar pH range of 2·5 to 8 for the PVC (Corning exchanger) and PVC (Orion exchanger) - nitrate electrodes at 10^{-2} M nitrate concentration (Table I). Electrode performance at pH values higher than 8 suffers because of longer response times.

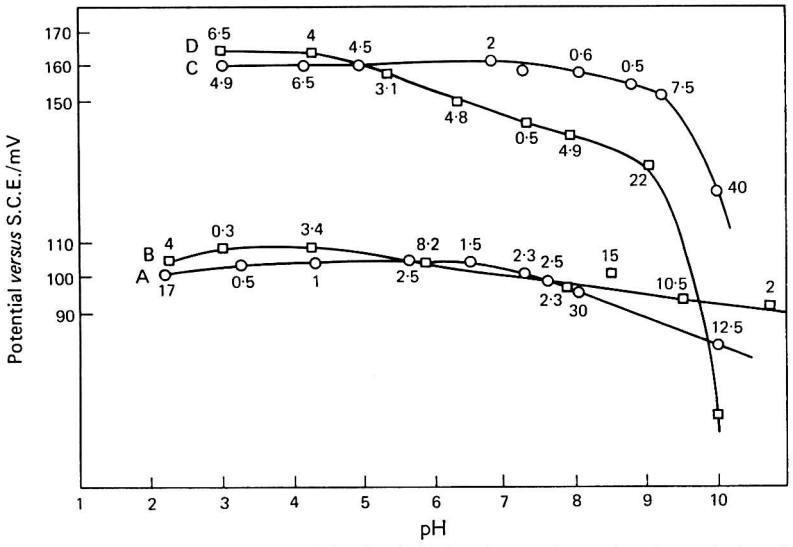

Fig. 4. Potential response of the PVC (Orion ion exchanger) - nitrate (○) and PVC (Corning ion exchanger) - nitrate (□) electrodes at various pH values: A and B, 10^{-2} M nitric acid; C and D, 10^{-3} M nitric acid. Figures adjacent to the symbols refer to the time in minutes required for steady potentials to be attained

RESPONSE TIMES—

Response times for liquid exchanger ion-selective electrodes have been variously recorded and, in general, exceed those for the solid-state selective ion-sensitive electrodes. Too much emphasis has been placed on response times of electrodes, certainly for times of less than about 2 minutes. Undoubtedly response times are generally more rapid when proceeding from dilute to concentrated systems than conversely. However, as shown in Fig. 1, this trend is not always observed and the same electrode can be temperamental with regard to response times. The important feature of both the PVC (Corning exchanger) and the PVC (Orion exchanger) electrodes is that an equilibrium (that is, 100 per cent.) response time is achieved within a few minutes for nitrate solutions between 10^{-4} and 10^{-1} M concentration. However, it is important to note that 95 per cent. response times are of the order of seconds. A similar trend is observed in most of the nitrate - interferent mixtures at least over the linear section of the calibration graphs (Fig. 3).

After studies with nitrate - perchlorate solutions, both PVC electrodes took up to 40 minutes before returning to normal behaviour with pure nitrate solutions. This pattern has also been noted for calcium-selective electrodes after exposure to zinc solutions.[29]

EFFECT OF COBALT-60 γ-RADIATION ON THE ORION AND CORNING PVC - NITRATE ELECTRODES—

Little work has been published on the radiation stability of selective ion-sensitive electrodes.[34,35] To this end, one functioning PVC (Orion exchanger) - nitrate electrode and one PVC (Corning exchanger) - nitrate electrode were exposed to a total γ-ray dose of $1{\cdot}6 \times 10^3$ rad over a period of 24 hours. The electrodes were immediately tested and gave essentially the same slope, constant, activity range and response times as the non-irradiated

electrodes. Evidently both PVC - nitrate electrodes can withstand short exposures to cobalt-60 γ-radiation without any great loss in performance. Kubota[34] has reported an Orion 92–07 nitrate electrode to be stable to a cumulative cobalt-60 γ-ray dose of 10^5 rad, although there was a small parallel shift, in a negative direction, of the calibration graph. Larger doses (up to 5×10^7 rad) caused non-linear responses. The major radiation effect is sustained by the internal reference solution.[34]

Conclusion

The selectivity coefficients for the seven anions listed in Table II are essentially identical for both PVC - nitrate electrodes, which suggests a close chemical similarity between the proprietary Orion[23–25] and Corning liquid ion exchangers. However, there are differences and thin-layer chromatography on silica gel G with benzene - toluene (1 + 1 v/v) gave an R_F value of about 0·02 for the Orion exchanger non-volatile constituent compared with 0·65 for that of the Corning exchanger.

The general performances of both PVC (Corning exchanger) and (Orion exchanger) electrodes compare favourably with their commercial counterparts (Tables I and II). The recommended amount of Orion 92–07–02 or Corning 477316 exchanger for re-charging the respective electrodes, namely 0·4 ml, is also sufficient to cast a master PVC membrane. Several fresh electrodes can be prepared from the master membrane as required and, if desired, the active disc from the master membrane can be sealed to the original rigid PVC electrode body to provide a new fully functional PVC membrane electrode. This represents a considerable cost saving on ion exchanger alone, and the long lifetime of the PVC (Corning exchanger) electrode is a notable feature.

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The Determination of Ammonia in Boiler Feed-water with an Ammonium-selective Glass Electrode

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An investigation has been made into the accuracy of ammonium-selective (ammonium-responsive) glass electrodes for determining ammonia (10 to 1000 μg l^{-1}) in boiler feed-water and similar high-purity water samples from power stations. The electrode potential follows the Nernst equation in samples containing up to 10 000 mg l^{-1} of ammonia, the pH of which is controlled between 8·0 and 8·4 by the addition of triethanolamine - hydrochloric acid buffer solution. However, interfering species in the buffer solution cause a detectable deviation from Nernstian response at low ammonia concentrations (less than 1000 μg l^{-1}). By use of a calomel - 0·1 N hydrochloric acid reference electrode, reproducible results have been obtained in static buffered solutions containing 10 to 1000 μg l^{-1} of ammonia. Of the other impurities likely to be present in power-station waters only sodium caused a serious effect (100 μg l^{-1} of sodium is equivalent to 25 μg l^{-1} of ammonia). The within-batch standard deviations of analytical results were 2, 7, 17 and 33 μg l^{-1} at concentrations of 10, 100, 500 and 1000 μg l^{-1} of ammonia, respectively. Details of a recommended analytical procedure for discrete samples are given, and the application of the ammonium-selective electrode to continuous on-stream analysis is briefly discussed.

The pH of feed-water is maintained between 8·5 and 9·3 to reduce the corrosive attack on construction materials in the pre-boiler system of power stations. To attain this alkalinity ammonia is added directly to the system, or alternatively is produced in the system by the decomposition of compounds such as hydrazine, cyclohexylamine and morpholine. The determination of small concentrations (less than 1000 μg l^{-1}) of ammonia is, therefore, required for plant control purposes.

In 1964, Tetlow and Wilson[1] published a method suitable for use in power-station laboratories, based on the formation of indophenol blue. They chose an absorptiometric technique because a literature survey had indicated that only such methods had the necessary sensitivity. At about the same time, Mattock and Uncles[2] reported the use of a glass electrode that was selective for both ammonium and potassium ions in solution. More recent work[3] indicated that a good response to changes in ammonia concentration as low as 100 μg l^{-1} could be obtained with such an electrode in a continuously flowing sample whose pH was controlled between 8·0 and 8·5 by the addition of a triethanolamine - hydrochloric acid buffer solution.

A detailed evaluation of the glass electrode system has been carried out and a simple and rapid method developed for low levels of ammonia in condensate and feed-water. This work is described below.

Basis of technique—

The technique is essentially the same as that used for measuring the pH value of a solution with a glass electrode. When an ammonium-selective glass electrode is immersed in a solution, it assumes a potential determined by the activity of the ammonium ions in the solution. Thus, by measuring its potential against a reference electrode of constant potential, an estimate of ammonium activity can be obtained. If the electrode potential is affected only by the activity of ammonium ions, it should obey the Nernst equation—

$$E = E_0 + \frac{2{\cdot}3026RT}{F} \log a_{NH_4^+} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

where E is the potential of the electrode, $a_{NH_4^+}$ is the ammonium ion activity, R is the gas constant, T is the absolute temperature, F is the Faraday constant and E_0 is the standard electrode potential.

In power-station practice the total ammonia content (T_{NH_3}) of the sample has to be

determined and, as the changes in potential only indicate changes in the activity of the ammonium ion, measurements must be made under conditions when the relationship—

$$T_{NH_3} = Ka_{NH_4^+} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

is valid, K being a constant characteristic of the experimental conditions.

From consideration of the hydrolysis of ammonia in aqueous solutions and of the effect of ionic strength on the activity coefficient of the ammonium ion, it can be shown that equation (2) is valid if measurements are made in a solution of constant pH and of constant ionic strength. By use of the triethanolamine - hydrochloric acid buffer solution we have calculated that both the pH and ionic strength remain essentially constant for samples containing a concentration of ammonia up to 5×10^{-4} M (8500 μg l^{-1}). At greater ammonia concentrations a significant increase in the ionic strength of the solution occurs and equation (2) is no longer valid.

The effect of K is allowed for by standardisation against a solution of known total ammonia content, and hence the measurement of $a_{NH_4^+}$ will give an unbiased estimate of the total ammonia concentration in the sample in the concentration range of interest, *i.e.*, less than about 10 000 μg l^{-1}.

Experimental

All experimental conditions and techniques were as described under Method, except when otherwise stated. The types of instrumentation used were as follows. The flow-through system consisted of an Electronic Instruments Ltd. laboratory sodium monitor, Model 8980, modified to pump one part of buffer solution to ten parts of sample. A Pye, Model 290, pH meter, an ammonium-selective glass electrode, Electronic Instruments Ltd., Type GKN 33, and a calomel reference electrode, Electronic Instruments Ltd., Type RJ 23/1, were used.

For the flow-through system the internal electrolyte was a saturated solution of potassium chloride, but for the majority of the static* measurements this was replaced with 0·1 N hydrochloric acid.

All chemicals were of analytical-reagent grade, except triethanolamine (laboratory-reagent grade), cyclohexylamine and morpholine (general-purpose reagent grades) and n-octadecylamine (technical grade).

The temperature in the laboratory varied between 18 and 21 °C.

Comparison of flow-through and static systems—

The response of an ammonium-selective electrode to changes in ammonia concentration from 10^2 to 10^7 μg l^{-1} was measured under both continuously flowing and static conditions. For this test a calomel - saturated solution of potassium chloride reference electrode was used.

Table I

Response of an ammonium-selective electrode to changes in ammonia concentration

Concentration of ammonia/μg l^{-1}	Change in electrode response compared with a 10^7 μg l^{-1} standard	
	Static/mV	Flowing/mV
1×10^2	—	270·8
1×10^3	216·5	222·1
1×10^4	162·6	165·7
3×10^4	134·8	137·8
1×10^5	105·8	107·7
3×10^5	79·4	80·8
1×10^6	51·4	51·8
3×10^6	26·6	27·0
1×10^7	0·0	0·0

The results given in Table I show reasonable agreement between the two procedures at concentrations of ammonia greater than 10 000 μg l^{-1}. At lower concentrations the response in the static test was markedly less than in the flowing system and no real equilibrium

* The term "static" refers throughout this paper to the system in which the electrodes are immersed in a magnetically stirred, buffered solution of the sample contained in a polythene beaker.

was established in the former at concentrations of 1000 $\mu g\ l^{-1}$ or less. The effect in the static system was probably caused by a gradual increase in potassium ions in the vicinity of the ammonium-selective electrode caused by leakage from the reference electrode. No such effect could occur with the flow-through system as the reference electrode was placed downstream with respect to the ammonium-selective electrode, and any potassium ions were continuously removed in the sample stream.

A preliminary investigation of the flowing system gave encouraging results (which are discussed later) and indicated that no inherent difficulties were apparent that would prevent an automatic procedure from being developed. However, the static system offered many advantages for routine laboratory analysis and a full investigation was made.

ALTERNATIVE ELECTROLYTES FOR USE WITH THE CALOMEL ELECTRODE—

The results in Table I indicated that potassium ions from the reference electrode caused significant interference in the electrode response at low ammonia concentrations. An alternative electrolyte for use with the calomel electrode was therefore sought.

A calomel electrode with 0·1 N hydrochloric acid as internal electrolyte was reported to be "capable of admirable behaviour" by Ives and Janz[4] and one based on their description was prepared and tested. The results were compared with a commercial calomel electrode [Electronic Instruments Ltd. (E.I.L.) RJ 23] in which the potassium chloride electrolyte solution had been replaced with 0·1 N hydrochloric acid. With both electrodes good equilibrium was quickly established in the concentration range from 100 to 10 000 $\mu g\ l^{-1}$, and the results given in Table II show that an essentially Nernstian response was obtained. At higher ammonia concentrations the response became non-Nernstian, owing to changes in the potential of the reference electrode, which were in turn caused by changes in the ammonia concentration. The calomel - 0·1 N hydrochloric acid sytem in the E.I.L. electrode was used for all subsequent work.

TABLE II

COMPARISON OF COMMERCIAL AND LABORATORY-CONSTRUCTED CALOMEL - HYDROCHLORIC ACID ELECTRODES

	Commercial electrode		Laboratory-constructed electrode	
Concentration of ammonia/$\mu g\ l^{-1}$	Reading/mV*	Response to concentration changes/mV	Reading/mV*	Response to concentration changes/mV
10 000	−95·0	—	−98·7	—
1000	−151·7	56·7 (0·5)†	−154·6	55·9 (0·7)
100	−203·6	108·6 (0·6)	−208·0	109·3 (0·8)

* Mean of four results.
† The figures in parentheses are the standard deviations of individual results (mV).

COMPARISON OF AMMONIUM-SELECTIVE ELECTRODES—

Four electrodes (Electronic Instruments Ltd., Type GKN 33) were obtained and the response of each to changes in ammonia concentration was measured. Four concentrations of ammonia were used and four determinations were made at each concentration with each electrode. The results are summarised in Table III and show no significant differences in equilibrium response. The time required to reach equilibrium varied between 2 and 4 minutes.

TABLE III

RESPONSE OF FOUR AMMONIUM-SELECTIVE ELECTRODES

	Concentration of ammonia/$\mu g\ l^{-1}$			
	1000	500	200	100
		Change in reading/mV		
Electrode	Reading/mV			
1	−156·2 (0·3)*	16·1 (0·6)	37·2 (0·9)	53·8 (1·1)
2	−157·1 (0·2)	15·8 (0·3)	36·7 (0·8)	52·8 (0·7)
3	−157·1 (0·2)	16·3 (0·4)	37·4 (0·6)	52·6 (0·8)
4	−151·2 (0·3)	16·2 (0·4)	37·6 (0·3)	52·5 (0·4)

* The figures in parentheses are the standard deviations of individual results (mV).

EFFECT OF pH—

Mattock and Uncles[2] found that high concentrations of hydrogen ions decreased the response of the electrode to very low concentrations of ammonia. The selection of a suitable pH value therefore depends on a compromise between this factor and the dissociation of ammonia into ammonium ions. In view of the later work by Electronic Instruments Ltd.,[3] it was decided to control the pH in the range from 8·0 to 8·5, where 95 and 85 per cent., respectively, of the ammonia will be present as ammonium ions.

The effect of small changes in pH within this range on the linearity of the calibration graph was investigated. The response of the electrode system to three standard ammonia solutions (containing 10 000, 1000 and 100 $\mu g\ l^{-1}$) was measured by use of each of three buffer solutions (at pH values of 8·0, 8·2 and 8·4). Differences in e.m.f. between the three ammonia solutions varied by less than 1 per cent. over the pH range involved.

EFFECT OF TRIETHANOLAMINE—

It was observed that the response of the electrode increasingly diverged from the theoretical values as the concentration of ammonia decreased. Assuming that some interfering species in the water or buffer solution was causing this effect, then all nominal concentrations would be subject to a constant error. To test this assumption measurements of the response of an electrode system to standard ammonia solutions of different concentrations were made and the observed concentrations were calculated from the change in e.m.f. from the 1000 $\mu g\ l^{-1}$ of ammonia standard, assuming Nernstian response. Constant errors of 30 and 60 $\mu g\ l^{-1}$ were then assumed and the concentrations re-calculated as if the standard solution contained 1030 and 1060 $\mu g\ l^{-1}$ of ammonia. The results of the calculations, given in Table IV, show that by using the value of 30 $\mu g\ l^{-1}$ the response virtually agreed with the theoretical values.

TABLE IV

COMPARISON OF NOMINAL AND CALCULATED AMMONIA CONCENTRATIONS

Nominal concentration, of ammonia/$\mu g\ l^{-1}$	Difference between nominal and calculated* ammonia concentrations in $\mu g\ l^{-1}$, assuming the constant error, also in $\mu g\ l^{-1}$, to be—		
	0	30	60
800	+14	+8	+3
500	+7	−8	−21
200	+28	+3	−18
100	+24	−3	−24

* Calculated assuming Nernstian response of the electrode.

The results indicated the presence of about 30 $\mu g\ l^{-1}$ of ammonia above the nominal amount added to each solution. This excess could have been in the water used to prepare the solutions or introduced with the buffer solution. Sodium, at a concentration of about 120 $\mu g\ l^{-1}$, introduced into one or other of the solutions would have had a similar effect. The ammonia content of the water used was determined and found to be less than 3 $\mu g\ l^{-1}$. The sodium content of each solution was determined flame-photometrically and was found to be less than 10 $\mu g\ l^{-1}$. It was concluded that the buffer solution contained either ammonia or some other species (*e.g.*, ethanolammonium ions) which affected the response of the glass electrode. (It was not possible, because of interference, to determine ammonia in triethanolamine by the method of Tetlow and Wilson.[1])

Buffer solutions prepared from samples of triethanolamine from four different suppliers all gave similar satisfactory electrode responses. Later work has shown that samples of triethanolamine that were yellow in colour gave unacceptably high blanks.

EFFECT OF TEMPERATURE—

Changes in temperature will affect the theoretical response of the electrode system by changing the value of the RT/F factor in the Nernst equation; this will alter the slope of the calibration graph.

The effect of temperature was tested by measuring the response of the electrode system to changes in ammonia concentration at two temperatures, 21·5 and 28·5 °C. The solutions and the whole electrode assembly were equilibrated at the relevant temperature before measurements were made.

The results, corrected to 20 °C by multiplying by the appropriate factor, $\frac{58{\cdot}2}{58{\cdot}5}$ and $\frac{58{\cdot}2}{59{\cdot}9}$ for 21·5 and 28·5 °C, respectively, showed good agreement and indicated that the major effect of temperature is only that which is to be expected from theoretical considerations.

METHOD

APPARATUS—

To avoid the contamination of solutions by sodium leached from glassware it is advisable to use plastic apparatus whenever possible. The apparatus can be cleaned by soaking it for several days in de-ionised water.

Polythene bottles—Polythene bottles with polythene screw-caps are convenient for storing samples, dilute standard solutions of ammonia and the buffer reagent.

Polythene beakers—100-ml polythene beakers are convenient for containing the samples *plus* buffer solutions during measurement.

Ammonium-selective glass electrode—Our work has shown that Type GKN 33 electrodes (Electronic Instruments Ltd.) are suitable. (Other manufacturers' electrodes may be satisfactory, but none of these was tested.) Immerse the bulb of the electrode in 0·2 M ammonium chloride solution for at least 24 hours before initial use. Between analyses, leave the electrode bulb immersed in a solution containing 1000 μg l^{-1} of ammonia *plus* buffer. Do not allow the bulb of the electrode to become dry.

Reference electrode—Use a calomel electrode with 0·1 N hydrochloric acid as internal electrolyte. Type RJ 23 electrodes (Electronic Instruments Ltd.) with a ceramic plug were found to be suitable but Type RH 23 electrodes are recommended. The latter electrodes have a ground-glass sleeve junction and were observed to reach equilibrium more rapidly than the ceramic plug type.

pH, or mV, meter—A high-impedance meter should be used that is capable of discriminating to within 0·005 pH unit (0·3 mV).

Stirrer—A magnetic stirrer is convenient although care must be taken to thermally insulate the sample solution from the heat produced by the electric motor. The magnetic follower should be coated with plastic. Other forms of mechanical stirrer should be suitable but agitation with a stream of bubbles should not be used.

REAGENTS—

Water—Water of very low ammonia content (preferably less than 20 μg l^{-1}) should be used for the preparation of the buffer solution and standard ammonia solutions. Such water can be consistently obtained by passing distilled water through a column of strongly acidic cation-exchange resin in the hydrogen form. The concentration of ammonia in the water can be determined, when necessary, by the method described by Tetlow and Wilson.[1]

Triethanolamine - hydrochloric acid buffer solution—Dissolve 15 g ($\pm$0·2 g) of triethanolamine (laboratory-reagent grade) in about 800 ml of water. Add 3·0 ml ($\pm$0·05 ml) of hydrochloric acid (sp.gr. 1·18) and dilute the solution to 1 litre with water. The pH of this solution should be 8·2 ($\pm$0·2); if the pH is outside this range adjust it by adding triethanolamine or hydrochloric acid. This solution should be stable for at least 4 weeks.

Hydrochloric acid, 0·1 N—Prepare this solution by suitable dilution of the contents of an ampoule of concentrated volumetric solution with water.

Standard ammonium chloride solution A—Dry some ammonium chloride at 100 °C. Dissolve 3·141 g of the dried ammonium chloride in water in a 1-litre calibrated flask, dilute the solution to the mark with water and mix. Store the solution in a glass bottle. This solution should be stable for at least 20 weeks. (1 ml of solution A is equivalent to 1000 μg of ammonia.)

Standard ammonium chloride solution B—Transfer 50 ml of standard solution A with a pipette into a 500-ml calibrated flask, dilute to the mark with water and mix. Store the solution in a polythene bottle. This solution should be stable for at least 4 weeks. (1 ml of solution B is equivalent to 100 μg of ammonia.)

Standard ammonium chloride solution C—Transfer 10 ml of standard solution B with a pipette into a 1-litre calibrated flask, dilute to the mark with water and mix. Transfer the solution immediately into a polythene bottle. Prepare this solution freshly each day as required. (1 ml of solution C is equivalent to 1 μg of ammonia.)

PROCEDURE—

Sample collection—The temperature of the water leaving the sampling device should be less than 30 °C. Polythene bottles are convenient sample containers; they should be completely filled with sample, and then stoppered. Aliquots of the sample should be analysed as soon as possible after sampling.

Analysis of samples—Place 50 ml of a standard ammonium chloride solution, the concentration of which is within a factor of two of the expected concentration of ammonia in the sample (see Discussion), into a 100-ml polythene beaker and add 5·0 ml ($\pm$0·1 ml) of buffer solution. Remove the electrodes from the solution in which they have been immersed and remove droplets of the solution from the tips of the electrodes by lightly touching them with a soft tissue or filter-paper. Immerse the electrodes in the prepared solution and stir gently until the electrode system reaches equilibrium. Depending on the type of meter used, either note the millivolt reading, V_1, or set the reading on the pH scale to an arbitrary value, R_1. Measure the temperature of the solution. The temperatures of subsequent sample solutions should not vary by more than $\pm$1 °C from this temperature.

Place 50 ml of sample in a 100-ml polythene beaker and add 5·0 ml ($\pm$0·1 ml) of buffer solution. Transfer the electrodes from the standard solution to the sample, removing droplets of solution from the electrodes as before. Stir the solution gently until equilibrium is established. Again note either the millivolt reading, V_2, or the reading on the pH scale, R_2.

Calculation of results—Calculate the difference in readings between the sample and standard ammonium chloride solutions from the appropriate equation, either $\Delta mV = V_2 - V_1$, or $\Delta pNH_4 = R_2 - R_1$. From this difference, and the calibration graph, calculate the ammonia concentration in the sample.

PREPARATION OF CALIBRATION GRAPH—

By means of a series of successive dilutions of freshly prepared standard ammonia solution C (1000 μg l^{-1}), prepare solutions containing 500, 100, 50 and 10 μg l^{-1} of ammonia. Place 50 ml of solution C in a 100-ml polythene beaker, add 5·0 ml ($\pm$0·1 ml) of buffer solution and immerse the electrodes in the mixture to obtain a reading (V_1 or R_1). Repeat the procedure with each of the other standard solutions to obtain readings V_2 or R_2. Subtract the readings (V_1 or R_1) of the standard solution C from the readings (V_2 or R_2) of each of the other solutions and plot the differences against the logarithm of the ammonia concentration. These determinations should be repeated until the calibration graph has been defined with the required precision.

Theoretically, the calibration graph should be linear. However, a slight curvature is introduced in practice, due predominantly to some interfering species in the buffer solution.

RESULTS

PRECISION—

On each of ten occasions the calibration procedure was carried out by using concentrations of 10, 25, 50, 100, 250, 500 and 1000 μg l^{-1} of ammonia, with duplicate solutions at each concentration; the order in which the determinations were made was chosen at random on each occasion.

The total standard deviation for any one concentration was calculated from the difference between the reading at that concentration and the corresponding reading at the concentration immediately above it; this method of calculation was adopted to reduce errors and is similar to that recommended under Method. For the calculation at 1000 μg l^{-1}, the differences between the readings at 1000 and 500 μg l^{-1} were used with, in this instance, the 500 μg l^{-1} reading as the datum.

From the Nernst equation, and assuming that the response of the electrode agrees with theory, the apparent ammonia content of each solution was calculated from the difference in readings from the 1000 μg l^{-1} solution. An almost constant error of about 30 μg l^{-1} was found with each solution. When the standard deviations, calculated in millivolts, were converted to ammonia concentrations in micrograms per litre, this effect was allowed for,

e.g., the within-batch standard deviation of 1·9 μg l^{-1} at a nominal 10 μg l^{-1} of ammonia is actually the standard deviation at 40 μg l^{-1} of ammonia.

A summary of the results is given in Table V.

TABLE V

PRECISION OF ANALYTICAL RESULTS

Ammonia added/μg l^{-1}	Calculated* ammonia concentration/μg l^{-1}	Standard deviation† Within-batch/μg l^{-1}	Between-batch/μg l^{-1}	Total‡/μg l^{-1}
1000	(1030)	33	54	65
500	539	17	27	32
250	287	11	N.S.	12
100	128	7·0	N.S.	7·1
50	78	4·0	N.S.	4·8
25	50	2·3	5·1	5·7
10	39	1·9	2·2	2·8

* Calculated from 1030 μg l^{-1}, assuming Nernstian response of the electrode.

† Each batch of within- and between-batch standard deviations has 20 and 9 degrees of freedom, respectively.

‡ The total standard deviation is the estimate of the standard deviation of any one result in any one batch.

N.S. means not statistically significant at the 5 per cent. probability level.

"CRITERION OF DETECTION"—

As blank determinations were not made during the precision tests, a separate experiment was carried out in which the response of the electrode to solutions containing 0, 10 and 20 μg l^{-1} of added ammonia was measured. Five aliquots of each solution were measured in random order. The standard deviations at each concentration did not differ significantly and the results were combined to give a mean standard deviation of 1·5 mV. This is equivalent to 0·026 pNH_4 unit which, at the estimated value of the blank solution (calculated assuming Nernstian response of the electrode and found to be 20 μg l^{-1}), is equivalent to 1·3 μg l^{-1} of ammonia.

