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Summaries of Papers in this Issue

An Evaluation of the 3-Methyl-2-benzothiazolinone Hydrazone Method for the Determination of Phenols in Water and Waste Waters

Friestad's method for the determination of phenol with 3-methyl-2-benzothiazolinone hydrazone (MBTH) has been evaluated. The Friestad manual and automated MBTH methods were modified to lower the detection limit from 1 mg l^{-1} to 1 μg l^{-1}. The values for phenol obtained by this method on river water and industrial waste samples were equal to or greater than those obtained by the 4-aminophenazone method.

MORRIS E. GALES, Jun.

Methods Development and Quality Assurance Research Laboratory, National Environmental Research Center, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268, U.S.A.

Analyst, 1975, **100**, 841–847.

A Rapid Method for the Simultaneous Determination of Paraquat and Diquat in Pond and River Waters by Pyrolysis and Gas Chromatography

A rapid method is described for the determination, in aqueous systems, of two widely used herbicides known commercially as paraquat and diquat. Pyrolysis of these herbicides under carefully controlled conditions, followed by gas-chromatographic analysis of the pyrolysate, allows detection of the herbicides down to 0·01 p.p.m. At the lowest concentration levels (0·01–0·1 p.p.m.), there is some loss of linearity of response, possibly as a result of adsorption of the herbicides on the glass surfaces of the vessels used. This effect may have resulted in substantial errors in previously reported low levels of paraquat and diquat and may also occur in the determination of other ionic herbicides.

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Analyst, 1975, **100**, 848–853.

Determination of Dimetridazole in Feedstuffs and Pre-mixes by High-speed Liquid Chromatography

A method is described for the determination of dimetridazole (1,2-dimethyl-5-nitroimidazole) in feedstuffs and pre-mixes by high-speed liquid chromatography. Dimetridazole is extracted from the sample with methanol - water (1 + 2). After liquid - liquid extraction into dichloromethane, an aliquot of the solution is injected into a high-speed liquid chromatograph.

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Analyst, 1975, **100**, 854–856.

A Rapid Method for Monitoring Low Levels of Di-(2-ethylhexyl) Phthalate in Solutions

A simple gas - liquid chromatographic method using a nickel-63 electron-capture detector for the determination of di-(2-ethylhexyl) phthalate (DEHP) in blood and aqueous solutions is described. The DEHP is extracted into n-hexane in a single-step extraction and is injected directly, using 5 per cent. SE-30 as liquid phase. The detection limit was 2 ng and a linear detector response was found in the range 2–20 ng. The method was used to monitor DEHP in blood and aqueous or lipophilic solutions.

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Analyst, 1975, **100**, 857–861.

The Determination of Oxygen-18 to Oxygen-16 Ratios in Inorganic Phosphates by Gas - Liquid Chromatographic - Mass Spectrometric Examination of the Tri-n-butyl Derivative

Aqueous solutions containing inorganic orthophosphates from biological materials were purified on ion-exchange columns and the phosphate was precipitated as its silver salt. The dried silver phosphate was made to react with 1-bromobutane in dimethylformamide, yielding tri-n-butyl phosphate. This solution was suitable for injection directly into a combined gas chromatograph - mass spectrometer for analysis, enabling the oxygen-18 to oxygen-16 ratio of the original inorganic orthophosphate to be determined.

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Analyst, 1975, **100**, 862–864.

Procedures for the Deoxygenation of Liquids

Procedures are described for the deoxygenation of pure liquids, liquid mixtures and solutions of solids in liquids using tris(2,2′-bipyridyl)cobalt(II) perchlorate {$[Co(bpy)_3](ClO_4)_2$} and sodium tetrahydroborate ($NaBH_4$) in a colour-indicating reaction. The efficiency of the procedures is assessed by reference to the ^1H spin - lattice relaxation times of a variety of materials. The values obtained show that the procedures are efficient, reproducible and time saving.