The "criterion of detection"[5] (taken as 2·326 times the within-batch standard deviation of the blank determination) was therefore about 3 μg l^{-1}.

ACCURACY—

Analysis of samples of feed-water—Samples of feed-water from five power stations were analysed by the electrode procedure and, for comparison, by the indophenol blue method.[1] The results are summarised in Table VI. The differences in results between the two methods were less than 5 per cent., except for samples A2 and E2. These two samples were found

TABLE VI

AMMONIA CONTENT OF FEED-WATER SAMPLES ANALYSED BY THE ELECTRODE AND INDOPHENOL BLUE METHODS

Power station	Sample	Concentration of ammonia: Electrode method/μg l^{-1}	Indophenol blue method/μg l^{-1}*	Percentage difference
A	1	0·128 (2)†	0·125	+2·4
	2	0·142 (2)	0·127	+11·8
		0·133‡	—	+3·6
B	1	0·624 (6)	0·649	+4·0
	2	0·574 (5)	0·588	−2·5
C	1	0·226 (2)	0·219	+3·2
	2	0·220 (3)	0·210	+5·0
D	1	0·777 (2)	0·763	+1·6
E	1	0·262 (5)	0·251	+4·4
	2	0·229 (2)	0·203	+12·8
		0·209‡	—	+3·0

* Each result is the mean of three determinations.

† The figures in parentheses are the number of determinations.

‡ Result corrected for the sodium content of the sample.

to contain 35 and 80 $\mu g\ l^{-1}$ of sodium, and when the ammonia concentrations measured by the electrode were corrected for the effect of the sodium content (*i.e.*, equivalent to about 9 and 20 $\mu g\ l^{-1}$ of ammonia, respectively), the corrected results were within 5 per cent. of the results obtained by the indophenol blue method. The other feed-water samples all contained less than 10 $\mu g\ l^{-1}$ of sodium and no correction of the results was made.

INTERFERENCES—

The effects of several other impurities were tested at two concentrations of ammonia, *viz.*, 100 and 1000 $\mu g\ l^{-1}$. The results in Table VII show that the electrode responded to many other ions, although the magnitudes of the responses are considered to be inappreciable for normal power-station analyses. The concentration of sodium expected in most samples will cause a negligible response, but under certain circumstances, *e.g.*, during condenser leakage, there may be enough to cause a significant error; the concentration of potassium at any time is expected to be insignificant.

From previous work[6] it was expected that n-octadecylamine would affect the response of the electrode, but during the one batch of analyses in which it was present no effect was observed. However, if the electrode is exposed continuously to n-octadecylamine an effect is likely to develop and strict control should be exercised under these conditions.

TABLE VII

EFFECT OF OTHER IMPURITIES

Impurity	Concentration of impurity/$\mu g\ l^{-1}$	Interference (expressed as $\mu g\ l^{-1}$ of ammonia) at ammonia concentrations of—	
		100 $\mu g\ l^{-1}$	1000 $\mu g\ l^{-1}$
Li^+	1000	61	72
K^+	1000	930	1100
Na^+	1000	210	310
Mg^{2+}	1000	15	40
Ca^{2+}	1000	20	<33
Cu^{2+}	1000	<7	<33
Ni^{2+}	1000	10	<33
Fe^{2+}	1000	7	<33
Zn^{2+}	1000	24	<33
Hydrazine	4000	190	160
Hydrazine	1000	50	40
Cyclohexylamine	4000	19	<33
Morpholine	10 000	<7	<33
n-Octadecylamine	300	<7	<33

DISCUSSION OF THE METHOD

CALIBRATION OF THE ELECTRODE - MEASURING SYSTEM—

The results in Tables IV and V show that the potential of the ammonium-selective glass electrode was proportional to the pNH_4 value of a solution for concentrations of ammonia between 1000 and 10 $\mu g\ l^{-1}$, if it is assumed that the buffer solution, added to each solution of ammonia, contained a species that had a constant effect on the electrode. Because of this effect it is not possible to use the theoretical Nernstian slope of the calibration graph for calculating results, and it is therefore necessary to prepare a calibration graph as described under Method.

In the procedure described under Method it is recommended that the concentration of ammonia in the solution used to set the apparatus for a batch of analyses should be within a factor of two, *i.e.*, within $\pm 0{\cdot}3$ pNH_4 unit of the expected ammonia concentration in the sample. Under these conditions the effect of temperature on the response of the electrode is small; if the temperatures of the standard and sample solutions differ by no more than 1 °C, and are within 5 °C of the temperature at which the calibration graph was prepared, the maximum error introduced by temperature variations will be less than 0·005 pNH_4 unit (*i.e.*, 1 per cent. error).

To check that the electrode gives the expected response it is recommended that a second standard ammonia solution, differing from the first by a factor of two, should be analysed with each batch of determinations.

PRECISION AND BIAS—

The results in Table V show that at ammonia concentrations between 250 and 1000 $\mu g\ l^{-1}$

the relative within-batch standard deviation was from 3 to 4 per cent., while at 100 and 10 $\mu g\ l^{-1}$ the standard deviations were 7 and 2 $\mu g\ l^{-1}$, respectively. However, these results were obtained by measuring the responses of the electrode to changes in ammonia concentrations over a range of 2 pNH_4 units. If measurements are made over a much smaller concentration range (not greater than $\pm 0{\cdot}3$ pNH_4 unit is recommended in the Method) more precise results can be obtained.

The results in Table VII show that effects of various magnitudes were caused by a number of other ions, and this lack of specificity of the electrode makes it unsuitable for many types of analysis. However, for high-purity water samples from power stations the only serious effect expected would be caused by sodium (1000 $\mu g\ l^{-1}$ of sodium is equivalent to 250 $\mu g\ l^{-1}$ of ammonia), which enters the system adventitiously via condenser leaks. The results in Table VI show that of nine power-station water samples, two contained significant amounts of sodium. Comparison of the results (corrected when necessary for the sodium) obtained by using the electrode with those obtained by using the indophenol blue method gave differences of less than 5 per cent., and this was considered to be satisfactory for routine application in power-station laboratories.

Although n-octadecylamine did not affect the response of the electrode during the tests described, continuous exposure of the electrode to this amine is likely to cause an effect similar to that found with the sodium-selective glass electrode.[6]

RESPONSE TIMES—

The time taken for an electrode to reach an equilibrium response depended on a number of factors, these being the concentration of ammonia in the sample, the change in concentration between the sample being measured and the previous sample, and the individual electrode being used. The average response time during the precision tests was about 5 minutes, and it varied between 2 minutes for the 1000 $\mu g\ l^{-1}$ solution and 8 minutes for the 10 $\mu g\ l^{-1}$ solution.

During the comparison of four ammonium-selective glass electrodes (see Table III), it was noticed that electrodes 1 and 2, which had had much prior use, gave a faster response than electrodes 3 and 4, which had not been used previously.

APPLICATION TO ON-STREAM ANALYSIS—

The results in Table I on a continuously flowing system with a calomel - saturated potassium chloride solution reference electrode indicated that such a system would be suitable for on-stream analysis. Some preliminary laboratory tests confirmed that the results obtained had a precision similar to that given by the static system, although the response time was appreciably longer because of the time required to displace the previous solution in the flow cell.

A comparison of the laboratory work carried out on the ammonium-selective electrode with that on the sodium-selective electrode[6] shows some similarity between the two electrodes. It is, therefore, reasonable to assume that the results obtained with the on-stream application of the sodium-selective electrode[7] could be applied with some degree of confidence to the ammonium-selective electrode. However, with a direct-reading commercial instrument the non-Nernstian response caused by the effect of the buffer solution on the electrode cannot be compensated for completely. This error can be markedly reduced by altering the gain on the amplifier, so that the scale reads correctly at two different concentrations of ammonia that span the concentration range of interest.

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The Use of Atomic-absorption Spectrophotometry for the Determination of Copper, Chromium and Arsenic in Preserved Wood

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The application of atomic-absorption spectrophotometry to the determination of copper, chromium and arsenic in preservative-treated wood is described. The preserving compounds are rapidly leached from wood samples with a mixture of dilute sulphuric acid and hydrogen peroxide solution. Copper, chromium and arsenic in the leach solution are determined by atomic-absorption spectrophotometry and the results are compared with those obtained by colorimetric analysis.

With this method an analytical sensitivity for arsenic of 0·10 μg ml^{-1} has been achieved by use of an argon (entrained air) - hydrogen flame and a 10-cm propane burner.

Timber in service or in storage is subject to deterioration by decay-producing fungi and wood-boring insects, but it can be protected by treatment with chemical compounds that are toxic to these organisms. In recent years interest in waterborne preservatives containing copper, chromium and arsenic salts has rapidly increased, and it has become necessary to have a rapid method for determining these metals in preserved wood and treating solutions, both for routine control purposes and for research studies. Present methods of chemical analysis[1–4] for the determination of copper, chromium and arsenic in treated wood are slow, tedious and often create bottlenecks in routine analysis, or in research projects requiring many determinations. Also, because of variations in the treatability of wood, many separate determinations are often required to ensure that significant results are obtained. Recent work at the Forest Products Research Laboratory has shown that fixed copper, chromium and arsenic compounds can be rapidly and quantitatively leached from sawdust samples[5] and thin sections of wood,[6] and the elements determined by colorimetric analysis.

Although such a procedure is rapid it was thought that a more rapid finish could be accomplished by using atomic-absorption spectrophotometry. An atomic-absorption spectrophotometric method[7] has been described in which the preservative compounds are leached from the sample by Soxhlet extraction with dilute hydrochloric acid, but the extraction stage is lengthy and takes between 4 and 8 hours. Modern instrumental techniques, such as atomic-absorption spectrophotometry, allow rapid finishes to determinations, but this advantage is lost when dissolution of the wood or extraction of the preservative from the matrix is time consuming. In the proposed procedure the preservative compounds are quantitatively leached from the wood in 20 minutes. The test and calibration solutions are 0·5 M with respect to sulphuric acid and 0·3 per cent. w/v with respect to sodium sulphate, but this causes no apparent detrimental interference or problems at the atomic-absorption stage. The high analytical sensitivity for copper, chromium and arsenic can be an embarrassment on occasions but can be overcome by the use of less sensitive resonance lines, dilution of the test solutions or the use of a burner with a shorter path-length.

The popular air - acetylene flame does not give good sensitivity for the determination of arsenic because it absorbs a large proportion of the resonance radiation emitted by the hollow-cathode lamp. Therefore, because of its high transmission of short wavelength radiation, the argon (entrained air) - hydrogen flame was used.[8] In previous work a multi-slot burner and an acetylene burner have been used, but in the present work better sensitivity was obtained with a propane burner.

It has been stated by Lambert[7] that the absorbance of chromium is depressed in 0·5 M sulphuric acid solution. In this work sodium sulphate is added to the sulphuric acid solution;

the absorbance of chromium is enhanced, and sensitivity similar to that claimed by the instrument makers, which can be obtained from their literature, has been attained. It is necessary to be able to determine copper, chromium and arsenic in dilute sulphuric acid solution and in the presence of sulphate. This is because digestion procedures for the decomposition of wood, in which the use of sulphuric acid is essential, are often favoured, and many treating solutions contain copper(II) sulphate.

The proposed procedure has been applied to preservative-treated ash, *Fraxinus excelsior*; Baltic redwood, *Pinus sylvestris*; birch, *Betula* species; Douglas fir, *Pseudotsuga menziesii*; grand fir, *Abies grandis*; Japanese larch, *Larix leptolepis*; lodgepole pine, *Pinus contorta*; Norway spruce, *Picea abies*; obeche, *Triplochiton scleroxylon*; sequoia, *Sequoia sempervirens*; silver fir, *Abies alba*; Sitka spruce, *Picea sitchensis*; and western red cedar, *Thuja plicata*.

Experimental

Equipment—

The atomic-absorption equipment consisted of a Pye Unicam, Model SP90A, Series 2, single-beam spectrophotometer, fitted with an EMI No. 9662A photomultiplier, a turret to hold three hollow-cathode lamps and a Pye Unicam, Model AR25, linear recorder. Standard Pye Unicam copper, chromium and arsenic hollow-cathode lamps were used.

Atomic-absorption conditions—

The analytical performance of atomic-absorption spectrophotometry for the determination of copper and chromium offers no problems of sensitivity when the technique is applied to wood preservatives. However, the sensitivity for arsenic, 2·0 μg ml^{-1}, is poor when the recommended air - acetylene flame is used and is of limited use for the direct determination of arsenic in leach solutions. Arsenic can be concentrated by solvent extraction[9] to provide a detection limit of 0·1 μg ml^{-1}, but this is time consuming and defeats the object of rapid analysis. The argon (entrained air) - hydrogen flame used in conjunction with a 10-cm acetylene burner has been described by Johns,[8] who obtained a sensitivity of 0·65 μg ml^{-1}. This technique was tried and was found to improve the lower limit of determination. Following this, the acetylene burner was replaced with a standard Pye Unicam 10-cm propane burner, which has a slit 1 mm in width compared with a 0·5-mm wide slit in the acetylene burner. With the propane burner a sensitivity of 0·10 μg ml^{-1} of arsenic was achieved when using a wavelength of 197·2 nm. Calibration graphs, corrected for blank values, resulting from the use of air - acetylene or argon (entrained air) - hydrogen flames with acetylene and propane burners are shown in Fig. 1. The absorbance readings were made with the instrument set at a scale expansion of $\times 1$ and at wavelengths of 193·7 and 197·2 nm, and the solutions were 0·5 M with respect to sulphuric acid, and contained an excess of sodium sulphate.

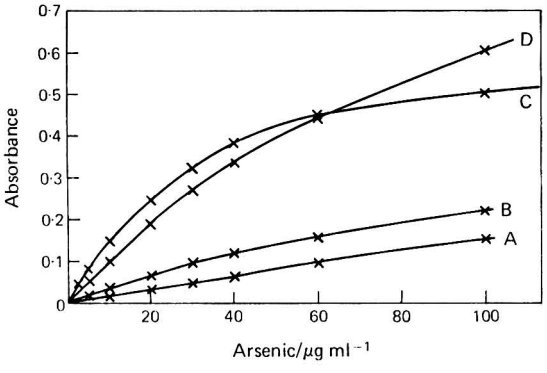

Fig. 1. Calibration curves for arsenic at scale expansion $\times 1$. A, acetylene burner, air - acetylene flame, wavelength 193·7 nm; B, acetylene burner, argon (entrained air) - hydrogen flame, wavelength 193·7 nm; C, propane burner, argon (entrained air) - hydrogen flame, wavelength 193·7 nm; and D, propane burner, argon (entrained air) - hydrogen flame, wavelength 197·2 nm

A wide range of arsenic concentrations occur during batch analysis. For this reason a wavelength of 197·2 nm was often used as, although it gave poorer analytical sensitivity than a wavelength of 193·7 nm, it gave a more linear calibration graph. This is shown by the calibration graphs in Fig. 1. By using a wavelength of 193·7 nm, scale expansion up to ×5 was possible, but at a higher scale expansion accurate measurement of the absorbance signal became difficult because of the increased noise level. With a wavelength of 197·2 nm a lower noise level was obtained and scale expansion of ×10 was possible. Calibration graphs, corrected for the blanks, at scale expansions ×5 and ×10, are given in Fig. 2.

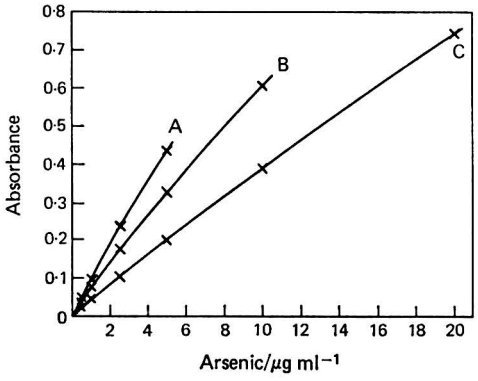

Fig. 2. Calibration curves for arsenic obtained by using a propane burner and an argon (entrained air) - hydrogen flame. A, wavelength 197·2 nm at scale expansion ×10; B, wavelength 193·7 nm at scale expansion ×5; and C, wavelength 197·2 nm at scale expansion ×5

Johns' flame-ignition procedure[8] for the operation of the argon (entrained air) - hydrogen flame with an acetylene burner was found to be suitable for the propane burner. Argon is introduced from a cylinder, via a reducing valve, into the air inlet. Similarly, the hydrogen supply is connected to the fuel inlet. Care should be taken to ensure that the gas lines are free from leaks, especially the hydrogen line, and that the spray chamber is flushed with hydrogen before ignition. To light the flame, adjust the hydrogen flow to 1500 cm^3 min^{-1} and ignite it with a lighted taper. The argon supply is then turned on and adjusted to a flow of 5 l min^{-1}. Great difficulty will be experienced in igniting the mixture if argon and hydrogen are introduced together.

Wood extractives in the leach solutions caused no interference during the determination of copper and chromium with an air - acetylene flame and they enhanced the absorbance signal for arsenic at certain burner observation heights. The adjustment of the observation height for arsenic with the argon (entrained air) - hydrogen flame and the propane burner is critical. No enhancement of the signal occurred at observation heights of between 1·3 and 1·5 cm, and an observation height of 1·4 cm was therefore used for the determination of arsenic.

INTERFERENCES—

Although dilute sulphuric acid is not an ideal medium for atomic-absorption spectrophotometry, it is useful as a reagent for rapidly dissolving and leaching the preservative from wood. Many digestion procedures require sulphuric acid to assist in the decomposition of wood. Also, preservative solutions containing copper(II) sulphate, potassium or sodium dichromate and arsenic pentoxide undergo fixation with the formation of potassium and sodium sulphates as by-products. The concentration of potassium or sodium is variable, especially if the treated timber is exposed to water, when the salt will be leached out from the wood. Therefore, interference by potassium and sodium sulphates with the absorbance signals of copper, chromium and arsenic was investigated.

Two sets of three series of solutions were prepared, containing 10 μg ml^{-1} of copper, 20 μg ml^{-1} of chromium or 20 μg ml^{-1} of arsenic; each set contained increasing amounts of

either potassium or sodium sulphate. All solutions were 0·5 M with respect to sulphuric acid. The solutions were aspirated and the recorded absorbances plotted against the concentrations of either potassium or sodium sulphate to give a series of graphs. A small blank correction was made for arsenic. The relationships, shown in Fig. 3, show that the presence of potassium or sodium sulphate has no effect on the copper absorbance signal, curve A. The curve D, for chromium, shows that in this instance the signal is enhanced and rapidly reaches a plateau. The curves B and C, for arsenic, are different for potassium and sodium sulphate additions. At first they both decrease with increasing concentrations of salt. The signal for potassium, curve B, is depressed and then becomes constant, but the signal for sodium, curve C, increases and reaches a plateau. Next, amounts of potassium from 100 up to 800 μg ml^{-1} in the presence of solutions containing 10 μg ml^{-1} of arsenic were found to have no effect on the arsenic absorbance signal provided excess of sodium sulphate was present. The conclusion is that any interference is overcome if an excess of sodium sulphate is added to both the test and calibration solutions. The experiments were repeated with 0·005 M sulphuric acid solution. Only chromium showed any change in the absorbance signal, see curve E in Fig. 3. A 100-fold increase in sulphuric acid concentration gives a slightly reduced signal for chromium which is an acceptable loss in sensitivity.

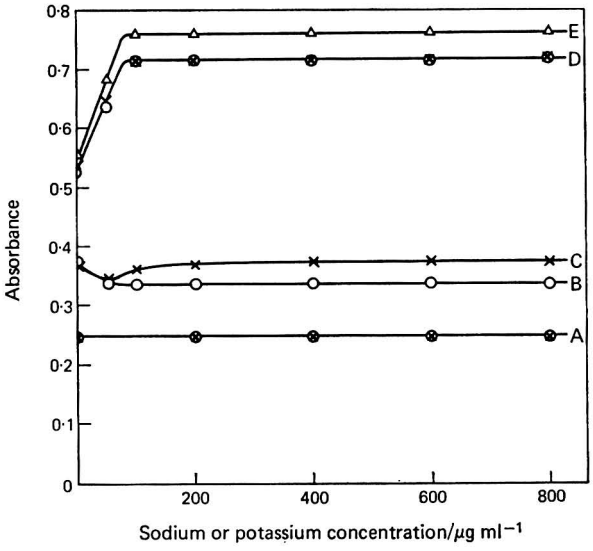

Fig. 3. Effects of sodium sulphate, potassium sulphate and sulphuric acid on the absorbance signals of copper, chromium and arsenic. A, copper, 10 μg ml^{-1} in 0·5 M sulphuric acid *plus* increasing amounts of sodium (×) and potassium (○); B, arsenic, 20 μg ml^{-1} in 0·5 M sulphuric acid *plus* increasing amounts of potassium; C, arsenic, 20 μg ml^{-1} in 0·5 M sulphuric acid *plus* increasing amounts of sodium; D, chromium, 20 μg ml^{-1} in 0·5 M sulphuric acid *plus* increasing amounts of sodium (×) and potassium (○); and E, chromium, 20 μg ml^{-1} in 0·005 M sulphuric acid *plus* increasing amounts of sodium

Wood contains appreciable amounts of calcium, magnesium and phosphate. Experiments similar to those described above were carried out in the presence of excessive amounts of calcium, magnesium and phosphate, but no interference occurred in 0·5 M sulphuric acid and 0·3 per cent. w/v sodium sulphate solution. It should be noted that phosphate does not interfere in the direct colorimetric determination of arsenic.[5] The blank on untreated wood is so small that it is negligible, which shows that an insignificant amount of the phosphate present is ionic.

The possibility of inter-elemental interference was investigated. Standard solutions were prepared containing different ratios of copper, chromium and arsenic in excess of each other and of the concentration found in treated wood. The recoveries of the elements sought were similar to the amounts added, demonstrating that no inter-elemental interference had occurred.

COMPARISON WITH COLORIMETRIC METHOD—

Two sawdust samples were cut on a sampling machine[10] from the cross-section of each specimen of treated timber. One sample was analysed by the atomic-absorption spectrophotometric procedure and the other by colorimetric methods.[5] The samples were dried, weighed and transferred to calibrated flasks, and the preservative compounds were extracted with a mixture of 2·5 M sulphuric acid solution and hydrogen peroxide solution for 20 minutes at 75 °C. The chromium compound is completely soluble only in the presence of hydrogen peroxide.[5] The leach solutions were cooled, sodium sulphate solution was added, and the mixture was diluted to give a solution 0·5 M with respect to sulphuric acid and 0·3 per cent. with respect to sodium sulphate. The sawdust was filtered off before aspirating the leach solutions. Water was aspirated between each test or calibration solution. Sodium sulphate solution was not added to the leach solutions for colorimetric analysis.

In the proposed procedure the leach solution containing sawdust was diluted to a standard volume in a calibrated flask. The volume displacement caused by the sawdust, which reduces the actual volume of the solution in the calibrated flask, was determined and taken into account when calculating the results. The volume displacement for 1 g of oven-dry wood was found to be 0·6 ml of solution.

RESULTS

The proposed procedure and the colorimetric methods were applied to sawdust samples. Adjacent samples, cut within 5 mm of each other, were analysed by the different techniques to reduce the effect of any variations in loading due to the anatomical structure of the wood. The results, given in Table I, are in good agreement and show that the proposed procedure is quantitative and has the required precision. The usual loading of copper, chromium and arsenic in the cross-section of treated wood is usually in the range from 0·10 per cent. upwards. In research projects it is necessary to be able to determine copper, chromium and arsenic at lower levels, especially when investigating the loadings achieved with impermeable species of timber and in distribution studies when only small samples are available. The proposed procedure was found to be suitable for the determination of copper and chromium down to 0·0002 per cent. and arsenic down to 0·0005 per cent. At these low levels, naturally occurring copper, chromium and arsenic must be taken into account. Standard deviations, each based on six determinations, were ±0·0026 per cent. for copper at the 0·20 per cent. level, ±0·0038 per cent. for chromium at the 0·10 per cent. level and ±0·0031 per cent. for arsenic at the 0·10 per cent. level.

TABLE I

COMPARISON OF RESULTS OBTAINED BY ATOMIC-ABSORPTION SPECTROPHOTOMETRY WITH THOSE OBTAINED BY COLORIMETRIC ANALYSIS

Species	Content (based on oven-dry wood), per cent.					
	By atomic absorption			By colorimetric analysis		
	Cu	Cr	As	Cu	Cr	As
Ash	0·074	0·13	0·13	0·071	0·12	0·12
Baltic redwood	0·14	0·22	0·19	0·14	0·22	0·19
Birch	0·13	0·21	0·24	0·14	0·21	0·24
Douglas fir	0·34	0·62	0·40	0·34	0·63	0·39
Grand fir	0·31	0·58	0·51	0·33	0·58	0·51
Japanese larch	0·067	0·12	0·12	0·074	0·12	0·11
Lodgepole pine	0·29	0·49	0·46	0·30	0·49	0·45
Norway spruce	0·20	0·33	0·24	0·21	0·33	0·23
Obeche	0·061	0·11	0·061	0·061	0·11	0·061
Sequoia	0·10	0·15	0·15	0·11	0·16	0·16
Silver fir	0·21	0·35	0·37	0·22	0·35	0·35
Sitka spruce	0·20	0·34	0·32	0·21	0·33	0·32
Western red cedar	0·27	0·29	0·32	0·28	0·30	0·31

The extraction procedure, coupled with atomic-absorption spectrophotometry using a relatively inexpensive single-beam instrument, for the quantitative determination of copper, chromium and arsenic in treated wood enables these elements to be determined rapidly and accurately. A complete analysis, including leaching, can be accomplished in less than 1 hour. It is possible to use the sulphuric acid - hydrogen peroxide digestion procedure instead of the leaching technique to increase the speed of analysis. The reaction of sulphuric acid - hydrogen peroxide solution with wood is very vigorous and can sometimes be violent. For this reason the leaching procedure is preferred.

Method

Instrument operating conditions—

Instrument control	Copper	Chromium	Arsenic
Wavelength/nm	324·8	357·9 or 429·0	193·7 or 197·2
Slit width/mm	0·08	0·06 to 0·10	0·30
Attenuator setting	1	2 or 3	1 or 3
Lamp current/mA	4	8	7
Scale expansion	up to × 10	up to × 10	up to × 10
Burner	10-cm acetylene	10-cm acetylene	10-cm propane
Burner height/cm	1·0	0·5	1·4
Acetylene flow-rate at a pressure of 0·7 kg cm^{-2}/cm^3 min^{-1}	1000	1800	—
Air flow-rate at a pressure of 2·1 kg cm^{-2}/l min^{-1}	5	5	—
Hydrogen flow-rate at a pressure of 0·7 kg cm^{-2}/cm^3 min^{-1}	—	—	1800
Argon flow-rate at a pressure of 2·1 kg cm^{-2}/l min^{-1}	—	—	5

Reagents—

Sulphuric acid, 2·5 M—Cautiously add, with stirring and cooling, 280 ml of AnalaR concentrated sulphuric acid to 1600 ml of water, cool and dilute to 2 litres with water.

Hydrogen peroxide solution, 100-*volume*—This reagent was of AnalaR grade.

Sodium sulphate solution, 3 *per cent. w/v*—Dissolve 30 g of AnalaR sodium sulphate in water and dilute to 1 litre.

Sulphuric acid, 0·5 M - *sodium sulphate*, 0·3 *per cent. w/v solution*—Dilute 200 ml of 2·5 M sulphuric acid solution *plus* 100 ml of 3 per cent. w/v sodium sulphate solution to 1 litre with water and mix.

Standard solution—Dissolve 0·1985 g of AnalaR copper(II) sulphate in water and transfer the solution to a 100-ml calibrated flask. Dissolve 0·2828 g of AnalaR potassium dichromate in water, add 10 ml of 2·5 M sulphuric acid solution and 2 ml of 100-volume hydrogen peroxide solution, boil, cool and transfer the mixture to the calibrated flask. Then dissolve 0·1320 g of AnalaR arsenic trioxide by boiling it in a solution consisting of 10 ml of 2·5 M sulphuric acid solution, 2 ml of 100-volume hydrogen peroxide solution and 10 ml of water, cool the solution and transfer it also to the calibrated flask. Add 10 ml of 3 per cent. w/v sodium sulphate solution to the calibrated flask, dilute to the mark with water and mix. (1 ml of standard solution is equivalent to 500 μg of copper, 1000 μg of chromium and 1000 μg of arsenic.)

Calibration solutions—

Transfer by pipette, taking suitable precautions, portions of the standard solution to calibrated flasks, dilute each to the mark with 0·5 M sulphuric acid - 0·3 per cent. w/v sodium sulphate solution and mix.

Procedure—

Transfer the weighed sample to a 250-ml calibrated flask, add 50 ml of 2·5 M sulphuric acid solution and 10 ml of 100-volume hydrogen peroxide solution, and heat in a water-bath at 75 °C for 20 minutes with occasional swirling to mix the contents of the flask. Remove the flask from the water-bath, add 150 ml of water and 25 ml of 3 per cent. w/v sodium sulphate solution, swirl to mix, cool the flask and contents to room temperature, dilute to the mark with water and again mix. Mix twice more at 5-minute intervals. Filter 25 ml of the leach solution through a dry 9·0-cm Whatman No. 44 filter-paper, discarding the first 5 ml of filtrate.

By using the operating conditions given above, aspirate the 0·5 M sulphuric acid - 0·3 per cent. w/v sodium sulphate solution to obtain the blank absorbance, then a suitable range of calibration solutions followed by the filtrate from the sample solution. Check the calibration solutions after the last sample has been run. Plot calibration graphs of the concentration (μg ml^{-1}) of copper, chromium and arsenic against absorbance. To determine the copper, chromium and arsenic contents of the test solution compare the absorbance readings with the calibration graphs.

This procedure is suitable for samples in the weight range from 3 to 8 g. For smaller samples, use 100, 50 or 25-ml calibrated flasks and corresponding volumes of reagents.

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Spectrophotometric Determination of Phosphorus in Biological Samples after Dry Ashing without Fixatives

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Simple dry ashing of samples without the use of fixatives can be used in the determination of phosphorus, in addition to metals, in biological materials. Two alternative spectrophotometric methods can be applied directly to a hydrochloric acid solution of the ash.

Dry ashing is often preferred to other methods for the decomposition of biological materials as it enables many samples to be treated rapidly and simultaneously. Some problems are created by the loss of certain elements; however, other methods for the decomposition of samples also have some disadvantages, *e.g.*, the use of plasma oxygen[1] is expensive and wet oxidation is potentially hazardous. The concentrated acids used in the latter procedure often interfere in subsequent determinations of minerals (*e.g.*, lower values for sodium were obtained by flame emission and absorption in the presence of sulphuric acid[2,3]). The loss of many elements can be diminished by ashing[4] at temperatures below 450 °C or by the use of fixatives (magnesium nitrate for trace elements[5] and sodium carbonate when determining non-metals including phosphorus[6]). As phosphorus is often required to be determined in addition to other minerals, it is frequently necessary to ash separate samples for this element and for metals. The time taken for the analysis is therefore considerably prolonged. Some experiments had shown, however, that it may be possible to determine phosphorus in a hydrochloric acid solution of the ash as accurately as after ashing with sodium carbonate. Therefore, it was thought desirable to carry out a detailed study of this problem.

Ten samples of animal feedingstuffs, representing all types of matrices of biological materials, were ashed with and without sodium carbonate at 500 °C to establish if dry ashing without fixatives is adequate for the satisfactory recovery of phosphorus. Both series of ashings were carried out simultaneously, so that all samples were exposed to the same conditions. The ash from the untreated samples was dissolved in hydrochloric acid, while that from the sodium carbonate fusions was treated with nitric acid. Phosphorus was determined in the solutions by the spectrophotometric molybdovanadate[7] and molybdenum-blue[8] methods, and by the standard gravimetric molybdophosphate procedure.[6] Therefore, the two spectrophotometric methods, as well as the ashing procedures, were compared. The purpose of the present study was to find a reliable and rapid procedure for determining phosphorus in various biological samples.

Experimental

Reagents—

Sodium carbonate solution, 15 *per cent.*

Hydrochloric acid, 4 N.

Nitric acid, concentrated.

Ammonium molybdovanadate solution.[7]

Ammonium molybdate solution—Dissolve 25 g of ammonium molybdate in 200 ml of water at 60 °C. Add to this solution a mixture of 280 ml of concentrated sulphuric acid and 280 ml of water and, after cooling, dilute the solution to 1 litre.

Tin(II) chloride solution—Dissolve 0·25 g of $SnCl_2.2H_2O$ in 10 ml of concentrated hydrochloric acid and dilute to 100 ml with water. Prepare fresh daily.

Standard phosphate solution—Dissolve 439·4 mg of potassium dihydrogen phosphate, KH_2PO_4, in water and dilute to 1 litre with water.

1 ml of solution ≡ 100 μg of phosphorus.

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APPARATUS—

Samples were ashed in porcelain crucibles (32 mm in diameter) in a muffle furnace at 500 °C. Solutions were evaporated on a steam-bath. Absorbances of the yellow molybdovanadophosphate and molybdenum-blue colours produced were measured on a spectrophotometer, Model SF-4A (U.S.S.R.), at 436 and 680 nm, respectively, in 1-cm cells against blank solutions.

PROCEDURE—

Samples (0·5 g) were divided into halves. One half of each sample was evaporated to dryness with 1 ml of sodium carbonate solution *plus* a little water. Ashing of both halves of the samples was carried out with a slowly rising temperature, a 10 per cent. solution of ammonium nitrate being added if necessary. After ashing at 500 °C for 5 hours, the ash from the untreated samples was evaporated to dryness with 1 ml of 4 N hydrochloric acid and the ash from the samples treated with sodium carbonate was evaporated to dryness with 1 ml of concentrated nitric acid. Similar amounts of the respective acids were also used to transfer the residues into 25-ml stoppered flasks, the solutions in the crucibles being first diluted with a few millilitres of hot water. When cool, the solutions were diluted to 25 ml with water, and were further diluted if necessary.

For the spectrophotometric determination involving the yellow colour, add to an aliquot 7·5 ml of molybdovanadate reagent, dilute the solution to 25 ml with water and, after 2 hours, read the absorbance at 436 nm in 1-cm cells against a blank. Analytical values were obtained from a calibration graph constructed from measurements on standards in the range 0 to 300 μg of phosphorus per 25 ml. For the determination with the blue colour, dilute an aliquot to 15 ml, add 0·5 ml of ammonium molybdate solution, mix, after 5 minutes add 0·5 ml of tin(II) chloride solution, dilute to 25 ml with water and mix well. After 5 minutes, measure the absorbance at 680 nm in 1-cm cells against a blank. The phosphorus content is read from a standard graph constructed in the range 0 to 25 μg of phosphorus per 25 ml. The standard solution is treated in the same way as the samples. All determinations were made in triplicate.

RESULTS AND DISCUSSION

Both spectrophotometric procedures gave strictly linear graphs of absorbance against the amount of phosphorus for the ranges 0 to 300 μg for the yellow colour and 0 to 25 μg for the blue colour. The reduced molybdophosphate provided much better sensitivity than the procedure involving the formation of the molybdovanadate complex (the molar extinction coefficients were 27 800 and 1030, respectively). The molybdenum-blue method is preferred when rapid measurements of low concentrations of phosphorus are required, while the other method, the reagent for which is more stable, is more useful for wider ranges.

As the spectrophotometric methods are applied in the presence of acids, the yellow colour produced by 100 and 200 μg of phosphorus and the blue colour given by 5 and 15 μg of phosphorus in the presence of the respective amounts of hydrochloric and nitric acids expected in the final solutions were examined; 0·5 ml of hydrochloric acid and 1·0 ml of nitric acid were each diluted to 25 ml with water and 1, 2, 5 and 10 ml of these solutions were added to standards. No changes in absorbance occurred; therefore, aliquots of samples can be analysed without the need for extra precautions (moreover, aliquots containing less phosphorus than the amounts examined can be used and the solution considerably diluted prior to application of the molybdenum-blue method). The evaporation with nitric acid to remove chloride from the hydrochloric acid residue can therefore be omitted; this evaporation was carried out to prevent chloride interference, which is sometimes serious in the molybdovanadate method.

The two spectrophotometric methods were tested on ten samples of animal feedingstuffs, which were characteristic of a wide variety of matrices, the samples being ashed and the ash dissolved as described above. In the samples ashed with sodium carbonate, some difficulties occurred because of insufficient penetration by air. Samples without fixative ashed well, indicating that even lower temperatures can be used for ashing.

The analytical values obtained are presented in Table I. Certain differences can be observed both for the ashing procedures and for the spectrophotometric methods. The results obtained when using sodium carbonate fixative were higher, with the exception of

those for fish meal and wheat grain. The paired sample *t*-test showed that the difference in results is not significant, but this conclusion is not decisive as the range of phosphorus contents is very wide. Nevertheless, it seems that the difference between ashing procedures is negligible for practical purposes. Similar results were reported by Stuffins,[7] with good agreement between results obtained by Kjeldahl digestion and by simple dry ashing, especially in samples with higher calcium contents. In our experiments, poorer phosphorus recovery was obtained with samples with typically high contents of this element (*e.g.*, cakes). In other samples, the rôle of the excess of calcium is not unambiguous. Lower values obtained for fish meal when ashed with sodium carbonate are probably caused by difficulties occurring in the oxidation of this high-protein material.

TABLE I

DETERMINATION OF PHOSPHORUS IN BIOLOGICAL SAMPLES AFTER DRY ASHING

Sample	Ashed without fixative/mg g^{-1}			Ashed with Na_2CO_3 /mg g^{-1}		
	A	B	C	A	B	C
Barley grain ..	3·55	3·75	3·60	3·74	4·00	3·70
Blood meal.. ..	3·85	5·50	3·88	4·10	5·52	4·15
Bone meal	131·2	164·0	128·0	143·7	173·4	145·0
Cotton cake ..	9·50	10·25	9·80	10·50	10·52	10·42
Groundnut cake ..	5·30	6·25	5·32	6·00	7·00	6·08
Fish meal	31·50	35·20	31·44	29·20	33·80	29·20
Lucerne meal ..	3·37	3·25	3·42	3·54	3·39	3·48
Mixed feed for chick	6·02	7·00	5·96	6·35	7·29	6·50
Sugar beet pulps, dried	1·25	1·56	1·38	1·37	1·72	1·45
Wheat grain ..	3·05	3·50	3·02	2·78	3·28	2·84

A = Molybdovanadate method.
B = Reduced molybdophosphate method.
C = Standard gravimetric procedure.

It can be seen in Table I that much higher results are obtained with the molybdenum-blue method compared with those obtained by the procedure giving the yellow colour. The difference, however, is small for samples with lower phosphorus contents. Therefore, the former method is useful for the analysis of materials with low phosphorus contents. The procedure involving the production of the yellow colour is suitable for materials rich in phosphorus and, moreover, provides results similar to those given by the standard gravimetric method (Table I), which is in accordance with previous findings.[9]

The important conclusion to be drawn from this study is that simple dry ashing without fixative can be used for determining phosphorus as well as metals in biological materials. The application of fixatives or the use of more tedious wet digestions can therefore be avoided. For the final determination of phosphorus, two alternative spectrophotometric procedures can be used: either the accurate molybdovanadate method for wider ranges of phosphorus concentration, with which the reagent can be stored for about 6 months, or the reduced molybdophosphate method, which should be applied when rapid determination of very small amounts of phosphorus is required.

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Spectrophotometric Determination of Trace Amounts of Thorium in Lanthanum Oxide

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A spectrophotometric method for the determination of trace amounts of thorium in lanthanum oxide or lanthanum metal has been developed.

The method is based on the determination of thorium with thoron after its separation from lanthanum by extraction with a mixture of ethyl acetate and acetone. The efficiency of the separation was tested with a lanthanum-140 tracer. Other elements present in lanthanum oxide as impurities do not interfere.

Thorium contents down to 10 p.p.m. can be determined in a 1-g sample with 1-cm cells used for absorbance measurement. Errors of determination are within the range ±5 per cent. The relative standard deviations on samples containing 30 and 100 p.p.m. of thorium are 1·6 and 1 per cent., respectively.

LABORATORY-SCALE work on the production of pure lanthanum at the Institute for Technology of Nuclear Raw Materials has necessitated the development of a reliable and accurate spectrophotometric method for the determination of trace amounts of thorium in high-purity lanthanum oxide.

No spectrophotometric method for the determination of thorium as an impurity in lanthanum metal or lanthanum oxide has been described in the literature. However, data from the literature on the spectrophotometric determination of thorium in the presence of the rare earth metals would not be expected to indicate any probability of a successful direct determination of trace amounts of thorium in lanthanum oxide. Thoron and arsenazo reagents, which are the most widely used reagents for thorium owing to their sensitivity and selectivity when they are used in appreciably acidic solutions, are not sufficiently selective in the presence of a large excess of the rare earth elements.[1] Direct methods can be used only when microgram amounts, or, in the best instance, several milligrams of rare earths are present; otherwise, a separation prior to the thorium determination must be carried out.[1–4]

Savvin, Basargin and Makarova[1] examined the selectivity of the determination of thorium with thoron and arsenazo reagents compared with samarium as a representative of the rare-earth elements. They found that thoron was considerably more selective than arsenazo I, arsenazo II and especially arsenazo III, which is the most sensitive and most often used reagent from this group. Only dibromoarsenazo was found to be more selective than thoron, but this reagent was synthesised by the author and is not commercially available. In the present work, thoron was chosen as a reagent for thorium. However, in accordance with the above facts, preliminary experiments showed that in this particular instance, when lanthanum was the major component and thorium a trace impurity, the concentration ratio of these two elements did not permit the direct application of the thoron method. The extraction of thorium with an ethyl acetate - acetone mixture was used as a separation method, by which thorium can be separated from many elements, including lanthanum.[5]

EXPERIMENTAL

APPARATUS—

A Beckman Model DU spectrophotometer with 1-cm Corex cells was used for absorbance measurements.

A 256-channel Analyzer (RCL Inc., Skokie, Ill., U.S.A.) with a sodium iodide - thallium crystal was used for γ-activity measurements.

REAGENTS—

The reagents used were of analytical-reagent grade unless otherwise specified.

Lanthanum oxide, Specpure—Johnson Matthey and Co. Ltd.

Nitric acid, concentrated, sp.gr. 1·4, *and dilute* (3 + 8 *v/v*).

Aluminium nitrate, crystalline, $Al(NO_3)_3.9H_2O$.

Tartaric acid, crystalline and 5 *per cent. w/v aqueous solution.*

Mixture for extraction—Mix 2 volumes of ethyl acetate with 1 volume of acetone.

Wash solution—Dissolve by heating 90 g of aluminium nitrate in 33 ml of nitric acid (3 + 8 v/v) *plus* 33 ml of water.

Perchloric acid, concentrated.

Hydrochloric acid (1 + 1 *v/v*).

Hydroxylammonium chloride solution, 10 *per cent. w/v.*

Thoron [1-(o-*arsonophenylazo*)-2-*naphthol*-3,6-*disulphonic acid, sodium salt*], 0·2 *per cent. w/v aqueous solution.*

Thorium stock solution—Dissolve 2·53 g of thorium nitrate, $Th(NO_3)_4.6H_2O$, in distilled water and dilute to 1 litre to obtain a solution containing 1 mg ml^{-1} of thorium. Standardise the solution gravimetrically by oxalate precipitation and ignition to the oxide.

Thorium standard solution—Dilute the thorium stock solution with water to obtain a standard solution containing 20 $\mu g\ ml^{-1}$ of thorium.

PREPARATION OF CALIBRATION GRAPH—

Transfer by pipette volumes of standard thorium solution containing from 0 to 200 μg of thorium to a series of 25-ml calibrated flasks. Add 2 ml of hydrochloric acid (1 + 1 v/v), 1 ml of 10 per cent. w/v hydroxylammonium chloride solution, 1 ml of 5 per cent. w/v tartaric acid solution and 2 ml of 0·2 per cent. w/v thoron solution to each flask, make up to volume with water and mix. Read the absorbances at 545 nm against a reagent blank. Construct the calibration graph by plotting absorbance values against thorium concentration.

PROCEDURE—

Weigh 1 g (or some other suitable amount) of sample into a 150-ml beaker. Add several millilitres of water and nitric acid and dissolve by heating gently. Evaporate to dryness. Add 15 g of aluminium nitrate and 9 ml of dilute nitric acid to the dry residue and dissolve by heating. Cool the solution to room temperature and transfer to a 125-ml separating funnel. Rinse the beaker with 2 ml of dilute nitric acid and add the washings to the solution in the funnel. Rinse the beaker with 20 ml of mixture for extraction and transfer to the funnel. Add 1 g of solid tartaric acid and shake the mixture for 2 minutes. Allow the layers to separate and transfer the aqueous phase to a second separating funnel. Add 20 ml of mixture for extraction and extract again for 2 minutes. Separate and discard the aqueous phase. Combine the organic layers and rinse them by shaking them twice with 10-ml portions of the wash solution for 2 minutes. Discard the washings each time. Add 20 ml of water to the washed organic phase and shake for 2 minutes. Draw off the aqueous layer into a 250-ml beaker. Repeat the re-extraction of thorium in the same manner and add the aqueous layer to the solution in the beaker. Evaporate to dryness on a hot-plate. Add 2 ml of nitric acid and 1 ml of perchloric acid to oxidise the organic matter. Evaporate until white fumes are no longer emitted, avoiding an excessively high temperature in order to prevent baking. The sides of the beaker should be dry. Cool, add 2 ml of hydrochloric acid and, after several minutes, add 6 ml of water and 1 ml of 10 per cent. hydroxylammonium chloride solution. Boil the mixture and transfer with water to a 25-ml calibrated flask. Add 1 ml of 5 per cent. tartaric acid solution and 2 ml of 0·2 per cent. thoron solution, dilute to volume with water and mix. Measure the absorbance at 545 nm against a blank that has been taken through the entire procedure together with the sample.

RESULTS AND DISCUSSION

INFLUENCE OF LANTHANUM—

Results on the influence of lanthanum in various procedures in which thoron was used show considerable variations.[6–8] Experiments were therefore carried out in order to obtain information on the interference of lanthanum under the conditions used in the present work.

TABLE I

INFLUENCE OF LANTHANUM ON THE DETERMINATION OF THORIUM

Lanthanum added/ mg	Absorbance in the presence of—			
	0 μg of Th	50 μg of Th	100 μg of Th	200 μg of Th
0	0·000	0·122	0·245	0·480
1	0·001	0·123	0·247	0·482
5	0·011	0·132	0·256	0·488
10	0·021	0·141	0·261	0·497

According to the procedure described, colour was developed in the solutions that contained 0 to 10 mg of lanthanum, as well as in the solutions that contained the same amount of lanthanum and various amounts of thorium. The absorbances against a reagent blank are given in Table I.

It can be seen that the positive effect of lanthanum is proportional to its concentration and that a given amount has an equal effect on different concentrations of thorium. About 500 μg of lanthanum correspond to an absorbance of 0·001, so that the presence of lanthanum in amounts up to 500 μg can be neglected in thorium determinations. In other words, when the thoron method is applied to the determination of trace amounts of thorium in lanthanum, an efficient separation must be used in order to reduce the lanthanum, which is present in the final solution, to a negligible amount.

SEPARATION OF LANTHANUM BY EXTRACTION WITH ETHYL ACETATE - ACETONE—

It was established in earlier work[5] that thorium can be separated successfully from lanthanum by extraction with a mixture of ethyl acetate and acetone. The absence of lanthanum in the solution that contains the extracted thorium was proved qualitatively by the lack of a reaction with oxalate. The largest amount of lanthanum tested was 10 mg.

As the purpose of this work was the determination of trace amounts of thorium in lanthanum oxide, much larger amounts of lanthanum had to be considered. Therefore, the separation of thorium from 1 g of lanthanum oxide was studied. The behaviour of lanthanum during extraction was examined with a lanthanum-140 tracer added to the inactive oxide. By measuring the γ-activities of organic layers at various stages of the extraction process, the results shown in Table II were obtained. Activities are expressed as the percentage of the lanthanum-140 added to the sample.

TABLE II

LANTHANUM PRESENT AT VARIOUS STAGES IN THE EXTRACTION OF THORIUM

Stage	Lanthanum-140 found in organic layer, per cent. of original
1st extraction	3·42
2nd extraction	2·60
1st washing	0·91
2nd washing	0·05

About 6 per cent. of lanthanum passes into the organic layers after two extractions, but it is subsequently removed by two washings.

The efficiency of the separation was further established by applying the extraction procedure to samples that consisted of 1 g of lanthanum oxide and comparing their absorbances with those of blanks that had been treated in the same manner. The same values of the absorbances were obtained in both instances, which indicates that the amount of lanthanum possibly present in the final solution was below that which interferes in the determination of thorium.

INFLUENCE OF OTHER ELEMENTS—

The separation of thorium from various elements by extraction with ethyl acetate - acetone was investigated earlier.[5] The corresponding results obtained then indicated that no effect was to be expected from other elements present as impurities in lanthanum oxide.

This was confirmed with synthetic samples that consisted of 1 g of lanthanum oxide to which known amounts of thorium and other elements were added in concentrations that commonly occur in pure rare earth metals or oxides.[9] Moreover, owing to the selectivity of the separation, thorium can be determined in samples of much lower purity.

ACCURACY AND PRECISION—

The accuracy and precision of the method were studied by applying the described procedure to synthetic samples having the same compositions as those above. The mean values of duplicate results for various concentrations of thorium are given in Table III.

TABLE III

DETERMINATION OF THORIUM IN SYNTHETIC SAMPLES

Lanthanum oxide taken/g	Thorium taken/μg	Thorium found/μg	Error, per cent.
1	20	19	−5
1	30	31	+3·3
1	50	50·5	+1
1	100	102	+2
1	150	147·5	−1·7
1	200	195	−2·5

The relative standard deviations obtained on two series of ten synthetic samples each, containing 30 and 100 p.p.m. of thorium, were 1·6 and 1 per cent., respectively.

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The Determination of Molybdenum in Geological Materials by a Combined Solvent-extraction - Atomic-absorption Procedure

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A method is described for the determination by atomic-absorption spectrophotometry of molybdenum, up to the 1000 p.p.m. level, in geochemical samples. After attack with acid, the molybdenum in dilute perchloric acid medium is complexed with benzoin α-oxime and extracted into chloroform. The extract is mixed with nitric and perchloric acids and evaporated to dryness and the residue is dissolved in ammonia solution. The ammonium molybdate formed is dissolved in a solution containing 1 per cent. v/v of perchloric acid *plus* 0·5 per cent. w/v of ammonium chloride and aspirated into a luminous air - acetylene flame.

THE molybdenum contents of soil, stream sediment and rock samples are becoming of increasing interest to geologists engaged in the search for mineral deposits by using geochemical prospecting techniques. To meet the rising demand for analyses for this element, a method was sought of incorporating its determination into an established rapid scheme of geochemical analysis by atomic-absorption spectrophotometry, in which the sample is dissolved in a mixture of nitric and perchloric acids (3 + 1) and the solution is diluted to a fixed volume, centrifuged and aspirated directly into an air - acetylene flame.

As indicated by David,[1] the sensitivity of molybdenum is inadequate for its direct determination in samples that contain less than 10 p.p.m. of molybdenum. As the size of the sample is restricted to about 1 g because of the limited amount of material that is normally available for analyses, pre-concentration is essential. Solvent extraction was investigated.

Butler[2] has demonstrated the increase in sensitivity obtained by aspirating an organic solvent, but our tests on pure solvents showed that contamination of the Unicam spectrophotometer was too great for the procedure to be combined with other routines; a method involving back-extraction into an aqueous phase was therefore preferred. Waterbury and Bricker[3] used this technique and extracted molybdenum and iron with isobutyl methyl ketone from 6 N hydrochloric acid followed by back-extraction into water. Iron, which interferes in the determination of molybdenum, was separated by precipitation with ammonia solution.

Because of the low concentrations of molybdenum encountered in geochemical samples, concentration of the filtrate resulting from the ammonia separation was necessary, but was found to be too time consuming. An alternative back-extraction of the ketone layer into 2 per cent. w/v ammonium chloride solution, which was shown by Mostyn and Cunningham[4] to suppress interference by iron, was attempted but at the concentrations of sample solution needed to produce reasonable sensitivity for molybdenum, iron(III) oxide was deposited on the burner slot.

A solvent-extraction procedure that would separate molybdenum from iron was required.

EXPERIMENTAL

The precipitation of molybdenum as the benzoin α-oximate from cold solutions of dilute acids has long been the basis of a gravimetric method. If the precipitate is extracted into chloroform, separation from iron and other major constituents is effected.[5–8] The chloroform can then be volatilised and the benzoin α-oximate decomposed to yield a molybdenum salt that is soluble in an appropriate aqueous medium.

Flame conditions greatly affect the sensitivity of molybdenum when a luminous air - acetylene flame is used. A maximum sensitivity of 0·4 p.p.m. of molybdenum was achieved

by using a full acetylene cylinder and about 4× scale expansion on a Unicam SP90 spectrophotometer. Without scale expansion a 100 p.p.m. solution of molybdenum gave an absorbance of 0·3.

As most geochemical samples contain only small amounts of molybdenum the final volume of solution containing the molybdenum obtained from a 1-g sample needed to be no greater than 5 ml for a satisfactory background level of 2 p.p.m. of molybdenum. For higher levels of molybdenum the final volume was increased. The method was tested on samples containing up to 1000 p.p.m. of molybdenum.

Towards the end of the work a Techtron AA5 spectrophotometer was purchased, which facilitated greater scale expansion, better sensitivities and the possible use of the preferred nitrous oxide - acetylene flame.

INSTRUMENT AND OPERATING CONDITIONS—

A Unicam SP90 spectrophotometer was used with the following operating conditions: wavelength, 313·2 nm; slit width, 0·1 mm; air flow-rate, 5 l min^{-1}; fuel rate (acetylene), 2·4 l min^{-1}; burner height, 0·6 cm; sensitivity, 0·4 p.p.m. of molybdenum for 1 per cent. absorption; and scale expansion, about 4×.