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Department of Chemistry, University of Aston in Birmingham, Gosta Green, Birmingham, B4 7ET.

Analyst, 1975, **100**, 865–872.

A Rapid Method for the Assay of Ascorbic Acid Tablets

A method is described for the rapid routine assay of ascorbic acid tablets by using a subtractive enthalpimetric technique. The ascorbic acid is oxidised by hexacyanoferrate(III).

The effects of excipients have been studied. A comparison of the results obtained by using this method and the BP method shows that there is no significant difference in the accuracy of the two techniques. The main advantages of the proposed method are the time taken, the potential for automation of the process and the ability to carry out the assay of single dosage forms.

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Analyst, 1975, **100**, 873–877.

DECEMBER 1975 Vol. 100 No. 1197

The Analyst

An Evaluation of the 3-Methyl-2-benzothiazolinone Hydrazone Method for the Determination of Phenols in Water and Waste Waters

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Friestad's method for the determination of phenol with 3-methyl-2-benzothiazolinone hydrazone (MBTH) has been evaluated. The Friestad manual and automated MBTH methods were modified to lower the detection limit from 1 mg l^{-1} to 1 $\mu g\ l^{-1}$. The values for phenol obtained by this method on river water and industrial waste samples were equal to or greater than those obtained by the 4-aminophenazone method.

The trend towards more stringent pollution abatement practices has heightened the need for a method that can be used for determining a greater number of phenolic compounds than can be measured by the 4-aminophenazone (4AP) method. These compounds are considered to be pollutants because they cause an unpleasant taste and odour in potable water supplies that have been chlorinated. In addition, phenols are often considered to be an indication of man-made organic contamination.

The most widely used method for determining phenols in aqueous samples is the 4AP method.[1,2] In this procedure the phenol is distilled and subsequently made to react with hexacyanoferrate(III) and 4AP to form a red complex that is measured colorimetrically. The reaction with 4AP has several deficiencies, the most significant of which is its inability to measure certain *para*-substituted phenols.[3] Friestad *et al.*[4] have described both a manual and an automated method, each of which determines phenols by oxidative coupling with 3-methyl-2-benzothiazolinone hydrazone (MBTH). Friestad *et al.* have not only shown that the MBTH method is more universal in reactivity to phenols than the 4AP method, but also that it leads to higher molar absorptivities. The MBTH method is based on the coupling of phenol with MBTH in an acidic medium using ammonium cerium(IV) sulphate as an oxidant. The coupling takes place in the *para* position; if this position is occupied, the MBTH reagent will react at a free *ortho* position. Therefore, the reaction of MBTH with phenolic compounds is less dependent on the position of the substituent group than is that with 4AP. One disadvantage of the MBTH method, however, is that colours produced by different phenolic compounds range from red to violet and thus do not have their absorbance maxima at the same wavelength. The colours obtained have maxima ranging from 460 to 595 nm.

Goulden, Brooksbank and Day[5] have modified Friestad *et al.*'s automated method for use on relatively clean waters and have extended the detection limit from 10 to 0·2 $\mu g\ l^{-1}$. Their method consists basically of the automated distillation and condensation of a large sample (at a rate of 6·06 ml min^{-1}). After the colour formation step the product is concentrated by extraction into a solvent. This method can be used to analyse 10 samples per hour. In the course of their work, 60 Lake Ontario samples, containing 0–15 $\mu g\ l^{-1}$ of phenol, were analysed by both the automated 4AP and the automated MBTH methods. The results of this study showed no statistically significant difference between the two methods.

Goulden *et al.*'s automated method was not evaluated in this study because the distillation equipment and separator required are not commercially available. Rather, this paper is concerned with the evaluation of Friestad *et al.*'s manual and automated MBTH methods to establish their applicability for U.S. Environmental Protection Agency use by accumulating comparative data for the two methods on phenolic compounds and on samples of refinery

waste water, surface water and domestic waste. In the majority of determinations the MBTH method gave higher results, even though readings were made at a fixed wavelength. Minor changes were made in the sample volume, the reagents, the volume of reagents and the manifold. With these modifications, phenol can be determined by using both of these methods over a range from 1 to 1000 $\mu g\ l^{-1}$.