The only refinement to the standard SP90 model was the use of a fume-extraction hood over the chimney. The signal was stable.

REAGENTS—

All the reagents were of analytical-reagent grade unless otherwise stated.

Hydrofluoric acid, 40 *per cent. w/w*.

Perchloric acid,* 60 *per cent. w/w*.

Nitric acid, sp.gr. 1·42.

Acid mixture—Three volumes of nitric acid *plus* 1 volume of perchloric acid.

Ammonia solution, 0·880 *g* ml^{-1}.

Hydrogen peroxide, 100-*volume*, 30 *per cent. w/w*.

Ethanol.

Benzoin α-oxime solution—Dissolve 2 g of benzoin α-oxime in 100 ml of ethanol. Store in a refrigerator and renew every few days.

Chloroform.

Ammonium chloride - perchloric acid mixture—Dissolve 5 g of ammonium chloride in water, add 10 ml of perchloric acid and dilute to 1 litre.

Stock molybdenum solution, 5000 *p.p.m.*—Dissolve 4·60 g of ammonium molybdate tetrahydrate in de-ionised water; make just ammoniacal and dilute to 500 ml in a calibrated flask.

PROCEDURE—

To a 1-g sample in a PTFE beaker add 10 ml of hydrofluoric acid. Stand the mixture for 1 hour and then evaporate to dryness. Add 10 ml of the mixture of nitric and perchloric acids (3 + 1) and again evaporate to dryness, washing down the sides of the beaker once during the evaporation to ensure complete removal of hydrofluoric acid.

To the dry residue add a few drops of ammonia solution and digest on a water-bath (or low hot-plate) until all traces of ammonia are removed. Add 2·5 ml of perchloric acid and 10 ml of water *plus* a few drops of 100-volume hydrogen peroxide and warm gently to dissolve solids. Heat until fumes of perchloric acid are just produced, to destroy the peroxide, and dilute with about 20 ml of water.

Transfer the mixture to a centrifuge tube and centrifuge for about 5 minutes at 3000 r.p.m. Pour the liquid into a 100-ml separating funnel previously calibrated at 50 ml and bulk to this mark with water.

Add 2 ml of the 2 per cent. ethanolic solution of benzoin α-oxime and shake the mixture. Allow 5 minutes for formation of the complex, then extract the precipitate into 5 ml of chloroform by shaking the mixture for 1 minute. Draw off the lower layer into a 25-ml beaker. Repeat the extraction twice more, drawing off each extract into the 25-ml beaker. Evaporate off the chloroform by standing the beaker on a warm surface.

* When dilutions of perchloric acid are made the solutions obtained are expressed as per cent. v/v of this 60 per cent. w/w acid.

Allow the tarry residue to cool and add 5 ml of the mixture of nitric and perchloric acids (3 + 1). Cover the beaker with a watch-glass and transfer it to a hot-plate. After subsidence of any vigorous reaction, remove the cover and evaporate the mixture to dryness. Treat the residue with a few drops of ammonia solution and again evaporate to dryness.

Transfer 5 ml of 0·5 per cent. w/v ammonium chloride - 1 per cent. v/v perchloric acid solution with a pipette on to the warm residue, replace the watch-glass on the beaker and warm carefully for a few minutes. For accurate work the final solution should be made up in a 5-ml calibrated flask, and for high values of molybdenum a larger final volume should be used.

Aspirate the sample into a luminous air - acetylene flame under the conditions described above.

Calibration—

Prepare accurately diluted solutions of the stock molybdenum solution in the 0·5 per cent. w/v ammonium chloride - 1 per cent. v/v perchloric acid mixture to cover the range of concentrations of the sample solutions. Aspirate standard solutions before and after each batch of samples, aspirating water after each standard - sample solution, and run one check standard after every fifth sample.

Convert transmittance readings to absorbance figures and plot a graph of absorbance against the concentration of molybdenum in the standard.

Results and Discussion

The choice of suitable samples for analysis by this method was restricted by the limited availability of accurate molybdenum data on international standard materials. Table I gives a comparison of molybdenum results on samples previously analysed by different techniques and gives the results obtained by the suggested atomic-absorption procedure on standards G-1 and W-1.

Table I

Comparison of molybdenum results (in parts per million) for previously analysed samples by different techniques

Sample material	Absorptiometry (as molybdenum - dithiol complex)	X-ray fluorescence	Emission spectrography	Atomic absorption spectrophotometry by this method with: Unicam SP90	Atomic absorption spectrophotometry by this method with: Techtron AA5
Marl Slates from Northumberland and Durham—					
Cullercoats, Northumberland ..	23	35	20	25	18
Fishburn, Durham	92	107	100	87	84
Claxheugh, Durham	54	70	50	55	50
Fishburn, Durham, MSA8	160	190[9]	170	150	165
Fishburn, Durham, MSB2	555	694[9]	490	500	495
Mainsforth, Durham, MSD10 ..	460	543[9]	440	440	445
Locality unknown, MSG9	755	900	680	720	715
Kangankunde Hill carbonatite complex, Malawi—					
Phlogopite rock (fenite), 2633A ..	200	—	170	180, 200	—
Orthoclase-calcite fenite, 2633B ..	90	—	80	80, 60	—
Carbonatite-orthoclase rock, 2633C	120	—	100	120, 120	—
Mixed fenite, 2633D	140	—	140	120, 110	—
Monazite carbonatite, 1203 ..	15	—	—	—	12, 12
Monazite-rich carbonatite, 1208 ..	6	—	—	—	5, 6
Oleolondo, near Gilgil, Kenya—					
Manganese ore (pyrolusite) ..	268	—	—	230	230
Granite, G1	(Recommended value 7)	—	—	—	4, 5, 5
Diabase, W1	(Recommended value 0·5)	—	—	—	0·5, 1, 1
Molybdenite - quartz mixture, IGS6	Accepted mean 1380,[10] median 1450)	—	—	1420	1360

PRECISION—

An estimate of the precision of the results was made by calculating the standard deviation, s, from the equation $s = [d^2/2k]^{\frac{1}{2}}$, where d is the difference for a pair of duplicate analyses and k is the number of samples.

The standard deviation is 6 over the range 50 to 250 p.p.m. of molybdenum.

CHOICE OF SAMPLE ATTACK—

Work on samples in which the molybdenum was determined absorptiometrically as the dithiol complex showed that the intended use of a mixture of nitric and perchloric acids (3 + 1) was inadequate and that a preliminary treatment with hydrofluoric acid was essential (Table II). The slightly lower results obtained for samples A, B and C when perchloric acid was used instead of sulphuric acid were attributed to the precipitation of some of the molybdenum as molybdic acid. Table III shows that digestion of the residue, from the attack by perchloric acid, with ammonia solution before final dissolution facilitated full recovery of the molybdenum.

TABLE II

DETERMINATION OF MOLYBDENUM BY USING DIFFERENT SAMPLE DECOMPOSITION PROCEDURES

	Molybdenum concentration, p.p.m.			
Sample	Attack by nitric acid - perchloric acid	Attack by hydrofluoric acid - nitric acid - sulphuric acid	Attack by hydrofluoric acid - nitric acid - perchloric acid	Alkaline fusion
A	1	24	22	23
B	1	91	84	94
C	1	54	50	54
P1	3	—	30	—
P2	7	—	20	—
P3	16	—	17	—
P4	13	—	15	—

In samples with high manganese concentration, the manganese was precipitated as manganese(IV) oxide during the attack by acid, carrying the molybdenum with it. The incorporation of a little hydrogen peroxide at the dilute perchloric acid dissolution stage decomposed the manganese(IV) oxide, releasing the molybdenum. Excess of hydrogen peroxide was destroyed by lightly fuming with perchloric acid.

TABLE III

EFFECT OF AN AMMONIA DIGESTION ON THE RECOVERY OF MOLYBDENUM AFTER AN ATTACK BY PERCHLORIC ACID

	Amount of molybdenum recovered, p.p.m.	
Molybdenum added, p.p.m.	Without an ammonia digestion*	With an ammonia digestion†
125	103	125
250	232	249
375	335	370
500	455	498
625	555	622

* Pure molybdenum solution evaporated to dryness with perchloric acid and residue leached with 1 per cent. v/v perchloric acid.

† Duplicate molybdenum solution evaporated to dryness with perchloric acid; residue dissolved in ammonia solution, evaporated to dryness again and leached with 1 per cent. v/v perchloric acid.

EFFICIENCY OF EXTRACTION FROM PERCHLORIC ACID—

Extraction tests on 250 μg of molybdenum in perchloric acid of various concentrations were performed by complexing the molybdenum with 2 ml of a 2 per cent. w/v solution of benzoin α-oxime in ethanol (allowing 5 minutes for formation of the complex) and then shaking

the mixture for 1 minute with two 5-ml volumes of chloroform. Molybdenum remaining in the aqueous phase was determined absorptiometrically as the molybdenum - dithiol complex. These tests showed that 5 per cent. v/v perchloric acid was a suitable medium from which to extract molybdenum benzoin α-oximate.

The amounts of molybdenum were then increased to 500 μg and 1000 μg and further extractions from 5 per cent. v/v perchloric acid were carried out with two or three 5-ml volumes of chloroform. The results suggested that 1000 μg of molybdenum could be handled satisfactorily provided three extractions with chloroform were used (Table IV).

TABLE IV

EFFICIENCY OF EXTRACTION FROM PERCHLORIC ACID

Amount of molybdenum used/μg	Concentration of perchloric acid, per cent. v/v	Number of extractions with 5 ml of chloroform	Amount of molybdenum left in aqueous phase/μg	Extraction, per cent.
250	2	2	9	96·4
			8	97·0
250	5	2	2	99·2
			3	99·0
250	10	2	4	98·4
			5	98·0
500	5	2	6·5	98·7
			6	99·0
500	5	3	1·5	99·7
			2·5	99·5
1000	5	2	17	98·3
			15	98·5
1000	5	3	2·5	99·8
			5	99·5

SELECTION OF FINAL MEDIUM FOR ASPIRATION—

Sinyakova and Glinkina[8] used a mixture of nitric, perchloric and sulphuric acids to decompose the organic material after extraction. However, as the presence of sulphuric acid in a solution for atomic-absorption analysis is often undesirable, its effect on the determination of molybdenum was investigated. Table V shows that an increase in the concentration of sulphuric acid produced a steady decrease in the absorbance of a 25 p.p.m. molybdenum solution. On the other hand, concentrations of perchloric acid of up to 2 per cent. v/v had no effect.

TABLE V

EFFECT OF SULPHURIC AND PERCHLORIC ACIDS ON THE ABSORBANCE OF A 25 p.p.m. MOLYBDENUM SOLUTION

Acid strength, per cent. v/v	Absorbance	
	Perchloric acid medium	Sulphuric acid medium
1	0·161	0·143
2	0·161	0·128
5	0·158	0·092
10	0·146	0·056

The absorbance of a 25 p.p.m. molybdenum solution in aqueous medium was 0·161.

INTERFERING IONS—

Hillebrand and Lundell[11] observed that silicon, niobium, tantalum, palladium, tungsten, quinquivalent vanadium and sexavalent chromium are co-precipitated with benzoin α-oxime.

Silicon was volatilised in the recommended method of sample attack and niobium and tantalum remained insoluble.

No interference was detected from vanadium, tungsten or chromium when they were added to a pure molybdenum solution.

Interference from small amounts of iron and other cations carried through in aqueous droplets during the organic extraction was eliminated by the use of ammonium chloride.

When assessing the amount of ammonium chloride required it was noticed that a molybdenum solution containing 2 per cent. w/v of ammonium chloride and 1 per cent. v/v of perchloric acid gave a very slow signal response, preventing the use of small volumes of sample. Reduction of the ammonium chloride concentration to 0·5 per cent. w/v produced an immediate response and effectively suppressed interferences.

Conclusions

Although it was not found possible to develop an atomic-absorption method for the determination of molybdenum that was amenable to full integration into an existing analytical scheme for certain other elements, nevertheless a reliable, accurate and reasonably rapid method has been evolved. It has the advantage of being applicable, without modification, to a wide range of materials encountered in a geological context.

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An Emission-spectrographic Method for the Determination of Boron in Nuclear-grade Graphite*

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A rapid, highly sensitive method is described for the spectrographic determination of boron in nuclear-grade graphite. Boron, present in the sample in the form of boron carbide (B_4C), is volatilised as the trifluoride by the addition of 1 per cent. of Kel-F powder (polychlorotrifluoroethylene) to the samples. The samples, containing 500 p.p.m. of silicon as the internal standard, are excited by a 10-A d.c. arc in the presence of an argon-controlled atmosphere. A linear relationship between the intensity ratio of the lines boron 249·773 nm and silicon 245·214 nm and the boron concentration is established in the boron concentration range 0·1 to 5 p.p.m. The relative standard deviation, calculated from twelve determinations on each sample, ranges from ±8 per cent. (at 5 p.p.m. of boron) to ±11 per cent. (at 0·1 p.p.m. of boron).

SEVERAL methods, or refinements of existing methods, have been proposed in the literature for the spectrochemical determination of traces of boron in graphite and related materials.[1–9] In general, such methods fall into two main categories, *viz.*, methods based on the direct excitation of the sample by a d.c. arc or high-voltage spark, or enrichment procedures involving the ashing of the carbonaceous material in the presence of a suitable compound (in most instances calcium hydroxide) in order to fix the boron oxide formed. However, the latter procedures require a rather large sample size; they are time consuming and do not show any definite advantages compared with colorimetric methods. On the other hand, direct methods are complicated by the stability and refractory character of boron carbide. It is accepted either that boron is present in graphite as boron carbide or that this compound will be formed when graphite that contains boron is heated at a temperature of 2500 °C. The work of Feldman and Ellenburg[1] is considered to be the most exhaustive treatment of this aspect from an analytical point of view. Their results showed that satisfactory sensitivities could be achieved only if the transport of a significant number of B_4C particles into the arc discharge zone could be assured. The highest attainable temperature at the surface of the supporting electrode would also be necessary in order to melt the particles.

To overcome these difficulties fluorinated carriers (usually sodium or copper fluorides) have been extensively used[4–8] to exploit the possible formation of a volatile compound (boron trifluoride) by the reaction of boron carbide with the halogens.[10] However, the mechanism of boron evaporation is not completely understood because in some instances the results of tests with boron oxide were considered to be valid also for boron carbide.

The results of studies on the system boron carbide - graphite - third component (fluoride and metal oxides) carried out by Rajic[11,12] led us to exclude the possibility that fluorination occurs. In fact, boron was found to begin to distil at a temperature at which the added fluorides were molten or just beginning to evaporate from the electrodes, and under these conditions the fluoride does not dissociate. Moreover, X-ray diffraction analysis of the compound resulting from the system boron carbide - graphite - sodium fluoride revealed the presence of two new, unidentified phases, which may indicate that compounds other than boron trifluoride are formed.

EXPERIMENTAL

PRELIMINARY CONSIDERATIONS—

From the foregoing discussion one can conclude that boron trifluoride could be formed if a stream of gaseous fluorine was introduced into the electrode while the sample was being heated at a sufficiently low temperature to avoid an unwanted secondary reaction. It was

* Presented at the XVth Seminario Spettrochimico, Sorrento, June, 1970.

felt that the best approach to the problem would be to separate the fluorination step from the excitation of the evolved boron trifluoride vapour (both processes occurring in the same electrode).

The excellent fluorination properties of Teflon powder (polytetrafluoroethylene), already exploited for spectrochemical purposes,[13,14] suggested its use for this purpose. This compound is decomposed at a temperature of about 250 °C with the evolution of a large volume of gaseous fluorine (0·45 l g^{-1} at S.T.P.). The greatest problem in the handling of Teflon is its decomposition temperature, which is very low compared with that normally attained inside a graphite crucible subjected to a d.c. arc discharge. In several instances the gas was evolved at such a high rate that the charge was blown out of the electrode. Moreover, the fluorination process could occur only to a limited extent because all the fluorine was evolved in a few seconds.

Preliminary tests showed that a higher efficiency could be achieved with Kel-F powder (polychlorotrifluoroethylene), which, having finer particles, could be more intimately mixed with the powder. The problem of controlling the sample temperature was not resolved by adjusting the parameters of the d.c. arc. Also, lowering the current intensity did not greatly affect the sample temperature and gave rise to undesirable drifting of the arc; moreover, a marked loss of spectrum intensity was noted. However, the d.c. arc was found to be the most suitable discharge source as the highest possible sensitivity was necessary.

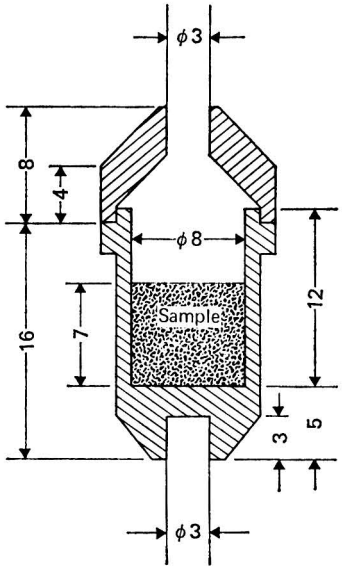

Fig. 1. Geometry of the electrode. Dimensions are given in millimetres

ELECTRODE GEOMETRY—

A better approach to the problem was felt to be offered by correct design of the electrode geometry and by the use of a stream of inert gas around the electrodes during the arcing.

Obviously a large electrode is required in order to provide a large surface for heat radiation. A large crucible must be used in order to retain a reasonable amount of sample remote from the discharge zone so that the fluorination and excitation processes can be kept apart. Finally, a small-diameter bore is necessary to permit the escape of the evolved gases at an optimum rate into the discharge zone. Extensive tests performed with several geometries led us to choose the electrode form shown in Fig. 1. This electrode,* normally used in the double-arc technique, has been slightly modified for our use.

* Ringsdorff Werke GmbH, Type RW1506.

A flow of argon was found to be effective in cooling the electrodes. Moreover, combustion of the graphite and consequently a change of electrode geometry during the arcing were avoided. The importance of the effect of the controlled atmosphere in producing a clear spectral background does not need to be stressed.

A further decrease in sample temperature could also be obtained by cathodic excitation. The excitation chamber (with controlled atmosphere) is shown in Fig. 2. The temperature of the sample during the arcing was measured with a chromel - alumel thermocouple contained in a stainless-steel tube of 0·75-mm diameter and insulated with magnesia. The signal from the thermocouple was fed to a 0·50-mV full-scale chart recorder (Hewlett and Packard Model 7127 A), which has a time response of 0·15 s.

The temperature was measured at three different levels in the sample (the upper, medium and lower levels) by boring three holes of 0·75-mm diameter at the corresponding heights in the wall of the electrode.

In Fig. 3 a record is shown of the temperature variation in the sample while a 10-A d.c. arc was operating and with an argon flow of 9 l min^{-1} in the excitation chamber (these experimental conditions corresponding to those used in the analytical procedure).

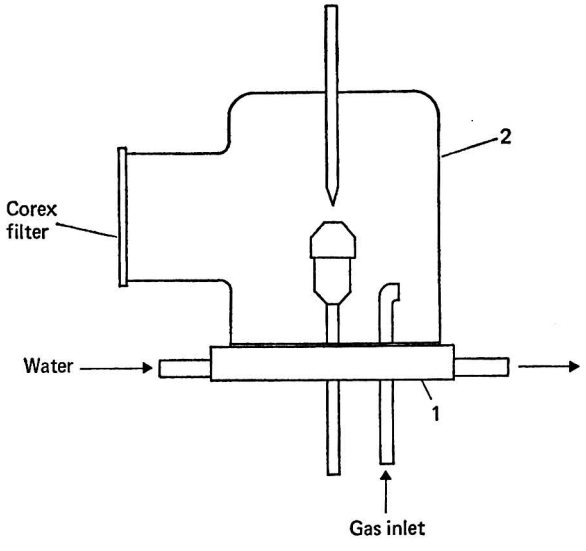

Fig. 2. Sketch of the excitation chamber. 1, water-cooled stainless-steel base plate; 2, glass dome

No significant differences were found in the curve shape or in the temperature recorded at the three levels in the sample. On the other hand, a more irregular temperature distribution and an over-all temperature about 300 °C higher were recorded when the argon flow in the excitation chamber was interrupted.

As soon as the arc was struck a rather steep temperature rise was observed, a temperature of about 250 °C being attained after a 15-s discharge; 50 s were required to reach a fairly constant temperature of about 650 °C. Under these conditions the Kel-F powder decomposed smoothly and continuously, the beginning of the reaction being indicated by the sudden change in colour of the discharge from blue to green.

BORON EVAPORATION—

In order to study the boron extraction process, two sets of graphite samples containing 400 p.p.m. of boron were prepared, boron carbide being used in one set and boron oxide in the other. Three samples of each set were separately mixed with 1 per cent. of Kel-F, sodium fluoride (melting-point 997 °C) or copper(II) fluoride (melting-point 950 °C).

Equal fractions of each sample were arced for 180 s under the above experimental conditions and the spectra were recorded by a falling-plate technique; the duration of each single exposure was 10 s. Because neither of the melting-points of the two inorganic compounds was reached, any fluorination process must be excluded. In Fig. 4 the distillation curves of boron (wavelength 249·773 nm) are shown for the various samples.

For the samples containing boron oxide, similar and almost constant rates of evaporation of boron were observed when either sodium or copper(II) fluoride was used as carrier, although a pronounced maximum occurred with the copper(II) fluoride. An extremely fast evaporation was achieved when the Kel-F was added, such that the boron line intensity could not be measured in the spectra corresponding to the highest evaporation rate, even when a 0·5 density neutral filter was placed in front of the spectrograph slit.

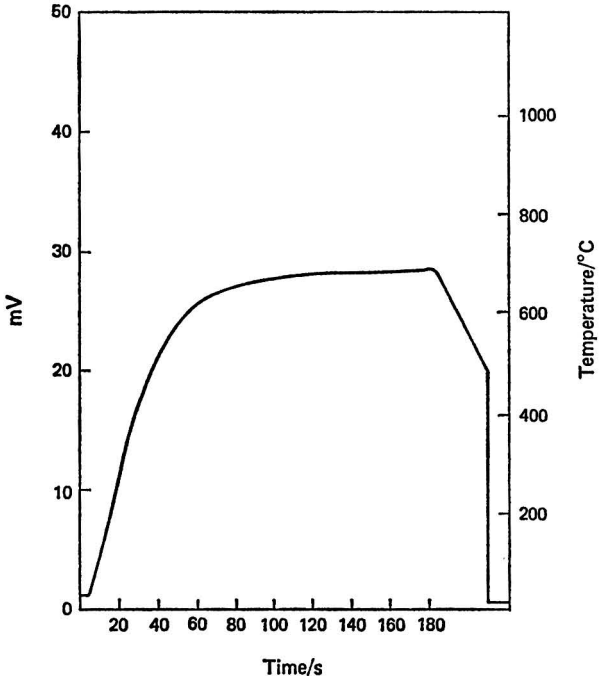

Fig. 3. Typical record of the temperature variation measured in the sample

In the samples containing boron carbide, no boron lines were recorded in the spectra when sodium fluoride was added, while very faint boron lines could be observed that lasted for the initial period when copper(II) fluoride was added. The addition of Kel-F powder promoted smooth evaporation of boron although after a discharge period of 180 s this process was not complete. In fact, with previously arced samples treated again with Kel-F powder and excited for a second time under the same conditions, very faint boron lines were recorded over long periods. However, the use of larger amounts of Kel-F was precluded by the tendency for the sample to be blown out of the electrode.

Even though further refinement of the experimental conditions could be envisaged the present conditions were considered to be satisfactory for analytical purposes.

PREPARATION OF THE STANDARD SAMPLES—

The lack of suitable graphite samples with a known concentration of boron led us to prepare synthetic standard samples from boron-free graphite powder* and boron carbide powder (150 mesh). By subsequent dilution with graphite powder a series of samples was

* Ringsdorff Werke GmbH, RWO grade.

prepared from a sample containing 1000 p.p.m. of boron so as to cover the concentration range from 0·1 to 5 p.p.m.

The samples were thoroughly mixed in plastic vials for 30 minutes with a mechanical mixer (Mixer Mill, Spex Industries, Metuchen, N.J., U.S.A.). Each sample (10 g) was analysed by the curcumin colorimetric method, after ashing in the presence of calcium oxide followed by fusion with sodium carbonate and distillation with methanol.[15] The mean value of five determinations on each sample was used as the certificate value for the prepared standard.

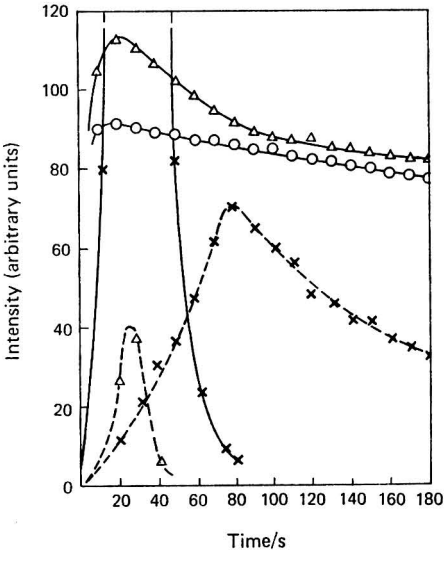

Fig. 4. Distillation curves of boron from graphite samples. Sources of boron: ——, boron oxide; ----, boron carbide. Sources of fluorine: ×, Kel-F; △, copper(II) fluoride; and ○, sodium fluoride

CHOICE OF THE INTERNAL STANDARD—

A critical survey of the elements that it is possible to use as the internal standard was made, taking into account the need to add a compound whose behaviour with fluorine was similar to that of boron carbide. Of course, it was considered important that the spectrum of the selected element should exhibit some lines in the region of the boron lines and that the amount added should be sufficiently high compared with the amount of it that may already be present in the sample.

Silicon seemed to be the most attractive choice because of its high reactivity with fluorine; moreover, the silicon spectrum exhibits several lines in the wavelength region of 250·0 nm, and a concentration of silicon of 500 p.p.m. is required for recording the line at 245·214 nm at a suitable intensity level.

In order to check the usefulness of the selected compound, twelve samples, each containing 100 p.p.m. of boron, 500 p.p.m. of silicon and 1 per cent. of Kel-F, were excited under the conditions described except that the excitation times were varied. The shortest time was 15 s and the longest 180 s, with time increments of 15 s from one sample to the next. For each recorded spectrum the intensity ratio between the boron and the silicon lines was measured, taking into account the density of the background.

These experiments showed that although the rate of evaporation of silicon was slightly higher than that of boron during the initial period of excitation, a fairly constant intensity ratio could be obtained if the duration of the exposure was from 100 to 150 s. Because 15 s are required to reach the decomposition temperature of the Kel-F, an excitation time of 150 s and an exposure time of 135 s were selected.

ANALYTICAL PROCEDURE AND PRECISION—

Twelve 400-mg standard samples containing 0·1 to 5 p.p.m. of boron were added to a mixture of silicon dioxide and Kel-F so that the resultant mixture contained 500 p.p.m. of silicon and 1 per cent. of Kel-F. These samples were introduced into the electrodes, gently compressed with a Plexiglass rod and arced under the conditions summarised in Table I. The intensities of the boron 249·773 nm and silicon 245·214 nm lines were calculated in a conventional manner from the emulsion calibration graph drawn from each plate at 250·0 nm. A background correction was always applied to both lines.

TABLE I

EXPERIMENTAL CONDITIONS

Spectrograph	Bausch and Lomb "Research Dual Grating"
Grating	15 000 grooves per inch blazed at 500 nm
Wavelength range	220–320 nm in the 2nd order
Slit width	10 nm
Illumination	Field lens in front of the slit
Optical filter	Corex
Excitation	d.c. arc, 10·8 A
Electrodes—sample (cathode)	See Fig. 1
upper (anode)	3-mm diameter graphite rod
Analytical gap	4 mm
Pre-arc time	15 s
Exposure time	135 s
Emulsion calibration	7-step rotating sector (log R = 0·2)
Plates	Kodak SA 1
Development	4 minutes at 20 °C in Kodak LX 24 developer

The working graph obtained from the mean values of the twelve determinations for each sample showed a pronounced curvature in the lower concentration range caused by the presence of a blank from the electrodes. From the graphical correction for straightening the curve a value of 0·09 p.p.m. of boron was established for this blank.

The standard deviation (1σ) calculated on the twelve determinations for each sample ranged from ± 11 per cent. at the 0·1 p.p.m. level to ± 8 per cent. at the 5 p.p.m. level of boron.

It must be pointed out that the concentration of 0·1 p.p.m. of boron does not represent the analytical limit of sensitivity of the method described. However, a rather high blank value from the electrodes used at present does not permit determinations to be carried out at lower concentrations. To substantiate the validity of the proposed method a series of five graphite samples of different origin was analysed in triplicate by the described procedure as well as by colorimetry. The results (Table II) showed an agreement within ± 20 per cent. and ± 15 per cent., respectively, for the two procedures.

TABLE II

COMPARISON BETWEEN SPECTROGRAPHIC AND CURCUMIN COLORIMETRIC RESULTS FOR THE ANALYSIS OF GRAPHITE

Graphite sample	Boron concentration*: By optical spectroscopy, p.p.m.	Boron concentration*: By colorimetry, p.p.m.
BA	0·24 ± 0·05	0·26 ± 0·04
A2	0·5 ± 0·09	0·68 ± 0·08
C6	0·65 ± 0·12	0·62 ± 0·09
KM7	0·75 ± 0·12	0·58 ± 0·07
Pechiney	0·34 ± 0·08	0·27 ± 0·05

* Results are given as a mean value of three determinations.

CONCLUSIONS

It has been shown that a fluorination process is effective for the extraction of boron present in graphite in the form of boron carbide (B_4C); the thermal decomposition of Kel-F powder provides a simple method of supplying the required gaseous fluorine to the sample.

A d.c. arc discharge can be successfully used for the excitation of the evolved vapours as well as for the decomposition of the Kel-F provided that the temperature of the sample is kept sufficiently low. Although a complete extraction of the boron could not be achieved, the method has a sensitivity comparable with that claimed for more sophisticated analytical procedures and is superior, particularly when the speed of analysis and the precision are of importance.

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The Analysis of Dental Amalgams by X-ray Fluorescence, by Using a Solution Technique

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A simple and comparatively rapid method is described for the analysis of dental amalgams that have the following range of concentrations in per cent. w/w: mercury 30 to 70, silver 20 to 50, tin 5 to 15, copper 0·5 to 1·5 and zinc up to 0·5. Mercury, silver, copper and zinc are determined in acidic solution after tin has been separated as metastannic acid and determined gravimetrically. By using a 0·5-g sample, precisions of ±0·26, ±0·20, ±0·04 and ±0·04 per cent. (2σ) are obtained on the mercury, silver, copper and zinc determinations, respectively.

SEVERAL methods have been published for the analysis of dental amalgams, and in many of these methods the mercury content is derived by difference after the chemical determination of silver, tin, copper and zinc.[1] Some workers have determined mercury by methods based upon evaporation in a closed system,[2] and have reported high precisions. A paper by Ryge[3] describes a procedure for the determination of mercury by X-ray fluorescence spectroscopy by using solid specimens, and gives accuracies to the nearest 1 per cent. An improved accuracy of ±0·30 per cent. (2σ) of mercury is obtained by using the method described below.

The method was developed primarily for the determination of mercury, but a complete analysis of the amalgam can be obtained with the expenditure of very little extra time and effort if desired. It involves the X-ray fluorescence determination of mercury, silver, copper and zinc in solution, after tin has been separated as metastannic acid and determined gravimetrically by a standard procedure.[4]

Previous experience with X-ray fluorescence solution techniques has enabled a standard procedure, with a sulphuric acid - phosphoric acid solution, to be used. It is based upon a procedure described in a BISRA report[5] dealing with the analysis of highly alloyed steels, and has been found satisfactory for many ferrous and non-ferrous alloys. Preliminary tests were therefore conducted with the object of using this standard technique for the analysis of dental amalgams. Initial dissolution of the amalgam was effected with nitric acid, which resulted in the quantitative precipitation of tin as metastannic acid. After removal of the metastannic acid precipitate, it was hoped to make the normal addition of the sulphuric acid - phosphoric acid mixture, and to use the resulting solution for the X-ray fluorescence determination. However, the solutions were unstable with respect to the precipitation of salts, especially if allowed to stand after the final dilution to a standard volume, and were therefore unsuitable for X-ray fluorescence analysis. Satisfactory solutions were finally obtained when sulphuric acid was added without phosphoric acid.

In the original method devised for the determination of mercury, it was found convenient to prepare calibration solutions from standard amalgams, which were readily made by mixing mercury with a commercial alloy of silver, tin, copper and zinc (the standard dental practice for preparing amalgams). While this technique was completely satisfactory for the determination of mercury alone, slight variations in the composition of the commercial alloy made the calibrations for silver, copper and zinc uncertain, and standard solutions of these elements are therefore recommended for accurate calibration of these constituents (Method B). Method A is recommended when a mercury determination alone is required.

PREPARATION OF SOLUTIONS FOR X-RAY FLUORESCENCE ANALYSIS—

Two methods for preparing calibration solutions are available, and are given in detail below. Amalgams of unknown composition are prepared for analysis by weighing 0·50 g into a beaker, and following the procedure given in Method A.

Method A—In this method mercury and a silver - tin - copper - zinc alloy are used.

Prepare standard amalgams by transferring the appropriate amount of mercury and the commercial alloy (total weight 0·50 g) into a beaker, and mixing them with a glass rod

to produce an amalgam. Slowly dissolve the amalgam in 10 ml of nitric acid (sp. gr. 1·42), and evaporate the resulting solution to a paste. After cooling, add 50 ml of water and gently boil the solution to re-dissolve precipitated salts. Allow the solution to stand overnight at room temperature before filtering off the metastannic acid precipitate on a paper-pulp pad and washing it with 2 per cent. v/v nitric acid solution. Ignite the residue at 800 °C in a silica crucible and, after cooling in a desiccator, weigh it as tin(IV) oxide.

To the filtrate add 10 ml of 15 per cent. v/v sulphuric acid solution and evaporate to fumes. After cooling, dilute with water and heat the mixture gently to re-dissolve precipitated salts. Cool and dilute to 50 ml in a standard flask.

Method B—In this method standard solutions are used.

Prepare two stock solutions: (*i*) containing 10 g of mercury per litre of 10 per cent. v/v nitric acid solution; and (*ii*) containing 7·2 g of silver, 0·24 g of copper and 0·06 g of zinc per litre of 10 per cent. v/v nitric acid solution.

Mix suitable volumes of the two stock solutions to provide calibration solutions of similar compositions to those prepared by Method A. For example, 30 ml of solution (*i*) *plus* 20 ml of solution (*ii*) will give a solution containing the equivalent of a 0·5-g sample of an alloy of composition: mercury 60, silver 28·8, tin 10·0, copper 0·96 and zinc 0·24 per cent., after removal of tin. The final solution for X-ray fluorescence analysis is completed as for Method A from "add 10 ml of 15 per cent. v/v sulphuric acid solution. . . ."

X-RAY FLUORESCENCE ANALYSIS—

The instrument used for this work was a Philips PW1220 semi-automatic X-ray fluorescence spectrometer with operating conditions as shown in Table I.

TABLE I

INSTRUMENTAL CONDITIONS FOR X-RAY FLUORESCENCE ANALYSIS

Excitation	Chromium target X-ray tube, 80 kV, 24 mA
Crystal	Lithium fluoride
Detectors	Scintillation and flow proportional
Counting time	200 s per element
Collimator	160 μm
Spin	The solution cups are rotated during excitation
Analysis peaks	Hg Lα and Ag, Cu and Zn Kα radiation

Transfer with a pipette 15-ml volumes of the amalgam solutions into solution cups fitted with Mylar windows, and record counts at the appropriate 2θ angles for mercury, silver, copper and zinc. No correction for spectrum background is necessary, as it remains constant over the concentration ranges examined, provided that the general rules for accurate X-ray fluorescence analysis are observed.[6]

RESULTS

As bias should be absent because synthetic standardisation was used, the accuracy of the method can be evaluated from the precisions given below. Table II shows the repeatability of the X-ray fluorescence analysis for mercury, silver, copper and zinc with aliquots of a standard stock solution treated as being of unknown composition. Precisions of ±0·26, ±0·20, ±0·04 and ±0·04 per cent. (2σ) obtained for mercury, silver, copper and zinc, respectively, are considered satisfactory for most analyses of this type of alloy.

TABLE II

REPEATABILITY OF A STANDARD SOLUTION ANALYSED AS A SOLUTION OF UNKNOWN COMPOSITION

Element	Calculated analysis, per cent.	Repeat X-ray fluorescence analysis of stock solution, per cent. 1	2	3	4	5	Average, per cent.	Standard deviation (σ)
Mercury	50·00	50·30	49·95	50·15	50·20	50·15	50·15	0·13
Silver	36·00	35·85	35·85	36·10	35·95	35·95	35·94	0·10
Copper	1·20	1·19	1·24	1·21	1·20	1·20	1·21	0·02
Zinc	0·30	0·28	0·32	0·32	0·29	0·31	0·31	0·02

Table III shows the results obtained for the determination of tin on standard amalgams, the theoretical tin contents of which have been calculated from the weights of mercury and silver - tin - copper - zinc alloy used to prepare the amalgams. The differences between the actual and theoretical values confirm the accuracy of $\pm 0{\cdot}20$ per cent. (2σ) for tin, which may be expected by the gravimetric method, although the use of the commercial alloy, which may not be completely homogeneous, can lead to greater discrepancies between actual and theoretical tin values.

Table IV gives the results obtained on a typical series of calibration solutions.

TABLE III

DETERMINATION OF TIN IN STANDARD AMALGAMS PREPARED BY USING MERCURY AND AN ALLOY OF COMPOSITION: Ag 71·2, Sn 25·9, Cu 2·30 AND Zn 0·60 PER CENT.

Alloy	1	2	3	4	5
Calculated tin, per cent.	18·3	15·6	12·6	10·0	7·76
Gravimetric tin, per cent.	18·2	15·7	12·8	10·2	7·63

TABLE IV

COUNTING RATES OBTAINED ON A SERIES OF CALIBRATION SOLUTIONS

Solution number	Mercury, per cent.	Hg Lα/ counts s^{-1}	Silver, per cent.	Ag Kα/ counts s^{-1}	Copper, per cent.	Cu Kα/ counts s^{-1}	Zinc, per cent.	Zn Kα/ counts s^{-1}
1	70·00	7374	21·60	5167	0·72	1529	0·18	461
2	60·00	6435	28·80	6724	0·96	1718	0·24	533
3	50·00	5498	36·00	8282	1·20	1919	0·30	604
4	40·00	4512	43·20	9927	1·44	2119	0·36	686
5	30·00	3462	50·40	11436	1·68	2322	0·42	736

DISCUSSION

The method of analysis described is considered to be satisfactory for the routine analysis of mercury amalgams. The chemistry involved is not complicated, the features that require particular care being the formation and handling of the metastannic acid precipitate, and the accurate control of the sulphuric acid concentration to avoid solubility and counting problems.

In this work a 0·5-g sample was used, but smaller weights can be analysed satisfactorily providing that the solution concentration is maintained at 1 g per 100 ml. X-ray fluorescence analysis of volumes of less than 15 ml requires strict control of the volume transferred by pipette because of errors that may arise if the depth of solution in the sample cup is less than the critical depth[6] required by the given analysis parameters. Alternatively, smaller sample weights can be analysed at lower solution concentrations if preliminary calibration at these concentrations has been carried out. Use of the latter method obviously causes some loss in sensitivity, but precision may possibly be maintained with longer counting times, or by the use of a tungsten anode X-ray tube, or both, which will give higher count-rates for the elements being analysed. The use of small sample weights, however, minimises the advantage of a solution technique for the analysis of these alloys, whose invariable inhomogeneity provides problems in obtaining a representative analytical sample.

Errors in the X-ray fluorescence analysis arising from matrix effects, *e.g.*, absorption and enhancement, are considered not to be a problem because for practical purposes the amalgams may be regarded as binary systems (mercury - commercial alloy), and matrix effects are therefore constant for both standard and unknown solutions. In addition, dilution of the samples in acidic solution further minimises absorption and enhancement effects, so that all calibrations over the ranges of composition investigated exhibit a nearly linear relationship.

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A Non-aqueous Titrimetric Determination of Acid-evolved Carbon Dioxide in Silicate Rocks

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A method is described for the determination of carbonate in silicate rocks at concentrations, expressed as carbon dioxide, down to 0·01 per cent. After treatment with acid the carbon dioxide liberated is absorbed into a dimethylformamide solution and titrated directly with a solution of tetrabutylammonium hydroxide.

CARBONATE is a common constituent of silicate rocks, but the content can be very low; less than 0·1 per cent. (traditionally reported as carbon dioxide) is not unusual. In such circumstances the use of the conventional gravimetric procedure for carbonate determination, whereby the amount of liberated carbon dioxide is measured by the small increase in weight of relatively heavy soda-lime filled tubes, may lead to some inaccuracy. In this laboratory attention has been directed towards alternatives to the gravimetric finish for this determination, which would be especially applicable to the determination of lower levels of carbonate while still using simple apparatus. The determination of carbon dioxide by non-aqueous titrimetry appeared promising. By adapting previous work,[1] particularly that of Jones, Gale, Hopkins and Powell,[2] involving this last technique a suitable method has been developed in which the range of measurement for lower levels of carbon dioxide in the final determination has been extended compared with the gravimetric procedure. With allowance for blank values obtained in the procedure, carbonate in silicate rocks can be determined down to a level of 0·01 per cent. as carbon dioxide from a 1-g rock sample. More sensitive methods would probably require instrumental techniques, such as the gas-chromatographic procedure of Jeffery and Kipping.[3] In the method now proposed, carbon dioxide is liberated from carbonate in the rock sample by heating with dilute orthophosphoric acid, as recommended by Jeffery and Wilson.[4] The evolved gas is carried by nitrogen through absorbents to remove water and hydrogen sulphide and is then absorbed into a 5 per cent. solution of monoethanolamine in dimethylformamide containing thymolphthalein as indicator, as recommended by Braid, Hunter, Massie, Nicholson and Pearce.[5] The carbon dioxide is finally titrated directly with a solution of tetrabutylammonium hydroxide in toluene.

After this method had been adopted for routine use in this laboratory, Sen Gupta[6] described a comparable procedure for the determination of carbon in rocks, stony meteorites and metallurgical samples. This method required an absorption solution of acetone and monoethanolamine, containing a suitable excess of sodium methoxide and phenolphthalein as indicator, to absorb and react with the carbon dioxide evolved, the excess of base being back-titrated with a standard methanolic solution of benzoic acid. Although this procedure has not been investigated, it would appear to offer no particular advantage over the method described in this paper.

EXPERIMENTAL

APPARATUS—

This is shown in Fig. 1.

REAGENTS—

All reagents should be of analytical-reagent grade when possible.

Dilute orthophosphoric acid—Dilute 1 volume of orthophosphoric acid (sp.gr. 1·75) with 3 volumes of carbon dioxide free water.

Absorption solution—To 10 ml of monoethanolamine in a 200-ml calibrated flask add 4 ml of a 0·1 per cent. solution of thymolphthalein in anhydrous methanol and dilute to volume with dimethylformamide.

Titrant—Dilute 40 ml of commercially available, approximately 0·1 N tetrabutylammonium hydroxide solution to 200 ml with toluene.

Standard sodium carbonate solution—Dissolve 1·2045 g of sodium carbonate, previously heated at 260 to 270 °C for 30 minutes to free it from moisture, in carbon dioxide free water and dilute to 500 ml (1 ml of this solution is equivalent to 1 mg of carbon dioxide).

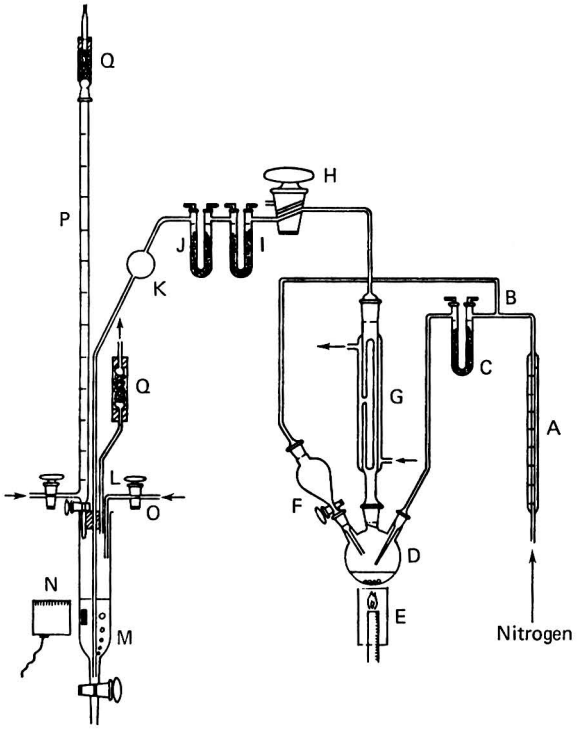

Fig. 1. Diagram of the apparatus. A, gas-flow meter, 0 to 450 ml min^{-1}; B, T-piece tube; C, absorption tube filled with soda-asbestos, 10 to 14 mesh; D, reaction flask, 3-neck, 100 ml (Quickfit FR100/35/11A); E, micro-scale Bunsen burner with shielded flame; F, tap funnel, 50 ml (Quickfit D2/11); G, condenser (Quickfit C 5/13); H, two-way tap; I, absorption tube filled with silica gel, self-indicating, 6 to 20 mesh; J, absorption tube, entrance half-filled with Anhydrone (dried magnesium perchlorate), 14 to 22 mesh, and exit half-filled with a mixture of equal parts of Anhydrone and copper(II) phosphate; K, safety trap, a glass bulb approximately 40 mm in diameter; L, stainless-steel delivery tubes, outside diameter approximately 3 mm; M, absorption vessel, a 100-ml cylindrical form dropping funnel, the open top being closed by a rubber bung through which pass the burette jet, delivery tubes and exit tube for the carrier gas and which is kept away from contact with non-aqueous solutions; N, magnetic stirrer (Grant Instruments, Type MSI) with polythene-covered stirring rod approximately 13 mm long; O, delivery tube connected to reservoir of absorption solution; P, 10-ml microburette fitted with side-arm connected to reservoir of titrant; and Q, guard tubes filled with soda-asbestos, 10 to 14 mesh

Notes—Taps in contact with non-aqueous solutions are of glass-impregnated PTFE. Flexible connections in contact with non-aqueous solutions are of polythene. All ground-glass joints except those containing taps are sealed with PTFE sleeves

PROCEDURE—

Weigh accurately 1 g of the powdered rock sample (previously ground to pass a 90-mesh sieve) into the reaction flask, add 2 or 3 glass beads (approximately 5 mm in diameter) to promote dispersion and 5 ml of water to cover the sample, and incorporate the flask into the apparatus. Add 20 ml of dilute orthophosphoric acid to the funnel, its tap being shut, and close the funnel with a cone adaptor connected to the nitrogen flow at the inlet side of the reaction flask to equalise the pressure.

Rinse the burette by alternately filling it and letting the titrant flow back into its reservoir, then fill the burette and its jet. Connect the absorption vessel into the assembly and to the vessel add about 25 ml of absorption solution from its reservoir. Flush out traces of carbon dioxide from the apparatus by passing nitrogen through it, first at a rate of about 400 ml min^{-1} for 20 minutes with the two-way tap open to the air, and then at a flow-rate of about 40 ml min^{-1} for 40 minutes with the two-way tap turned to connect with the absorption section of the apparatus. Stir the absorption solution continuously with the magnetic stirrer as the gas bubbles through. After flushing out, add sufficient titrant dropwise from the burette to turn the indicator in the absorption solution a definite blue colour. Continue the nitrogen flow for a further 10 minutes to ensure that the apparatus has been freed from carbon dioxide, shown by no reduction in intensity of the indicator colour, and note the burette reading. Check that the condenser water is flowing, then add the acid from the tap funnel to the reaction flask and close the tap. Heat the reaction flask with a small flame shielded from draughts and gently boil the contents. Adjust the flow of the nitrogen carrier gas to ensure steady, slow bubbling through the absorption solution with continued stirring by the magnetic stirrer. After boiling for 90 minutes, titrate the absorption solution until the indicator shows the same permanent blue colour as before. Determine the blank value for the determination by repeating the procedure under the same experimental conditions without the rock sample and using another portion of absorption solution.

Standardise the titrant by repeating the procedure under the same experimental conditions except for the addition of 5·00 ml of standard sodium carbonate solution to the reaction flask instead of the rock sample. Deduct the blank titration value from both sample and standardisation titration values in order to calculate the percentage of carbon dioxide in the sample.

RESULTS AND DISCUSSION

Results of applying the procedure to samples of a variety of silicate rocks are shown in Table I, where they are compared with those obtained from the same samples when using a conventional gravimetric finish. The gravimetric results were obtained by connecting a weighed soda-lime absorption tube, the exit end of which was packed with a short section of Anhydrone, immediately after the copper(II) phosphate - magnesium perchlorate tube in place of the absorption vessel. The reaction flask and condenser were flushed free from carbon dioxide, and the procedure was carried out in a manner similar to that described. The increase

TABLE I

COMPARISON OF THE RESULTS OF ANALYSIS FOR THE CARBONATE CONTENT OF SILICATE ROCK SAMPLES BY THE PROPOSED METHOD AND THE CONVENTIONAL GRAVIMETRIC PROCEDURE

Sample	Carbon dioxide found by— procedure described, per cent.	gravimetric method, per cent.
Biotitic green schist	0·78, 0·78, 0·77	0·77, 0·76
Granitised shale	0·02, 0·02, 0·02	0·05, <0·05, <0·05
Olivine basalt	0·13, 0·13, 0·14	0·14, 0·12, 0·12
Feldspar quartz porphyry	0·10, 0·11, 0·10	0·10, 0·05, 0·06
Rhyolite	0·06, 0·06, 0·06	0·07, 0·12, 0·10
Lamprophyre	3·20, 3·20	3.14, 3·17
Metamorphised basic lava	0·01, 0·01, 0·01	<0·05, <0·05
Pyroxenite	0·31, 0·31, 0·31	0·27, 0·28
Monzonite	0·05, 0·05	0·07, <0·05
Pegmatite	<0·01, <0·01	<0·05, <0·05

in weight of the soda-lime tube was compared with that of a similar tared tube and corrected for a gravimetric blank value determined in a similar way. The titrimetric finish gave a blank value of about 0·08 ml of titrant, equivalent to less than 0·01 per cent. of carbon dioxide, while the gravimetric finish gave a blank value of about 0·0005 g, equivalent to 0·05 per cent. of carbon dioxide, these percentages being expressed with reference to 1 g of sample. These results indicate the lower limit of detection of the titrimetric procedure and its better reproducibility at lower levels of carbonate content.

Some silicate rocks yield their carbon dioxide after a shorter period of boiling with dilute acid than that which is recommended. However, this time has been found to be generally applicable, except for samples that contain such minerals as scapolite, which are difficult to decompose with acid. Although the standardisation of titrant as described is preferred as it indicates the reproducibility of the procedure, the time required for a determination can be reduced by standardising the titrant directly with a known weight of pure organic acid introduced into the neutralised absorption solution. No blank value is deducted from the titration obtained in these circumstances. Benzoic acid has been proposed for this purpose,[2] but pure lauric acid (about 25 mg accurately weighed is a convenient amount) is recommended on account of its higher molecular weight, greater convenience in handling and good solubility in the absorption solution, in which respects it has been found preferable to either myristic or stearic acid.

The method can also be applied with advantage when non-carbonate carbon in silicate rocks has to be determined after generating carbon dioxide by a wet-oxidation procedure with an orthophosphoric acid - chromic acid mixture.[4]

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The Microbiological Assay of the "Essential" Amino-acids in Compound Feedingstuffs

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A re-examination has been made of the methods in current use for the microbiological assay of the "essential" amino-acids arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine, and improvements in basal media and methods of hydrolysis are suggested.

CONTINUING experience of microbiological assays, particularly those of "essential" amino-acids, has led the author to introduce into methods of extraction and in basal media modifications that are found to be improvements. The modified basal media, except that for tryptophan, are based on the formulations of Steele, Sauberlich, Reynolds and Baumann,[1] which are available in dehydrated form from Difco Laboratories. These modifications can easily be incorporated when the media are reconstituted. The Difco basal medium for tryptophan is based on the formulation of Greene and Black[2] but the author has found that his formulation, based on a previously described formulation,[3] is more flexible and sensitive.

The author was not fully satisfied with the method described earlier[3] for the extraction of tryptophan and has since discovered that the method of extraction described in this paper in which the powerful proteolytic enzyme pronase, isolated from *Streptomyces griseus*, is used is superior.

EXPERIMENTAL

ORGANISMS AND MAINTENANCE—

Three organisms are used for all these assays, *Streptococcus faecalis* R (for the assay of all amino-acids with the exception of phenylalanine and tryptophan), *Leuconostoc mesenteroides* P60 (for the assay of phenylalanine) and *Lactobacillus plantarum* (for the assay of tryptophan). The organisms are maintained as agar stab cultures.[3]

The basal medium for the assay of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine has the composition shown in Table I.

After mixing, adjust the pH of the medium to 6·8 with 30 per cent. sodium hydroxide solution, with bromothymol blue as external indicator, or electrometrically, and make the volume up to 500 ml with water.

The basal medium for the assay of tryptophan has the composition shown in Table II. It is possible to use *Lactobacillus plantarum* for the assay of tryptophan by using this medium, which omits tryptophan and includes an excess of nicotinic acid.

After mixing, adjust the pH of the medium to 6·8 with 30 per cent. sodium hydroxide solution, with bromothymol blue as external indicator, or electrometrically, and make up to 500 ml with water.

ASSAY PROCEDURE—

A separate standard graph *must* be established for each assay. In all instances the L-enantiomorph of the amino-acid and *not* the DL-isomer should be used for the construction of the standard graphs. The assumption that the DL-enantiomorph has exactly 50 per cent. of the activity of the L-acid is not correct in many instances, *e.g.*, methionine and tryptophan.[5]

The amounts of these amino-acids used to establish the standard graphs are given in Table III.

The standard solutions of the amino-acids, with the exception of lysine and tryptophan, should be prepared immediately prior to the setting up of an assay. Lysine and tryptophan standard solutions will maintain their activity for 7 days at a temperature of not greater than 4 °C.

TABLE I

BASAL MEDIUM FOR ASSAY OF ARGININE, HISTIDINE, ISOLEUCINE, LEUCINE, LYSINE, METHIONINE, PHENYLALANINE, THREONINE AND VALINE

Amino-acids	Amount/g	Other ingredients	Amount/g
DL-Alanine	0·40	Glucose	20·0
L-Arginine hydrochloride*	0·25	Sodium acetate	20·0
L-Asparagine	0·40	Ammonium chloride	3·0
L-Aspartic acid	0·10	Dipotassium hydrogen orthophosphate	5·0
L-Cystine	0·10	Sodium chloride	5·0
L-Glutamic acid	0·30	Adenine	$10·0 \times 10^{-3}$
L-Glutamine	0·10	Guanine	$10·0 \times 10^{-3}$
Glycine	0·10	Uracil	$10·0 \times 10^{-3}$
L-Histidine hydrochloride*	0·06	Xanthine	$10·0 \times 10^{-3}$
Hydroxylysine†	0·010	Thiamine hydrochloride	1000×10^{-6}
DL-Isoleucine*	0·25	Pyridoxine hydrochloride	1000×10^{-6}
DL-Leucine*	0·25	Pyridoxal hydrochloride	500×10^{-6}
Lysine hydrochloride*	0·25	Pyridoxamine dihydrochloride	500×10^{-6}
DL-Methionine*	0·10	Calcium *d*-pantothenate	1000×10^{-6}
DL-Phenylalanine*	0·10	Riboflavin	500×10^{-6}
L-Proline	0·10	Nicotinic acid	2000×10^{-6}
DL-Serine	0·10	*p*-Aminobenzoic acid	100×10^{-6}
DL-Threonine*	0·20	Biotin	$4·0 \times 10^{-6}$
DL-Tryptophan	0·10	Folic acid	100×10^{-6}
L-Tyrosine	0·10	Inorganic salt solution‡	5·0 ml
DL-Valine*	0·25	Water	to 500 ml

* Amino-acid to be assayed omitted from the medium.

† Addition essential for the assay of lysine.[4]

‡ Inorganic salt solution—Dissolve 10 g of magnesium sulphate ($MgSO_4.7H_2O$), 0·5 g of manganese(II) sulphate ($MnSO_4.4H_2O$) and 0·1 g of anhydrous iron(III) chloride in 250 ml of water and add 5 drops of concentrated hydrochloric acid. This solution will maintain its activity indefinitely at room temperature.

TABLE II

BASAL MEDIUM FOR ASSAY OF TRYPTOPHAN

Ingredient	Amount/g	Ingredient	Amount/g
Glucose	20	Ammonium chloride	3·0
Sodium acetate	20	Thiamine hydrochloride	1000×10^{-6}
Vitamin-free Casamino Acids (Difco)	10	Nicotinic acid	2000×10^{-6}
L-Cystine	0·1	Calcium *d*-pantothenate	1000×10^{-6}
Adenine	$10·0 \times 10^{-3}$	Pyridoxine hydrochloride	1000×10^{-6}
Guanine	$10·0 \times 10^{-3}$	Riboflavin	500×10^{-6}
Uracil	$10·0 \times 10^{-3}$	*p*-Aminobenzoic acid	100×10^{-6}
Xanthine	$10·0 \times 10^{-3}$	Biotin	$4·0 \times 10^{-6}$
Dipotassium hydrogen orthophosphate	2·5	L-Tyrosine	0·1
Potassium dihydrogen orthophosphate	2·5	L-Phenylalanine	0·2
Sodium chloride	5·0	Inorganic salt solution*	5·0 ml
		Water	to 500 ml

* For the composition of this solution, see the footnote to Table I.

TABLE III

AMOUNTS OF AMINO-ACIDS USED TO ESTABLISH STANDARD GRAPHS

Amino-acid	Amount/μg
L-Arginine hydrochloride (calculated as free acid)	10 to 50
L-Histidine hydrochloride (calculated as free acid)	5 to 25
L-Isoleucine	10 to 50
L-Leucine	10 to 50
L-Lysine (calculated as free acid)	50 to 250
L-Methionine	10 to 50
L-Phenylalanine	10 to 50
L-Threonine	10 to 50
L-Tryptophan	1 to 10
L-Valine	10 to 50

Set up the test preparation at three concentrations by taking 1, 2 and 4 ml of the test hydrolysate, making the volumes up to 5 ml with water and adding 5 ml of basal medium to each solution. Add the water and basal medium to each tube with a 5-ml calibrated B-D Cornwall Luer-Lok syringe, and the standard and test solutions with a 2-ml calibrated instrument. This will be found to be a more accurate and expeditious method than addition with a manual pipette.

Set up all concentration levels of the standard and test solutions in triplicate, cap the tubes with aluminium thimbles, with coloured thimbles for the blanks, sterilise by steaming for 30 minutes, cool in the dark and inoculate.

PREPARATION OF INOCULUM—

Make a transfer from a stab culture to a bottle of liquid stock medium[3] and incubate it for 18 to 20 hours at 30 °C, centrifuge aseptically, and suspend the deposit in 10 ml of sterile distilled water. Use this suspension directly for the assay of lysine by adding 1 drop of the inoculum with a sterile pipette to each tube, but omit to inoculate one of the blanks, so that it can be used to set the colorimeter. For the remaining assays, with the exception of phenylalanine, dilute the initial suspension with sterile distilled water (1 + 99) and add 1 drop of this final suspension to each tube, but again omit to inoculate one of the blanks. For the assay of phenylalanine with *Leuconostoc mesenteroides* P60, dilute the initial inoculum with sterile distilled water (5 + 95) and again add 1 drop of this final diluate to each tube.

Incubate the tubes at 35 °C for 48 hours, except for the assay of tryptophan with *Lactobacillus plantarum*, which should be incubated at 30 °C. After incubation, steam the tubes for 15 minutes, cool and determine the response nephelometrically. If the response is to be determined acidimetrically, the tubes should be incubated for 72 hours.

PREPARATION OF HYDROLYSATES FOR ASSAY—

Total acids (with the exception of tryptophan)—Weigh an appropriate amount of material and hydrolyse it with 50 ml of 3 N hydrochloric acid for not less than 8 to 10 hours at 15 p.s.i., cool, add 2 ml of 2·5 M sodium acetate solution and adjust the pH to 4·8 with 40 per cent. sodium hydroxide solution, with bromocresol green as external indicator, or electrometrically. Make the hydrolysate up to an appropriate volume, filter and then shake it in a separating funnel with diethyl ether. Re-adjust the pH of a portion of the solution to 6·8 and make it up to an appropriate volume for assay. Material containing appreciable amounts of lipids should be given a preliminary extraction with light petroleum in a Soxhlet extractor.

Tryptophan—Weigh accurately sufficient material to contain 1 g of protein into a 250-ml conical flask, add 50 ml of 0·2 N hydrochloric acid and autoclave the material at 15 p.s.i. for 30 minutes. This preliminary process denatures the proteins, destroys any trypsin inhibitors that may be present and hydrolyses starch, which would otherwise interfere with extraction and cause filtration difficulties later. After cooling, adjust the pH to 2·0 *electrometrically*, add 50 mg of (three-times crystallised) pepsin (1 to 60 000 potency) and incubate the flask overnight at 37 °C. Add 5 ml of a 0·1 M calcium chloride solution (1·11 g per 100 ml) then 1·2 g of dipotassium hydrogen orthophosphate and adjust the pH *electrometrically* to 8·0 with sodium hydroxide solution. Add 50 mg of pronase and incubate the mixture under a thin layer of sulphur-free toluene for 24 hours at 37 °C. Re-adjust the pH of the hydrolysate *electrometrically* to 8·4 with sodium hydroxide solution, add 20 mg of (three-times crystallised) trypsin and incubate the mixture at 40 °C for a further 24 hours. At the end of the incubation period steam the hydrolysate for 15 minutes to stop enzyme activity, cool and adjust the pH to 4·5 with hydrochloric acid, using bromocresol green as external indicator, make the volume up to 100 ml and filter the solution. Shake the enzymic hydrolysate twice with diethyl ether to remove indole, anthranilic acid and any lipid material that will interfere in the assay. Preliminary extraction with light petroleum in a Soxhlet extractor is desirable with material that has a high lipid content, *e.g.*, soya meal and wheat germ. Adjust the pH of a portion of the hydrolysate to 6·8 and make it up to a suitable volume for assay.

It is essential for a successful assay that the order of addition of the enzymes, pepsin, pronase and trypsin is followed strictly and that the pH of the hydrolysate is adjusted accurately at each stage of hydrolysis.

CALCULATION—

For calculating the results of these assays for routine purposes in the laboratory the older method of direct reading from the standard graph will be found to be satisfactory. The method has the advantage of being rapid and showing immediately whether an assay is valid or not. Provided that the values found at the three concentration levels recommended here do not differ among themselves by more than ± 10 per cent., the mean value will give the amount of amino-acid present in the test sample. A regular "drift," in either the upward or the downward direction, in the values of the different concentration levels indicates the presence of interfering substances and the assay must be regarded as invalid.

As the standard graphs are invariably curvilinear for all these amino-acids, it is strongly advised that after approximate values have been calculated by "direct" reading from the standard graph, exact values should be determined by the Wood[6] "log - log" procedure. If fiducial limits are required, they should be calculated by computer. The author now invariably calculates assay results by computer because of the added refinement of obtaining fiducial limits, and considers that fiducial limits greater than $\pm 6{\cdot}0$ per cent. are unacceptable. With well conducted assays the fiducial limits are generally between $\pm 2{\cdot}5$ and $\pm 3{\cdot}0$ per cent.

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An Improved Colour Reagent for the Determination of Blood Glucose by the Oxidase System

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A method involving the use of 4-aminophenazone as a colour coupler with sulphonated 2,4-dichlorophenol is described for determining the hydrogen peroxide produced from glucose with glucose oxidase.

The sensitivity of the method is such that 20 μg of glucose in a final volume of 4 ml give an optical density of 0·61 at 515 nm with 10-mm cells, corresponding to a molecular absorption of 22 000.

COLORIMETRIC techniques for the determination of glucose by the oxidase method are based on the following reactions in aqueous media—

$$\text{Glucose} + O_2 + \text{glucose oxidase} + H_2O \rightarrow \text{gluconic acid} + H_2O_2 \quad .. \quad (1)$$

$$H_2O_2 + \text{oxygen acceptor} + \text{peroxidase} \rightarrow \text{coloured product (oxidised acceptor)} \quad .. \quad .. \quad (2)$$

The oxygen acceptors originally used were benzidine, *o*-tolidine and *o*-dianisidine. Because of the carcinogenic nature of these three compounds, alternative acceptors are now being sought. One such acceptor system is phenol - 4-aminophenazone, which gives a purple colour in the presence of hydrogen peroxide and peroxidase.[1] This system has been in use in this laboratory for 2 years and has been found to be a most reliable, convenient and trouble-free reagent for the determination of glucose in blood by both manual and automated techniques.

The manual method has adequate sensitivity, the molar absorption of the colour formed being about 5100 according to results given in the original paper. It is therefore possible to use 0·1-ml blood samples. For paediatric work it would be an advantage to have a more sensitive method while retaining the advantages of the phenol - 4-aminophenazone system, a system in which only two solutions are used and which gives a stable and reproducible colour.

An investigation into the chromogenic properties of various substituted phenols established that 2,4-dichlorophenol when sulphonated gave a colour reaction with 4-aminophenazone, which is about four times as sensitive as the phenol - 4-aminophenazone system. By using sulphonated 2,4-dichlorophenol a method has been developed for the determination of glucose in blood.

METHOD

REAGENTS—

Glucose standards, 0 to 400 mg per 100 ml in 0·2 per cent. w/v benzoic acid solution.

Stock solution of sulphonated 2,4-dichlorophenol, 2 per cent.—For the preparation of this reagent, use the commercially available 2,4-dichlorophenol, the quality of which varies. If the solid is not white, it should be re-distilled from an all-glass apparatus with an air condenser.

To 10 g of 2,4-dichlorophenol add 20 ml of concentrated sulphuric acid and heat the mixture on a water-bath at 100 °C for 5 hours. Cool, add 400 ml of distilled water and neutralise the mixture with 10 N sodium hydroxide solution. Add 10 ml of N sulphuric acid and make the final volume up to 500 ml with distilled water. Filter, if necessary. This solution will keep indefinitely.

Protein precipitant solution—Dissolve 5 g of sodium tungstate, $Na_2WO_4.2H_2O$, 5 g of disodium hydrogen orthophosphate, Na_2HPO_4, and 4·5 g of sodium chloride in sufficient water to make 200 ml of solution. Add 250 ml of the 2 per cent. sulphonated 2,4-dichlorophenol solution and adjust the pH to 3 by adding N hydrochloric acid. Make the final volume up to 500 ml with distilled water. This solution keeps for at least 2 months.

Colour reagent—Dissolve 3 g of dipotassium hydrogen orthophosphate, K_2HPO_4, and 0·3 g of sodium azide in 290 ml of distilled water. Add 5 ml of Fermcozyme 653 AM* and 5 ml of 0·1 per cent. peroxidase, type 1, RZ approximately 0·6.† Finally, add 100 mg of 4-aminophenazone and stir until it has dissolved. This solution keeps for at least 2 months at 4 °C.

PROCEDURE—

Add 0·05 ml of blood to 4·95 ml of protein precipitant solution. Leave the suspended cells to stand for 5 minutes before centrifuging the solution. Transfer 1 ml of the clear fluid to a $\frac{5}{8}$-inch tube (U). Place 1 ml of protein precipitant solution in a second tube (B) and place 1 ml of standard, comprising a mixture of 0·05 ml of glucose standard solution (150 mg per 100 ml) and 4·95 ml of protein precipitant solution, in a third tube (S). Add 3 ml of colour reagent to each tube and incubate all the tubes at 37 °C for 15 minutes. Read the optical densities of S and U against B at 515 nm by using 10-mm cells.

$$\text{Blood glucose/mg per 100 ml} = \frac{\text{Optical density of U}}{\text{Optical density of S}} \times 150$$

The optical density of the 150 mg per 100 ml standard is about 0·45 (Table I). The colour is stable for 30 minutes after colour development and the subsequent fading of the colour is of the order of 10 per cent. h^{-1}.

TABLE I

RESULTS FOR CALIBRATION GRAPH

Number of observations	Glucose/mg per 100 ml	Optical density		
		Range	Mean	Standard deviation
10	50	0·145 to 0·151	0·149	0·001 77
10	100	0·298 to 0·303	0·301	0·001 55
10	200	0·600 to 0·617	0·609	0·005 75
10	300	0·920 to 0·927	0·921	0·002 95
10	400	1·250	1·250	—

Successive replicate analyses.

One millilitre of protein precipitant solution containing 0·01 ml of standard glucose solution *plus* 3 ml of colour reagent; incubation time 15 minutes at 37 °C; wavelength 515 nm; and cells with a 10-mm light path.

RESULTS AND DISCUSSION

It is stated by Henry[2] that the glucose oxidase reaction is not stoicheiometric because of the limiting rate of mutarotation. However, crude enzyme extracts such as Fermcozyme contain glucomutarotase and hence it is unlikely that the mutarotation rate is responsible for non-stoicheiometry. As the colour reaction with added hydrogen peroxide is almost instantaneous, any method that involves the use of a fading colour reaction will cause the colour produced by this hydrogen peroxide to fade more rapidly than the colour produced by the hydrogen peroxide formed in the oxidase reaction, the latter reaction being relatively slow. In the proposed reaction the colour developed is stable for sufficient time to minimise this effect, and hence the reaction is taken to completion and the actual yield of hydrogen peroxide according to equation (1) is stoicheiometric (Table II). A further factor may be the presence in the proposed reagent of sodium azide, a powerful catalase inhibitor. Crude glucose oxidase extracts contain relatively large amounts of catalase, and the destruction of hydrogen peroxide by catalase may be more marked with an initial high concentration of hydrogen peroxide.

When a known amount of glucose was added to whole blood from which all of the glucose originally present had been removed by dialysis, the recovery was quantitative (Table III). Although glucose oxidase is relatively specific for β-D(+)-glucose, the coupling reaction merely depends on the determination of hydrogen peroxide. This reaction is completely non-specific and many oxidising agents will give a colour with the 2,4-dichlorophenol - 4-aminophenazone system. In blood the only oxidants likely to interfere are peroxides liberated from erythrocytes by mineral acids. With the weakly acidic conditions under which protein precipitation

* Hughes and Hughes Ltd., 12A High Street, Brentwood, Essex.

† Sigma Chemical Co. Ltd., 12 Lettice Street, London, S.W.6. RZ=Reinheitszahl (purity number).

TABLE II

THEORETICAL AND ACTUAL YIELDS OF HYDROGEN PEROXIDE BASED ON EQUATION (1) SHOWING ONE MOLECULE OF HYDROGEN PEROXIDE PRODUCED PER MOLECULE OF GLUCOSE PRESENT

Glucose present/μg	Theoretical yield of H_2O_2/μg	H_2O_2 found/μg	H_2O_2 found as percentage of theoretical
20	3·78	3·67	97
		3·78	100
		3·78	100
		3·78	100
		Mean 3·75	Mean 99·25
10	1·89	1·83	97
		1·85	98
		1·92	102
		1·92	102
		Mean 1·88	Mean 99·75

The hydrogen peroxide used for calibration was the 20-volume solution diluted 1 + 39 with water and standardised with 0·116 N potassium permanganate solution that had been standardised with sodium oxalate. This solution was then diluted 1 + 999 with water and used immediately.

takes place there is no interference from erythrocyte peroxides in the proposed method. Reducing agents may reduce the amount of colour formed by reduction of the hydrogen peroxide. The most likely interfering reducing substances in protein-free blood filtrates are uric acid and creatinine. Uric acid and creatinine, both up to a concentration of 30 mg per 100 ml, when added simultaneously to whole blood prior to protein precipitation have no effect on the results (Table IV).

Ascorbic acid added to whole blood before protein precipitation interferes with colour development; 10 mg of ascorbic acid per 100 ml cause a reduction in colour equivalent to 8·2 mg of glucose per 100 ml. The reduction caused by the presence of 2 mg of ascorbic acid per 100 ml was equivalent to 1·5 mg of glucose per 100 ml. Ascorbic acid is unlikely to be present at concentrations higher than 1 mg per 100 ml in whole blood and it is considered that interference at this level would be too low to be measured (Table IV).

TABLE III

RECOVERY OF GLUCOSE ADDED TO GLUCOSE-FREE BLOOD

Number of observations	Glucose added/mg per 100 ml	Glucose found/mg per 100 ml		Recovery, per cent.	
		Range	Mean	Range	Mean
5	100	98·8 to 99·5	99·2	98·8 to 99·5	99·2
5	200	198·4 to 204·5	201·9	99·2 to 102·3	101
5	300	293·8 to 298·7	296·4	97·9 to 99·6	98·8
5	500	491·0 to 501·3	495·0	98·2 to 100·3	99·0

TABLE IV

EFFECT OF SOME POSSIBLE INTERFERING SUBSTANCES

Number of observations	Glucose found/mg per 100 ml							
	Sample		Sample + 30 mg of creatinine + 30 mg of uric acid per 100 ml		Sample + 10 mg of ascorbic acid* per 100 ml		Sample + 2 mg of ascorbic acid* per 100 ml	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
4	164·9 to 166·2	165·9	164·9 to 166·2	165·9	—	—	—	—
8	48·2 to 50·2	49·5	48·5 to 50·2	49·7	—	—	—	—
4	137·7 to 139·0	138·5	—	—	129·2 to 130·5	129·7	136·7 to 137·4	137·0
4	69·5 to 72·8	71·0	—	—	61·6 to 64·5	63·4	—	—
8	44·9 to 45·6	45·2	—	—	—	—	42·9 to 44·3	43·6

* Samples analysed immediately after addition of ascorbic acid.

Another reported source of error in glucose oxidase methods is inhibition of colour development by the fluoride used to preserve the blood. In the proposed method fluoride not only does not interfere when added to whole blood (Table V), but very large amounts of fluoride and potassium oxalate (2 and 6 mg, respectively) added to 1 ml of the protein-free fluid have no effect on the colour development. We conclude that fluoride is not a poison for either glucose oxidase or peroxidase, and any interference in other methods results from the effect of fluoride on the coupling reaction itself.

TABLE V

EFFECT OF FLUORIDE AND OXALATE

	Glucose/mg per 100 ml			
	Sample		Sample + 500 mg of sodium fluoride + 1500 mg of potassium oxalate per 100 ml	
Number of observations	Range	Mean	Range	Mean
4	217·4 to 219·7	218·5	217·4 to 220·0	218·2
8	60·7 to 64·6	62·6	61·0 to 63·9	62·1

Twenty successive analyses gave a mean result of 149·8 mg of glucose per 100 ml with a range from 148·0 to 151·0 mg per 100 ml and a standard deviation of 0·91 mg per 100 ml, when read on the SP600 spectrophotometer. To eliminate any bias the same solutions were also read on an Analmatic print-out colorimeter and then gave a mean value of 150·2 mg of glucose per 100 ml with a range from 147·7 to 153·1 mg per 100 ml and a standard deviation of 1·37 mg per 100 ml. Successive blood samples were analysed by the proposed method and by the copper reduction method of King and Garner.[3] For 100 specimens the proposed method gave a mean result of 131·0 mg of glucose per 100 ml with a range from 25 to 325 mg per 100 ml. The same specimens analysed by the King and Garner method gave a mean result of 138·0 mg of glucose per 100 ml with a range from 30 to 330 mg per 100 ml (ignoring the sign in each instance).

Statistical analysis of the pairs showed the average difference to be 8·3 mg per 100 ml with a range for the glucose oxidase method from 12 mg per 100 ml above that given by the King and Garner method to 15 mg per 100 ml below it, with a standard deviation of 5·47 mg per 100 ml. A significance *t*-test was applied to the results and as two completely different techniques were deliberately used it is not surprising that, with 99 degrees of freedom, p is less than 0·001.

The difference of 7 mg per 100 ml shown between the mean results of the two methods arises from (*i*) the falsely high results given by the King and Garner method, which are caused by reducing substances such as creatinine and uric acid and by reducing carbohydrates other than glucose, and which are the main cause of the difference, and (*ii*) the slightly low results given by the oxidase method, which are caused by unidentified reducing substances.

CONCLUSION

The method appears to combine the simplicity of the phenol - 4-aminophenazone method, with the advantage that smaller amounts of sample are used in the analysis. The method will be particularly useful when it is difficult to obtain blood samples, for example in paediatric work, or when samples containing low concentrations of glucose are required to be analysed. The system has no advantages in its application to macro-scale automated analyses as the phenol - 4-aminophenazone system is of adequate sensitivity, but it will be useful in micro-scale automated systems, and such a system is at present being devised.

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3-Propyl-5-hydroxy-5-D-arabinotetrahydroxybutyl-3-thiazolidine-2-thione, a Specific Colorimetric Reagent for the Determination of Copper in Water

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The 3-propyl homologue of 3-methyl-5-hydroxy-5-D-arabinotetrahydroxybutyl-3-thiazolidine-2-thione has been prepared and used as a reagent in the colorimetric determination of copper in water. Interference by other metals and by cyanide has been examined; inhibition of complex formation by cyanide appears to be almost quantitative.

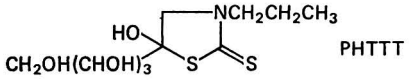
CORBETT[1] has described a sensitive, specific colorimetric reagent for copper, 3-methyl-5-hydroxy-5-D-arabinotetrahydroxybutyl-3-thiazolidine-2-thione (MHATT). The 3-propyl homologue (PHTTT) was prepared for this study because propylamine, being a liquid, is more easily handled than the gaseous anhydrous methylamine, and it was expected that PHTTT would have properties similar to those of MHATT.

PHTTT was prepared by the reaction of carbon disulphide with *N*-propylglucosylamine,[2] and the latter compound by the reaction of propylamine with glucose.[3]

$$CH_2OH(CHOH)_4CHO + CH_3CH_2CH_2NH_2 \longrightarrow CH_2OH(CHOH)_4CH{:}NCH_2CH_2CH_3 + H_2O$$

N-propylglucosylamine

$$CH_2OH(CHOH)_4CH{:}NCH_2CH_2CH_3 + CS_2 \longrightarrow$$



PHTTT

REPARATION OF PHTTT—

Add 9 g of glucose to a mixture of 4·5 ml (3 g) of propylamine and 5 ml of methanol. Heat the mixture in a beaker, with stirring, at a temperature of between 60 and 65 °C for 15 to 20 minutes. A viscous mass is obtained. Add to it 30 ml of hot ethanol and allow it to cool. *N*-Propylglucosylamine crystallises out on allowing the mixture to stand overnight, giving a yield of 11 g. Add 11 g of *N*-propylglucosylamine to a mixture of 7 ml (9 g) of carbon disulphide and 40 ml of ethanol and heat the mixture in a beaker at 60 °C for 20 minutes. Allow it to stand overnight at room temperature and then cool to 2 °C to enable the reaction product to separate. Re-crystallise the latter twice from ethanol - water solution (7 + 3). The final product is a white crystalline solid.

Elemental analyses of the product gave the following results: C 40·2, H 6·4, N 4·9 and S 22·2 per cent.; PHTTT requires C 40·4, H 6·4, N 4·7 and S 21·6 per cent.

PROPERTIES OF PHTTT—

PHTTT is very sparingly soluble in water but is more readily soluble in aqueous ethanolic solution. In solution a yellow complex is formed between copper and PHTTT at pH values higher than 6. The ratio of copper to PHTTT in the complex was shown by both the method of continuous variation and the mole ratio method to be 1:3. Complex formation is complete after 10 minutes and the yellow colour is stable for more than 4 hours within the pH range 6 to 8. At pH values higher than 8 the colour is less stable. For example, at a pH value of 8·6 the colour is stable for 15 minutes. This optimum pH range makes PHTTT useful for the determination of copper in natural waters without any preliminary adjustment of the pH. MHATT, on the other hand, has an optimum pH range of 4 to 7, which does not accommodate the pH range of most natural waters (6 to 8·5).

The absorption maximum of the complex is at 435 nm, at which wavelength the molar extinction coefficient of $Cu(PHTTT)_3$ is 13 900. Beer's law is obeyed at copper concentrations of up to at least 1000 $\mu g\ l^{-1}$ under the conditions used in the test. The range can be extended

by using higher concentrations of PHTTT. The practical lower limit for analytical purposes is 40 $\mu g\ l^{-1}$ of copper if 40-mm cells are used.

Method for the determination of copper in water

Reagent—

PHTTT reagent, 0·15 per cent.—Dissolve 0·15 g of 3-propyl-5-hydroxy-5-D-arabinotetrahydroxybutyl-3-thiazolidine-2-thione in a mixture of 15 ml of ethanol and 15 ml of water. Make the volume up to 100 ml with water.

Procedure—

To 100 ml of the filtered sample of water add 1 ml of 0·15 per cent. PHTTT solution, mix and allow it to stand for 10 minutes. Measure the absorbance of the solution at 436 nm, by using 40-mm cells and water as reference. Read the concentration of copper from a calibration graph prepared from standard copper solutions within the concentration range 0 to 1000 $\mu g\ l^{-1}$ of copper and with pH values within the range 6 to 8.

Ten replicate determinations of copper were made at concentrations of 100 and 1000 $\mu g\ l^{-1}$, each solution being 5×10^{-3} M with respect to sodium hydrogen carbonate solution and at pH 7. Standard deviations of ± 1 and ± 8 $\mu g\ l^{-1}$, respectively, were obtained.

Interferences—

Concentrations of 10 mg l^{-1} of the commonly occurring pollutant metals cadmium, nickel and zinc, and 3 mg l^{-1} of lead (the maximum possible concentration to avoid precipitation), were found neither to form a coloured complex with PHTTT nor to interfere with copper - PHTTT complex formation in tap water containing 200 $\mu g\ l^{-1}$ of copper. Mercury prevented the copper - PHTTT colour development, probably by competing with copper for the PHTTT and forming a colourless complex with it, but it is unlikely that mercury would interfere in copper determinations in practical situations. The degree of mercury interference was found to depend on the sum of the concentrations of mercury and copper. For example, with the specified amount of PHTTT reagent 3 mg l^{-1} of mercury interfered in the determination of 1 mg l^{-1} of copper but not in the determination of 0·2 mg l^{-1} of copper. Mercury interference can be overcome by the addition of excess of PHTTT reagent.

Cyanide solutions were shown to inhibit, almost quantitatively, copper - PHTTT complex formation, presumably because of the high stability of the copper - cyanide complex. This effect, which is illustrated in Table I, has been of use in enabling the concentration of copper - cyanide complexes present in polluted water to be determined.[4]

Other copper-complexing ligands often present in polluted fresh waters, for example carbonate, triphosphate, amino-acids and humic acids, were found not to interfere at the concentrations likely to be encountered.

Table I: Effect of cyanide on copper - PHTTT complex formation

Total copper 100 $\mu g\ l^{-1}$ ($1{\cdot}58 \times 10^{-6}$ M); PHTTT 15 mg l^{-1} (5×10^{-5} M); pH 7·0

Concentration of potassium cyanide/M	Concentration of copper by PHTTT/$\mu g\ l^{-1}$	Concentration of copper as cyanide complex/$\mu g\ l^{-1}$
0	100	0
1×10^{-6}	88	12
4×10^{-6}	56	44
9×10^{-6}	18	82

PHTTT was chosen for use in work to differentiate quantitatively between the chemical states of copper in polluted waters because it was the only reagent that was sufficiently selective. For example, nickel, at the concentrations commonly found in polluted water, formed interfering coloured complexes with all of the many other reagents considered.

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Microdetermination of Mercury in Biological Samples

Part III.* Automated Determination of Mercury in Urine, Fish and Blood Samples

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An extension of the use of the automatic mercury analyser published earlier is discussed. Wet-digestion procedures for blood and fish samples that are also applicable to other types of sample are described. With the standard procedures recommended it is possible to determine mercury concentrations in blood down to about the normal values for unexposed persons, *i.e.*, about 5 ng g^{-1}, and mercury in fish down to the 0·1 μg g^{-1} level, with an acceptable standard error. Comparative studies between our method and the methods of activation analysis and gas chromatography have shown good agreement.

In Part I of this series[1] a manual method was described for the determination of mercury in urine involving wet digestion at room temperature with acidic permanganate solution, reduction of the mercury(II) ions to metallic mercury with tin(II) chloride and final detection of the purged mercury vapour by means of flameless atomic absorption at 253·7 nm. The sensitivity was about 1 ng per sample and the precision was adequate for trace analysis. Also in this previous paper, the influence of some foreign substances on the analysis was discussed. The flameless atomic-absorption technique is the most attractive tool for the microdetermination of mercury available today, as has been pointed out in a review by Manning.[2]

In Part II[3] equipment by means of which the digested urine samples could be automatically analysed at a high speed without any manual work or supervision was described. The equipment dealt with one sample every 2 minutes, and the sensitivity was the same as for the manual method.

The present paper deals with the extended application of the automatic mercury analysis equipment to fish and blood samples, which are of great interest in the field of hygiene. Initially, two modifications to the urine analysis method previously described[3] are discussed, followed by some additional findings.

Urine samples

The following two modifications have been introduced into the procedure for urine analysis compared with the original description given in Part II.[3] Firstly, we have found that it is necessary for urine samples to be collected in vessels containing some preservative agent. This is because the growth of bacteria renders the digestion procedure less efficient, and also because there could be a danger of conversion into volatile mercury compounds by bacterial action.[4] The addition of 1-g portions of sulphamic acid and 0·5 ml of a detergent (Triton X-100) to the 500-ml polyethylene bottles used as sampling vessels was shown to work well in this respect. Urine samples obtained in the above manner were stable at room temperature for at least 1 month.

An alternative method was to make the urine samples alkaline with sodium hydroxide to a pH value of about 13, thus reducing the tendency, noted with acidic samples, for coarse agglomeration of the precipitate in the urine to occur. However, more inorganic precipitate resulted at this alkalinity. Moreover, as stated below, under alkaline conditions the mercury in urine is more displaced to the liquid phase, which minimises sampling errors. The reagent blank derived from analytical-reagent grade sodium hydroxide was found to be negligible.

Distribution studies conducted on centrifuged urine samples have shown that an appreciable fraction of the total mercury content could be contained in the precipitate. With

* For details of Parts I and II of this series, see reference list on p. 155.

some urine samples acidified to pH 2, results for the total amount of mercury in the precipitates ranged from 4 to 45 per cent. These findings stress the importance of adequate shaking of the urine sample before taking sub-samples for analysis. The corresponding range of results when batches from the original urine samples were made alkaline was 2 to 10 per cent.

With acidic urine samples the visible presence of a large amount of precipitate correlates well with a high total recovery of mercury from the precipitate. In alkaline solution the proteins are hydrolysed to a greater extent and are more soluble, so the lower mercury recoveries from these precipitates were to be expected.

Secondly, we have found it convenient to decrease the amount of urine taken for analysis from 1·0 to 0·5 ml and, at the same time, to increase the volume of the permanganate digestion solution added from 1·5 to 2·0 ml. The reason for these changes was that the urine samples were sometimes rich in proteins, which adversely affected the digestion efficiency and caused extensive foaming in the purgation tower. Another advantage of using a smaller volume of sample is that it is then possible to use a concentration range in which the calibration graph is more linear, *i.e.*, from 0 to 200 ng of mercury per sample. The resultant decrease in sensitivity (by a factor of two) is not a disadvantage as it is still possible to determine mercury concentrations at levels down to those normally found in urine taken from unexposed persons.

We have recently constructed a duplicate of the automatic equipment under discussion with the difference that the Hitachi spectrophotometer is replaced by a mercury detector (HGM 2300, constructed by the Incentive Research Development Co., Bromma, Sweden). This double-beam detector accepts 20-cm cylindrical gas cells. With this equipment the sensitivity can be increased to about 0·1 ng of mercury per sample.

Blood samples

In the field of hygiene the determination of mercury in urine has, to date, been the most common way of monitoring the exposure of man to mercury. However, urine analysis is lacking in response when controlling persons, for example, fish eaters, who are exposed to alkyl mercurials that are not converted to "inorganic" mercury in the body to a very great extent. In addition, the reliability of sampling methods for urine has been questioned. For these reasons mercury control will probably be principally based upon blood analysis in future. There is therefore a demand for rapid methods for trace determination of mercury in blood.

The application of our automatic mercury analysis equipment to biological samples other than urine is dependent only on whether the sample can be converted into a solution containing free mercury(II) ions.

Digestion—

With the amount of permanganate digestion solution used for urine samples, only about 0·05 g of blood could be completely digested.[3] The digestion efficiency was not appreciably improved by using stronger sulphuric acid or by replacing it with nitric acid; also a permanganate digestion at elevated temperature or the pre-treatment of the blood samples with alkali was not successful. This limitation of the maximum sample volume, together with a proportionately high reagent blank, restricts analysis by this method to blood samples containing high levels of mercury.

There was an obvious requirement for a more efficient digestion mixture. Many mixtures have already been described in the literature.[5–8] Most of the wet-digestion procedures described, however, make use of the individual treatment of samples in high-temperature apparatus, a technique that is incompatible with rapid automated analysis.

Several combinations of strong oxidants have been investigated in this laboratory under both acidic and alkaline conditions at room temperature and at elevated temperatures. For example, we have not been successful with chromium(VI) oxide, hypochlorite, or cerium(IV) - perchloric acid. More promising was oxidation with hot perborate or persulphate solutions, but the high salt concentrations required resulted in heterogeneous mixtures on cooling. Mixtures containing oxidants together with chloride ions were shown to work well, but for this purpose they must be excluded because of the extensive frothing in the test-tubes caused by the liberation of chlorine. The same problem occurred with hydrogen peroxide. At a later stage, when the proteins are partly decomposed, the addition of chloride ions or hydrogen

peroxide could be of value to finish the reaction. The most efficient digestion solution found consisted of a mixture of perchloric and nitric acids, the former acting as the principal oxidant and the latter, probably, as a catalyst. The digestion should be carried out at elevated temperature (70 to 75 °C) and with a fairly high strength of the acids in the final digestion mixture. It was concluded that no mercury is lost when such a digestion is carried out. This conclusion was drawn after comparison between digestions of blood samples with mercury standard added and digestions of blood samples with the same amount of mercury added just before the final determination on the next day.

REAGENTS—

Digestion solution—Mix 10 volumes of concentrated perchloric acid with 2 volumes of concentrated nitric acid. Both chemicals should be of analytical-reagent grade quality.

Hydroxylammonium chloride solution, 50 per cent. w/v.

Tin(II) chloride solution, 2 per cent. w/v.

Mercury stock standard solution.

Details of the above three solutions can be found in Part II of this series.[3]

PROCEDURE—

The blood samples are sent to the Institute in standard 10-ml disposable tubes with some anti-coagulant added, usually heparin. They are stored in a refrigerator before analysis.

Shake the blood sample thoroughly and transfer 0·2 g to a disposable test-tube (10 × 160 mm) by means of an Oxford sampler (see below). Change the disposable syringe tip after each sample. (CAUTION—Blood should be regarded as a poison when handling.)

Add 1·5 ml of the digestion solution to the tube and place it into a water-bath at 70 to 75 °C. After about half an hour, when the mixture is homogeneous, swirl the tube thoroughly and leave it to stand overnight loosely covered with a plastics stopper. Next day allow the tube to cool, add 1·0 ml of de-ionised water and five drops of the hydroxylammonium chloride reagent. Shake the mixture vigorously. The sample is now ready for the automated analysis procedure described in Part II.[3] With such a procedure it is possible to analyse one sample every two minutes.

CALIBRATION—

At the same time as the samples, standards should be made up consisting of blood from unexposed persons (the mercury concentration of which should be known) mixed with micro-litre volumes of the standard mercury(II) nitrate solution, giving 0 to 30 ng of mercury per sample, corresponding to 0 to 150 ng per gram of blood. The absorbance *versus* concentration graph is linear within this range. The reagent blank value for mercury is about 0·5 ng per sample, corresponding to 2·5 ng per gram of blood.

RESULTS AND DISCUSSION—

To avoid time-consuming weighing of blood in routine work we use a calibrated Oxford sampler. This is a pipette consisting of a piston (with fixed amplitude of movement) in an outer cylinder. The piston is spring-loaded and operated by means of a button at one end of the cylinder. The liquid is sucked into a disposable plastics cap attached to the other end of the cylinder. By using a 0·2-ml sampler 0·202 g $\pm$ 2 per cent. (one standard deviation) of blood is found by experience to reach the test-tubes for digestion, *i.e.*, a systematic relative error of +1 per cent. is introduced by substituting the nominal volume for the actual weight. This can be regarded as tolerable without correction. These results relate to whole blood; corresponding values for 0·2-ml samples of erythrocytes are 0·195 g $\pm$ 4 per cent., and for plasma 0·200 g $\pm$ 1 per cent.

By digestion of 0·2-ml aliquots of blood about 3 ng g^{-1} of mercury can be determined, which is sufficient to cover normal values in unexposed persons. The relative standard deviation of single determinations on the same blood sample on different days is about $\pm$15 per cent. at the 20 ng of mercury per gram of blood level. If necessary, the sample volume can be increased to 0·3 g without any further changes in the procedure. The addition of an equal amount of water to the blood before adding the acids facilitates the digestion process, but is not essential. Also, 1-g portions of blood can be digested if the overnight procedure is finished with hydrogen peroxide treatment in a boiling water bath for about half

an hour. [The normal digestion procedure also works well, giving a quantitative yield with organomercurials added to blood samples. We have successfully analysed phenylmercury nitrate, ethylmercury bromide, Diurgin (an R–S–Hg–C–R diuretic), methylmercury hydroxide and methylmercury dicyanamide.] After digesting and cooling the samples it is important to dilute them with water to diminish the oxidative power of the acids, otherwise the final tin(II) reduction of mercury can be affected. The addition of hydroxylammonium chloride solution removes traces of dissolved nitric acid fumes and free chlorine by reduction and also keeps the mercury(II) ions in solution by complexation.

NOTE—If the acid strength is too high at this stage, the addition of the chloride reagent can give rise to the liberation of free chlorine, sometimes in such large amounts that the pump efficiency of the machine can be eliminated.

To test the reliability of the method, nine blood samples from chlorine manufacturing workers exposed to mercury were cross-checked against the activation analysis method of Sjöstrand.[8] In Fig. 1, the mean values of triplicate determinations obtained by the activation analysis method* are compared with our corresponding mean values from single determinations on different days.

The results can be seen to be in good agreement. The slope of the regression line is 0·96 and the coefficient of correlation 1·0. The deviation of the slope of the line from unity can be explained as an effect of deviating standards between the laboratories.

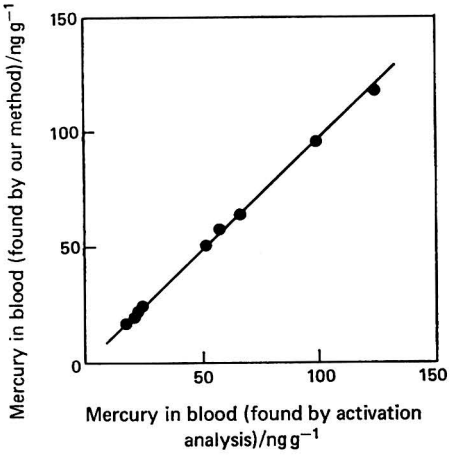

Fig. 1. Correlation between mercury determinations on blood samples by activation analysis and by the perchloric acid - nitric acid procedure

FISH SAMPLES

During the last few years it has become evident that consumption of fish must be regarded as being slightly hazardous to man in respect of mercury poisoning. In Sweden, and in other countries, these findings have given rise to recommendations concerning the maximum acceptable intake of contaminated fish corresponding to 30 μg of mercury per day with a restriction prohibiting the distribution of fish as a foodstuff if the mercury concentration exceeds 1 $\mu g\ g^{-1}$. In order to supervise these regulations there is a demand for rapid, routine methods that work well at about this hygienic limit value, but also at lower levels.

Analyses of fish have been carried out at several laboratories in Sweden. However, only two methods are frequently used, both of which are rather time consuming. One of these is that of Westöö,[9] which is based upon the gas-chromatographic detection of methylmercury by an electron-capture unit, the sample having previously been subjected to homogenisation, extraction and refining steps. It was concluded by Westöö that mercury occurs in fish almost exclusively as methylmercury and that the mercury is homogeneously distributed

* The activation analyses were performed at the Isotoptekniska Laboratoriet, Stockholm.

in the meat. The gas-chromatographic method has often been cross-checked with the activation analysis method of Sjöstrand.[8]

It was shown that the determination of mercury in fish was also quite easily accomplished by our automated method. Manual homogenisation and digestion must, however, be carried out before analysis. To avoid losing the gain with the use of automatic equipment, all manual steps should be carried out as quickly as possible.

HOMOGENISATION—

It was discovered that fish meat was easily homogenised satisfactorily by adding excess of alkali solution and, after occasionally shaking it, allowing the mixture to stand overnight at room temperature. Without any further mechanical treatment the extraction of mercury into the solution was then almost complete; with pike, perch and even herring less than 1 per cent. of the total mercury content was retained in the insoluble parts of the homogenised mixture. With eels, which are very rich in fat, this simple technique failed to separate the proteins, but after storage for several days only 5 per cent. of the total mercury content was left in the insoluble matter. By using a higher concentration of alkali and storing at 50 to 60 °C, the proportion of mercury in the solution was slightly increased, but the mixture was still very heterogeneous.

Alkaline fish homogenates are very stable with respect to the mercury concentration; if stored in a refrigerator they are probably indefinitely stable and even at room temperature they have a high degree of stability. Not even boiling the alkaline homogenates in open vessels brought about any noticeable changes in the mercury concentrations.

NOTE—If chloride ions were present in appreciable amounts some losses occurred on heating, probably because of the high volatility of methylmercury chloride.

On acidification of the alkaline homogenates large amounts of proteins were precipitated, but most of the mercury was still retained in the liquid phase. These findings, together with some dialysis-rate experiments, indicate that the methylmercury is linked to compounds of low molecular weight in the homogenates. The dialysis experiments were carried out with cod homogenates doped with methylmercury hydroxide as reference. The samples showed the same mercury dialysis rate as the methylmercury hydroxide standard. It has been further shown in this laboratory, by the method of Magos and Cernik,[4] that no conversion of methylmercury to inorganic mercury hydroxide takes place, even after storage of alkaline homogenates for several months.

DIGESTION—

As has been mentioned above, the mercury in fish is present as methylmercury. The mercury - carbon bond of methylmercury, as with other alkyl mercurials, is much stronger than the corresponding bond in methoxyalkyl or phenyl mercurials. The methylmercury bond was, however, easily split, giving free mercury(II) ions by contact with the digestion solutions described for urine or blood.

As it is convenient to work at room temperature, the permanganate - sulphuric acid digestion procedure was preferred when analysing fish samples with a mercury concentration above 0·25 $\mu g\ g^{-1}$. With this procedure it was possible to digest 0·2 ml of homogenate.

With the perchloric acid digestion, which operates at elevated temperatures, it was possible to digest 1 ml of homogenate. This technique facilitated the determination of mercury in fish down to at least the 0·10 $\mu g\ g^{-1}$ level with an acceptable relative standard error. By use of the original technique, however, and in contrast to blood samples, losses of methylmercury by evaporation occurred if precautions were not taken.

It was established that the evaporation of methylmercury was prevented by adding chloride ions to the homogenate before adding the perchloric acid digestion solution. This finding can be explained by assuming that the chloride ions associate with the mercury and on contact with the nitric acid the nascent chlorine produced rapidly breaks the mercury - carbon bond. Without the excess of chloride ions and while the temperature is rising, the oxidative power of this digestion solution is poor, so much of the mercury has already escaped before the temperature has become sufficiently high.

REAGENTS—

Homogenisation solution—Sodium hydroxide (40 g) is dissolved in water and diluted to 1 litre with water (pH 14). Store the solution in a polythene vessel.

Sodium chloride solution, 30 per cent. w/v—Sodium chloride (30 g) is dissolved in water and diluted to 100 ml with water.

Digestion solution—See under Blood samples.

Permanganate digestion solution.

Hydroxylammonium chloride solution, 50 per cent. w/v.

Tin(II) chloride solution, 2 per cent. w/v.

Mercury stock standard solution.

Details of the last four solutions can be found in Part II of this series.[3]

PROCEDURE—

Homogenisation—Weigh accurately into a polythene bottle about 1 g of fish meat containing not less than 0·1 $\mu g\ g^{-1}$ of mercury. Add a volume of homogenisation solution, measured in millilitres, nine times the numerical value of the weight of the sample in grams. Let the mixture stand at room temperature at least overnight with a few occasional vigorous shakings before taking out portions.

Digestion procedure I (for more than 0·25 $\mu g\ g^{-1}$ of mercury)—Remove 0·2 ml of the homogenised mixture with an Oxford sampler and transfer it to a test-tube (as for blood). Add 2·5 ml of the permanganate digestion solution and swirl the mixture. Close the tube loosely with a plastics stopper and let the mixture stand overnight at room temperature. On the following day, add 3 to 5 drops of the hydroxylammonium chloride reagent to reduce the excess of oxidising agent. The determination is then carried out according to the description given in Part II.[3]

Digestion procedure II (for more than 0·10 $\mu g\ g^{-1}$ of mercury)—Remove 1·0 ml of the homogenised mixture with an Oxford sampler and transfer it to a test-tube. Add one drop of the sodium chloride reagent and swirl the mixture. Add 1·5 ml of the perchloric acid - nitric acid digestion solution and keep the tube, loosely stoppered with a plastics stopper, in a water-bath at a temperature of 70 to 75 °C overnight. On the following day, and after cooling to room temperature, add 3 drops of the hydroxylammonium chloride reagent. The analysis is then carried out according to the description given in Part II.[3]

CALIBRATION—

Standard solutions are made up by adding microlitre portions of the standard mercury(II) nitrate solution to cod homogenates, the mercury content of which is known. The graph of absorbance *versus* amount of mercury is linear up to 100 ng per sample.

With 0·2-ml samples, treated in accordance with digestion procedure I, the range from 5 to 50 ng of mercury per sample should normally be covered, which is equal to between 0·25 and 2·5 μg of mercury per gram of the original fish meat. The reagent blank is 1·5 ng per sample, corresponding to 0·075 μg per gram of fish. With 1·0-ml samples, treated in accordance with digestion procedure II, a range of mercury contents from 10 to 50 ng per sample is equal to 0·10 to 0·50 μg per gram of fish. The reagent blank is 0·5 ng per sample, corresponding to 0·005 μg per gram of fish.

RESULTS AND DISCUSSION—

For routine purposes (to supervise the mercury limit value of 1 μg per gram of fish) we recommend the use of digestion procedure I. Analysis of sea fish is, however, best accomplished by using procedure II.

In calculating the results we omit the deviation from unity of the specific gravity of fish meat, and thus assume that the homogenates contain exactly one tenth of the mercury concentration of the original fish meat. This approximation gives an over-estimation of the actual value by about 1 per cent., but the conversion of nanograms per sample to micrograms per gram of fish is appreciably simplified by using constant factors of 0·05 and 0·01 for digestion procedures I and II, respectively. The relative standard deviation, as calculated from our day-to-day single determinations on the same homogenates, was tolerable (less than ± 15 per cent. down to about 0·25 μg per gram of fish with digestion procedure I and down to at least 0·10 μg per gram of fish with digestion procedure II). At the 1·0 $\mu g\ g^{-1}$ level this

relative standard deviation was ±5 per cent. with procedure I. The deviations between duplicates in the same series of analyses were, however, appreciably smaller.

The sensitivity of the two digestion procedures can be increased as follows.

Digestion procedure I—As the liquid part of a fish homogenate contains nearly all of the mercury in the homogenate, a filtration step can be introduced that allows a larger sample volume (0·5 ml) to be digested by avoiding the consumption of oxidant by proteins low in mercury. The filtration step is, however, time consuming.

Digestion procedure II—An increase in the sample volume, as for procedure I, would dilute the acids too much, thus lowering their digestion efficiency. It is therefore recommended that the homogenisation step be carried out with a smaller portion of alkali. At least one half of the proposed volume is satisfactory. Even the direct addition of the digestion acids to the fish meat could be a possibility; this would have the effect of omitting the homogenisation step.

Interference by foreign substances in the analysis has been discussed earlier.[1] The negative influence on peak heights of iodide ions was shown to be of the same magnitude with the digestion procedure with perchloric and nitric acids as for the permanganate procedure, *i.e.*, a maximum of 1 μg of iodide per sample could be tolerated. On analysing sea fish the ratio of iodine to mercury can sometimes be of such an order that this effect must be considered, as is apparent from Table I. The analysis of fish from lakes is, however, free from problems in that respect.

TABLE I

CONCENTRATIONS OF MERCURY AND IODINE IN FISH*

	Typical range of values		Range of ratio
	Hg/μg g^{-1}	I/μg g^{-1}	I : Hg
Fish from the sea.. ..	0·02 to 0·1	0·3 to 3	3 to 150
Fish from lakes	0·1 to 1	0·03 to 0·3	0·03 to 3

* The values are taken from various sources.

This work has been carried out on fish taken from Swedish lakes, which have already been analysed by activation analysis for total mercury (Isotoptekniska Laboratoriet, Stockholm) and by the gas-chromatographic technique for methylmercury (National Institute of Public Health, Stockholm).

Graphs of the correlation between our two methods and the methods mentioned above are shown in Figs. 2, 3 and 4. It is evident from the figures that there are very close relationships between, on the one hand, our two digestion procedures (Fig. 2), and, on the other, our values and the values from the activation analysis (Fig. 3) or the gas-chromatographic analysis (mercury determined as methylmercury) (Fig. 4).

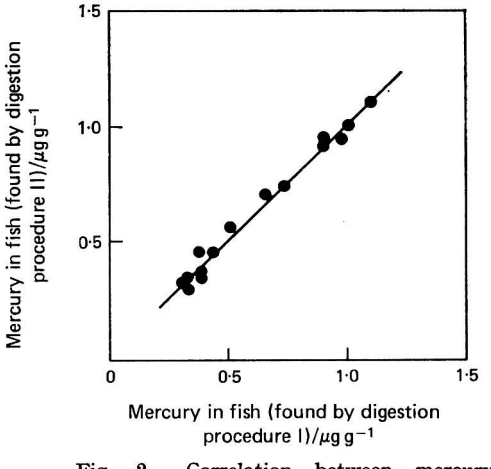

Fig. 2. Correlation between mercury determinations on fish samples by the two digestion procedures

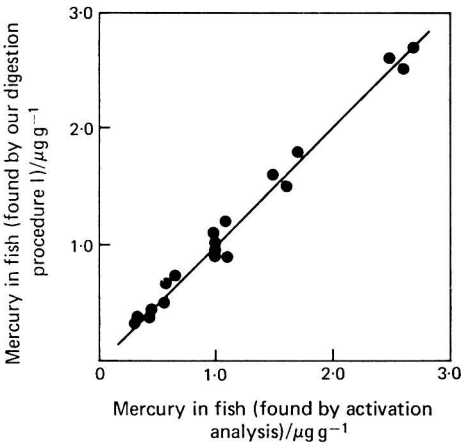

Fig. 3. Correlation between mercury determinations on fish samples by activation analysis and by digestion procedure I

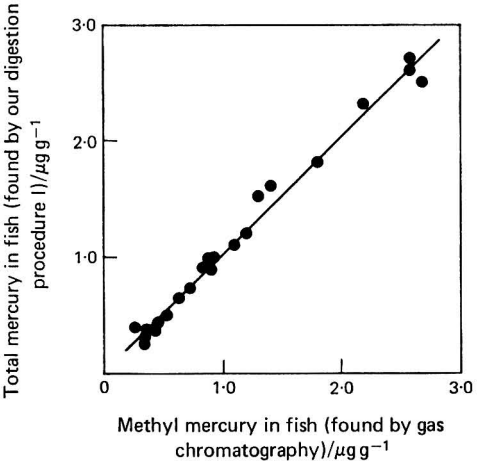

Fig. 4. Correlation between mercury determinations on fish samples by gas chromatography (determined as methylmercury) and by digestion procedure I

The correlation, r, and regression, b, coefficients were calculated as—

Digestion II $= f$ (Digestion I) $n = 15; r = 1{\cdot}0; b = 0{\cdot}98$ (Fig. 2)
Digestion I $= f$ (Activation analysis) $n = 19; r = 1{\cdot}0; b = 1{\cdot}0$ (Fig. 3)
Digestion I $= f$ (Gas chromatography) $n = 22; r = 1{\cdot}0; b = 1{\cdot}0$ (Fig. 4)
(n = number of samples)

Other sample types

The automated method[3] is applicable to all types of samples that can be digested in a similar way to urine, fish and blood. For example, we have digested 0·3 g of hair with the standard procedure for blood. This procedure also enabled us to digest 0·7 ml of milk or egg white, 0·4 g of apple or plum, 0·2 g of sausage, 0·1 g of bread or paper and 0·05 g of cheese or egg yolk, all without homogenisation. Foodstuffs and tissues should, however, normally be digested after some mechanical pre-treatment in alkali (homogenisation), possibly with a detergent added when working with fatty material. If homogenisation is not carried out the amount of sample used may be too small to be representative.

Natural water samples, normally too low in mercury to be analysed directly (*i.e.*, less than 0·1 ng ml^{-1}), could easily be concentrated by tin(II) reduction of a larger permanganate-digested sample, followed by the passing of the mercury vapour into a 2-ml volume of dilute acidic permanganate solution. The detection limit by this technique would depend only on the level of mercury in the reagent blank.

I thank Dr. G. Lindstedt for his valuable support and criticism of the manuscript, and my wife, L. Skare, for her skilful experimental work. I also thank Dr. G. Westöö of the National Institute of Public Health for her collaboration in the fish analyses.

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Note—References 1 and 3 constitute Parts I and II, respectively, of this series.

Inadmissibility of the "AnalaR" Method for the Determination of Metallic Zinc in Zinc Oxide

By V. J. NORMAN

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A chemical method for the quantitative determination of non-stoicheiometric zinc in zinc oxide, previously published by the author, has been incorrectly applied by AnalaR Standards Limited to the determination of free metallic zinc in zinc oxide. It is shown that, unless the free metal is extremely finely divided, large amounts can be present in the zinc oxide without being recorded in the analysis. Even with particle diameters as small as 1 μm, only 3 per cent. or less of the metallic zinc is detected by the method.

A PAPER by the author,[1] proposing a chemical method for the quantitative determination of non-stoicheiometric zinc in zinc oxide, entitled "The Photometric Determination of Excess of Zinc in Zinc Oxide," was published in 1964. It was stated in that paper that the proposed method was designed specifically for the determination of non-stoicheiometric zinc. Moreover, the expression "excess of zinc" has, for many years, been accepted as being synonymous with "non-stoicheiometric" or "interstitial" zinc.[2–10]

In the penultimate paragraph of the paper[1] it was stated that "Allsopp and Roberts have drawn attention to the fact that, in their analyses, the amount of hydrogen evolved may be taken as a measure of non-stoicheiometry only if the presence of free zinc or other free metal capable of displacing hydrogen can be excluded, and it is pointed out that this proviso also applies to the present method." While it was stated that the amount of non-stoicheiometric zinc determined by the Norman method was subject to interference from metallic zinc, it was not suggested that the method was suitable for the quantitative determination of free metals.

Notwithstanding the above, AnalaR Standards Limited[11] have incorrectly applied the method to the determination of metallic zinc in zinc oxide, and have set the acceptance limit at an optical density difference that is equivalent to approximately 8 p.p.m. of non-stoicheiometric zinc. This is, in itself, at variance with their specified maximum metallic zinc content of 0·002 per cent.

The inadmissibility of the application of the method to the determination of metallic zinc in zinc oxide has been demonstrated in the following manner. Guaranteed reagent grade zinc oxide was heated for several hours at 600 °C to reduce the non-stoicheiometric zinc content to less than 0·2 p.p.m.[12] To samples of this zinc oxide various amounts of metallic zinc were added in the form of Merck G.R. grade zinc granules, approximate diameter 0·05 cm, or zinc dust, particle diameter 1 to 3 μm. The metallic zinc was intimately mixed with the zinc oxide, and the samples were analysed by the method. The results of analysis are set out in Table I.

The results given in Table I show clearly that, unless the free metal is extremely finely divided, large amounts could be present in the zinc oxide without being recorded in the analysis. Even with particle diameters as small as 1 μm, only 3 per cent. or less of the metallic zinc is detected. This is due, in part, to the very slow rate of dissolution of metallic zinc in the sulphuric acid - phosphoric acid mixture specified, which was deliberately selected with a view to minimising the interference from any free metal.

Table I also shows that the optical density of reference solutions, as well as that of sample solutions, decreases with increasing additions of zinc dust. The rôle of the reference solution in the method is to compensate for oxidisable matter (other than non-stoicheiometric zinc) in the zinc oxide and reagents. Non-stoicheiometric zinc is present as zinc ions, which dissolve instantaneously in the acid mixture prior to the addition of dichromate and cannot, therefore, effect any reduction of reference solutions. However, when significant amounts

of free metallic zinc are present, complete dissolution of the free zinc does not take place before the addition of dichromate, and some reduction of reference solutions subsequently occurs.

TABLE I

METALLIC ZINC RECOVERED BY THE METHOD

Metallic zinc added, p.p.m.	Optical density: Reference solution	Optical density: Sample solution	Optical density: Difference	Zinc recovered by the method, p.p.m.
Merck G.R. zinc granules—				
500	0·730	0·730	0·000	<0·1
700	0·730	0·729	0·001	<0·1
2000	0·729	0·691	0·038	0·8
10000	0·723	0·612	0·111	2·2
Zinc dust—				
20	0·772	0·744	0·028	0·6
100	0·769	0·631	0·138	2·8
200	0·689	0·369	0·320	6·4
500	0·630	0·060	0·570	11·4
2000	0·473	0·021	0·452	9·0

There is another reason for the AnalaR method being unacceptable for the determination of metallic zinc in zinc oxide. AnalaR Standards Ltd. have specified that the difference between the optical densities of the reference and sample solutions must not exceed 0·385, which is equivalent to approximately 8 p.p.m. of non-stoicheiometric zinc. Any zinc oxide completely free from metallic zinc but in which the non-stoicheiometric zinc exceeded 8 p.p.m. would, therefore, fail the AnalaR test for metallic zinc. While the maximum non-stoicheiometric zinc content of analytical-reagent grades of zinc oxide encountered in these laboratories is 7·3 p.p.m., Allsopp[2] has reported values for excess of zinc in high-purity zinc oxide powder as high as 18 p.p.m.

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Book Reviews

ACTIVATED CARBON. SURFACE CHEMISTRY AND ABSORPTION FROM SOLUTION. By JAMES S. MATTSON and HARRY B. MARK, JR. Pp. viii + 237. New York: Marcel Dekker Inc. 1971. Price $19.75.

This book sets out to review the literature on the surface chemistry of activated carbons and the adsorption of a great variety of solutes from liquid solutions. While not being of direct analytical interest, it does indicate the wide uses of activated carbons in many industrial processes, and explains the physico-chemical theories of adsorption by these special reagents. Of considerable interest is the use of active carbons in waste-water treatment, which requires large amounts of highly active materials. Those interested in purification and adsorption from solution will find this a helpful book, and the analyst will doubtless see plenty of areas where his techniques can be of value in assessing the extent of purification. W. I. STEPHEN

PROGRESS IN NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY. Volume 6. Edited by J. W. EMSLEY, J. FEENEY and L. H. SUTCLIFFE. Pp. viii + 186. Oxford, New York, Toronto, Sydney and Braunschweig: Pergamon Press. 1970. Price £5.

The sixth volume in this well known series continues the tradition unchanged and has three unrelated review articles. Although the copyright is dated 1970, the edition is dated 1971, while internal evidence suggests that the articles were completed early in 1969, and in two cases additional material was added without proper integration at the proof stage in April, 1970. Such delays detract appreciably from the topicality of the two essentially bibliographical articles. That on "Spin - spin coupling between phosphorus nuclei" by E. G. Finer and R. K. Harris presents the information from over 200 references in essentially tabular form. The text provides a useful guide to directly and indirectly bonded P · · · P couplings. A more certain account will be possible only when the signs of a larger fraction of the coupling constants have been determined. The bibliography on "Nitrogen nuclear magnetic resonance" by E. W. Randall and D. G. Gillies also has over 200 references but the material is presented largely in running text rather than tables. This also discusses the difficulties associated with the line broadening due to the ^{14}N quadrupole moment and emphasises the advantages of using ^{15}N if possible.

The other article, "The theory of nuclear spin - spin coupling in high-resolution n.m.r. spectroscopy," by J. N. Murrell, is a very clear review of the theory designed to be read by the n.m.r. practitioner who wants to know the nature and state of the art. Research workers in any branch of n.m.r. would benefit from this article, whereas the other articles are intended for those who require information in the separate specialised fields. D. H. WHIFFEN

ELECTROCHEMICAL REACTIONS IN NONAQUEOUS SYSTEMS. By CHARLES K. MANN and KAREN K. BARNES. Pp. xii + 560. New York: Marcel Dekker Inc. 1970. Price $34.50; £16·40.

This book reviews many of the various chemical reactions in nonaqueous systems that have appeared in the literature up to the beginning of 1969. In reviewing a particular electrochemical reaction, the authors have generally dealt with the whole reaction from the starting material to the final products and in many cases have included not only the effects on the reactions of changes in solvents, supporting electrolytes or working electrodes, but also the effects brought about by slight modifications of the molecular structure of the reactants.

The authors are to be congratulated on their very efficient use of tables to summarise the various points. These tables allow the reader to make his own comparison of the various results available and also to use the book as a quick source of reference. For example, in the first chapter, which deals with experimental conditions, the table listing reference electrodes and their potentials is of immediate use to anyone proposing to work in this field, whilst one of the tables in the chapter dealing with the reduction of hydrocarbons lists values for the polarographic reduction of over two hundred compounds and is complete with well documented references.

The various discussions in the chapters dealing with purely organic compounds are lucid and fairly critical, giving reasoned indications of the present state of the topics; the final two chapters, which deal with the reduction of organometallic compounds and with the electrolysis of inorganic compounds in nonaqueous systems, respectively, cover such extensive ranges of compounds that critical reviews are not possible in the space allotted.

From the viewpoint of an analytical chemist, the book has severe limitations in that it does not discuss or indicate analytical applications and generally confines itself to the discussion of the reactions of single materials in pure solvents or mixtures of pure solvents, a situation rarely found in analytical chemistry laboratories. Nevertheless, for those who have to deal with electrochemical reactions in nonaqueous systems, this book will provide a useful addition to their library.

L. S. BARK

FLUORESCENCE ANALYSIS. A PRACTICAL APPROACH. By CHARLES E. WHITE and ROBERT J. ARGAUER. Pp. x + 389. New York: Marcel Dekker Inc. 1970. Price $18.75; £8·90.

Over the last few years, relatively great progress has been made in the use of fluorimetry in analysis and it is therefore not surprising that authors from one of the main schools of fluorimetric analysis should collect into book form much of the expertise gathered over years of practice.

The authors aim to present a book that will provide the teacher, student, research scientist and clinical chemist with a readily available source of the basic theory and techniques of fluorescence analysis. This is indeed a very big target for a book of less than 400 pages. In some respects they have done this; there are seven chapters dealing with organic compounds and in some of these there are sufficient details to enable one to make a determination of the required substance. The same may be said concerning the chapter dealing with clinical procedures and to a lesser extent that dealing with methods for metals and non-metals. However, the chapters dealing with the basic theories and with instrumentation leave much to be desired; they are not sufficiently detailed or explicit to allow an understanding of the basic concepts used in this type of analysis.

The worth of the book is somewhat lessened for me by several minor errors including typographical slips as well as several inconsistencies in the naming of compounds. For example, in some cases formal names are given in the text but common or trivial names are used in the corresponding formula diagrams. These are to some extent compensated for by the large number of references included in the text; these make the book a much more balanced entity. L. S. BARK

LABORATORY HANDBOOK OF METHODS OF FOOD ANALYSIS. Second Edition. By R. LEES. Pp. xii + 192. London: Leonard Hill. 1971. Price £3·80.

As with the first, the second edition of the Laboratory Handbook of Methods of Food Analysis aims to present methods, in a readable and readily understandable form, which are the most useful to the control analyst working in the food factory. The usefulness and reliability of such a book can best be judged by workers who regularly use it at the bench. Unfortunately, the present reviewer was unable to find any person who used the previous edition who could advance such an opinion. Nevertheless, the fact that a second edition has appeared only 3 years after the first suggests that the book has been a decided commercial success.

The methods are set out as a simplified sequence of numbered steps, apparently designed to be used mainly by junior laboratory technicians. Some of the earlier sections include points on reporting, accuracy and organoleptic examination, which will interest the more senior man. In view of the almost complete absence of interpretation of results, however, lecturers in food technology are unlikely to agree with the claim on the dust cover that ". . . the book will prove an invaluable aid for . . . students of food technology."

Lees deserves credit for applying a new format in books on food analysis by listing the chosen methods for a wide range of foods and giving cross-references to the procedures described in the following section. The system reduces the repetition found in other books, but ingenuity and experience are called for when the recommended methods are applied in some instances.

The author states in the Preface that "All the methods given in the first edition have been reviewed and, where appropriate, revised in accord with modern control laboratory practice." Comparison of the two editions appears to suggest that an attempt has been made to eliminate the errors in the previous edition (see Bush, *J. Ass. Publ. Analysts*, 1968, **6**, 135). The ". . . additional methods of food analysis which have been requested by factory control chemists" appear to be those for determining copper, zinc, potassium and saccharin, together with an alternative procedure for assessing jelly strength. An index is also now included and better quality paper is used. Whether such additions and amendments will be sufficient to induce owners of the first edition to purchase the new one is questionable and one gets the general impression that many readers of this journal will classify it as a "cookery book."

D. PEARSON

AN INTRODUCTION TO GAS - LIQUID CHROMATOGRAPHY. By R. A. JONES. Pp. xiv + 202. London and New York: Academic Press. 1970. Price £2·75; $8.00.

The techniques of gas - liquid chromatography are now well established and during the past few years the major developments have been the association of other techniques such as mass spectrometry with gas - liquid chromatography, the advent of pyrolysis gas chromatography and to some extent the development of high-pressure gas chromatography. There have not been many significant advances in the theories of chromatography or in the types of instrumentation in use.

Therefore, to be of interest, any new book must not only have a good presentation of fundamental techniques, etc., but must also present in a critical fashion the new works, the new applications, the latest in equipment and general apparatus as well as indicate that the author has some expertise to give to his readers. To a creditable extent this book fulfils these criteria. It is fairly concise but has more than an adequate coverage of the modern literature and techniques. It has a commendable clarity of presentation. The appendix—about a quarter of the book—includes a glossary of terms (a very useful piece of information for any beginner), tables of instrument specifications and addresses of manufacturers and, even more useful, a list of operational procedures and a very creditable section on fault finding.

At its price, this book is a good buy. L. S. BARK

THE ANALYTICAL CHEMISTRY OF SULPHUR AND ITS COMPOUNDS. PART I. Edited by J. H. KARCHMER. Pp. xviii + 534. New York, London, Sydney and Toronto: John Wiley & Sons. 1970. Price £16·50.

This book is one of the series of monographs dealing with analytical chemistry and its applications, originally intended to be one volume. The work on sulphur has been divided into two volumes, of which this is the first. It presents six chapters and is concerned primarily with inorganic aspects of sulphur chemistry although it includes necessary reference to some organic compounds and also a chapter dealing with thiols.

Each chapter is written by experts in the particular field and provides information from widely scattered sources including information about the various methods listed. The stated purpose of this particular method of treatment is to provide the analytical chemist with the means of devising his own methods to solve his particular problems and so to avoid the disastrous effects of possible interfering materials. In this respect the book is reasonably successful, there are sufficient references to enable the analyst to find the relevant literature and there is also enough background material to allow him to have a better appreciation of the material because of his background reading. In some of the methods given there are enough practical details to enable the chemist to use the book as a practical textbook. In many of the other methods the details are so sparse that it is necessary to consult the literature. In general, it will be necessary to use the latter procedure.

Nevertheless, the book is a very useful source book and in these days when the proliferation of literature is so great, any well written book that enables one to reach the relevant literature more quickly is welcome. This is such a book. L. S. BARK

TEXTBOOK OF POLYMER SCIENCE. Second Edition. By F. W. BILLMEYER, JR. Pp. xvi + 598. New York, London, Sydney and Toronto: Wiley-Interscience. 1971. Price £7·50.

Anyone new to the business and wanting some basic information about "plastics" can do little better than start with this book. It will not provide analytical chemists with full details of how to analyse their samples but, being written by a professor of analytical chemistry, it does give, in a very readable fashion, the kind of background information that is invaluable to anyone who must go at all deeply into the analysis of such materials.

After a brief introductory description of what polymers are, the author discusses polymer solutions and measurement of molecular weight and then briefly surveys analysis and testing, particularly the physical methods. He then describes the structure and properties of bulk polymers, goes into some detail about methods and mechanisms of polymerisation, and, after surveying the properties of the numerous commercial polymers available, concludes with chapters on plastics, fibre and elastomer technology.

At a time when it is customary for reviewers to complain of the rising price of books, I think this book is good value for money for any technical library. A. G. JONES

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ELEMENTS OF INSTRUMENT ELECTRONICS FOR THE ANALYST

For particulars of this Workshop Course (10–14 April), write to Wolfson Bioanalytical Centre, University of Surrey, Leapale Lane, Guildford.

An Emission-spectrographic Method for the Determination of Boron in Nuclear-grade Graphite

A rapid, highly sensitive method is described for the spectrographic determination of boron in nuclear-grade graphite. Boron, present in the sample in the form of boron carbide (B_4C), is volatilised as the trifluoride by the addition of 1 per cent. of Kel-F powder (polychlorotrifluoroethylene) to the samples. The samples, containing 500 p.p.m. of silicon as the internal standard, are excited by a 10-A d.c. arc in the presence of an argon-controlled atmosphere. A linear relationship between the intensity ratio of the lines boron 249·773 nm and silicon 245·214 nm and the boron concentration is established in the boron concentration range 0·1 to 5 p.p.m. The relative standard deviation, calculated from twelve determinations on each sample, ranges from ±8 per cent. (at 5 p.p.m. of boron) to ±11 per cent. (at 0·1 p.p.m. of boron).

G. ROSSI and G. SOLDANI

Chemistry Division, Euratom-CCR, ISPRA (Varese), Italy.

Analyst, 1972, **97**, 124–130.

The Analysis of Dental Amalgams by X-ray Fluorescence, by Using a Solution Technique

A simple and comparatively rapid method is described for the analysis of dental amalgams that have the following range of concentrations in per cent. w/w: mercury 30 to 70, silver 20 to 50, tin 5 to 15, copper 0·5 to 1·5 and zinc up to 0·5. Mercury, silver, copper and zinc are determined in acidic solution after tin has been separated as metastannic acid and determined gravimetrically. By using a 0·5-g sample, precisions of ±0·26, ±0·20, ±0·04 and ±0·04 per cent. (2σ) are obtained on the mercury, silver, copper and zinc determinations, respectively.

G. GLOSSOP

Department of Metallurgy, University of Sheffield, Sheffield, Yorkshire.

Analyst, 1972, **97**, 131–133.

A Non-aqueous Titrimetric Determination of Acid-evolved Carbon Dioxide in Silicate Rocks

A method is described for the determination of carbonate in silicate rocks at concentrations, expressed as carbon dioxide, down to 0·01 per cent. After treatment with acid the carbon dioxide liberated is absorbed into a dimethylformamide solution and titrated directly with a solution of tetrabutylammonium hydroxide.

J. I. READ

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

Analyst, 1972, **97**, 134–137.

The Microbiological Assay of the "Essential" Amino-acids in Compound Feedingstuffs

A re-examination has been made of the methods in current use for the microbiological assay of the "essential" amino-acids arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine, and improvements in basal media and methods of hydrolysis are suggested.

E. C. BARTON-WRIGHT

Galloway and Barton-Wright, Haldane Place, London, SW18 4NA.

Analyst, 1972, **97**, 138–141.

An Improved Colour Reagent for the Determination of Blood Glucose by the Oxidase System

A method involving the use of 4-aminophenazone as a colour coupler with sulphonated 2,4-dichlorophenol is described for determining the hydrogen peroxide produced from glucose with glucose oxidase.

The sensitivity of the method is such that 20 μg of glucose in a final volume of 4 ml give an optical density of 0·61 at 515 nm with 10-mm cells, corresponding to a molecular absorption of 22 000.

DENISE BARHAM and P. TRINDER

Biochemistry Department, Central Laboratory, Royal Infirmary, Sunderland.

Analyst, 1972, **97**, 142–145.

3-Propyl-5-hydroxy-5-D-arabinotetrahydroxybutyl-3-thiazolidine-2-thione, a Specific Colorimetric Reagent for the Determination of Copper in Water

The 3-propyl homologue of 3-methyl-5-hydroxy-5-D-arabinotetrahydroxybutyl-3-thiazolidine-2-thione has been prepared and used as a reagent in the colorimetric determination of copper in water. Interference by other metals and by cyanide has been examined; inhibition of complex formation by cyanide appears to be almost quantitative.

M. J. STIFF

Water Pollution Research Laboratory of the Department of the Environment, Stevenage, Herts.

Analyst, 1972, **97**, 146–147.

Microdetermination of Mercury in Biological Samples

Part III. Automated Determination of Mercury in Urine, Fish and Blood Samples

An extension of the use of the automatic mercury analyser published earlier is discussed. Wet-digestion procedures for blood and fish samples that are also applicable to other types of sample are described. With the standard procedures recommended it is possible to determine mercury concentrations in blood down to about the normal values for unexposed persons, *i.e.*, about 5 ng g^{-1}, and mercury in fish down to the 0·1 μg g^{-1} level, with an acceptable standard error. Comparative studies between our method and the methods of activation analysis and gas chromatography have shown good agreement.

I. SKARE

Chemistry Department, National Institute of Occupational Health, Fack, S-104 01 Stockholm 60, Sweden.

Analyst, 1972, **97**, 148–155.

Inadmissibility of the "AnalaR" Method for the Determination of Metallic Zinc in Zinc Oxide

A chemical method for the quantitative determination of non-stoicheiometric zinc in zinc oxide, previously published by the author, has been incorrectly applied by AnalaR Standards Limited to the determination of free metallic zinc in zinc oxide. It is shown that, unless the free metal is extremely finely divided, large amounts can be present in the zinc oxide without being recorded in the analysis. Even with particle diameters as small as 1 μm, only 3 per cent. or less of the metallic zinc is detected by the method.

V. J. NORMAN

Australian Defence Scientific Service, Department of Supply, Defence Standards Laboratories, P.O. Box 1935P, Adelaide, South Australia 5001.

Analyst, 1972, **97**, 156–157.

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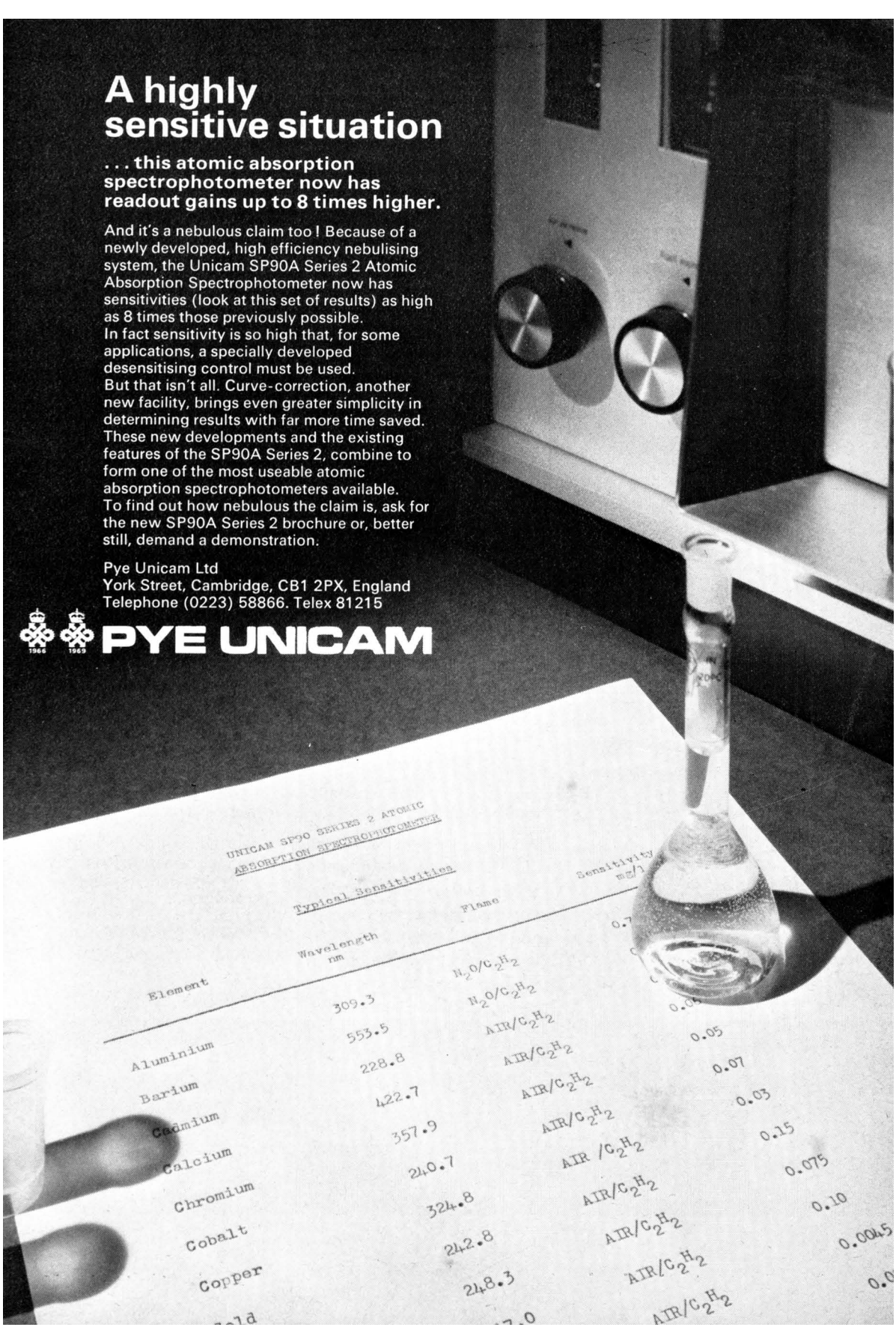

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