Manual Method

Apparatus

Samples were measured on a spectrophotometer at a wavelength of 520 nm and with a light path of 5 cm. A 750- or 1000-ml separating funnel was used for the extraction with chloroform.

Reagents

MBTH solution, 0·05 per cent. Dissolve 0·1 g of 3-methyl-2-benzothiazolinone hydrazone hydrochloride in 200 ml of distilled water.

Ammonium cerium(IV) sulphate solution. Add 2·0 g of ammonium cerium(IV) sulphate $[Ce(SO_4)_2.2(NH_4)_2SO_4.\ 2H_2O]$ and 1·5 ml of concentrated sulphuric acid to 150 ml of distilled water. After the solid has dissolved, dilute to 200 ml with distilled water.

Buffer solution. Dissolve, in the following order, 8 g of sodium hydroxide, 2 g of EDTA (disodium salt) and 8 g of boric acid in 200 ml of distilled water. Dilute to 250 ml with distilled water. Using this stock solution, make a working solution by mixing an appropriate volume with an equal volume of ethanol.

Procedure

Distillation

If the sample has not been preserved, add 0·5 g of copper sulphate and lower the pH to approximately 4 with concentrated sulphuric acid. If the sample has been preserved with orthophosphoric acid and copper sulphate at the time of collection, add 0·5 ml of sulphuric acid. Distil 450 ml of sample, add 50 ml of warm distilled water to the flask and resume distillation until 500 ml have been collected.

Colour development

Concentrations above 50 $\mu g\ l^{-1}$. To 100 ml of distillate, or an aliquot diluted to 100 ml, add 4 ml of MBTH solution. After 5 min add 2·5 ml of ammonium cerium(IV) sulphate solution. Leave the solution for another 5 min, add 7 ml of buffer and then, after 15 min, read the absorbance at 520 nm against a reagent blank. The colour is stable for 4 h.

Concentrations below 50 $\mu g\ l^{-1}$. To 500 ml of distillate add 4 ml of MBTH solution. After 5 min add 2·5 ml of ammonium cerium(IV) sulphate solution and after an additional 5 min add 7 ml of buffer. Then, after 15 min, add 25 ml of chloroform and extract the coloured product, shaking the separating funnel at least 20 times. Allow the layers to separate and pass the chloroform layer through filter-paper in order to remove any water. Read the absorbance at 490 nm against a reagent blank.

Discussion

Friestad *et al.*'s manual method has a detection limit of 1 mg l^{-1} of phenol. The colour is developed by the addition of 1 ml of MBTH solution, 1 ml of a 0·2 per cent. ammonium cerium(IV) sulphate solution and 2 ml of buffer solution to 1 ml of sample. For this study a sensitivity of 1 $\mu g\ l^{-1}$ was desired, therefore the following changes were made: the sample volume was increased from 1 to 100 ml and the same ratio of sample to reagent used in the automated method was used for colour development. This procedure resulted in a detection limit of 50 $\mu g\ l^{-1}$. Samples that contain less than this concentration are extracted with chloroform following development of the colour, and in this way a detection limit of 1 $\mu g\ l^{-1}$ is achieved.

Automated Method

Apparatus

The Technicon AutoAnalyzer that was used consisted of a sampler, a manifold, a proportioning pump III, a heating bath with distillation coil, a distillation head, a colorimeter equipped with 50-mm flow cell and 520-nm filter, and a recorder.

Reagents

MBTH solution, 0·05 per cent. As described under Manual Method.

Stock ammonium cerium(IV) sulphate solution. Dissolve 1·0 g of the solid in 150 ml of distilled water, add 3·0 ml of concentrated sulphuric acid and dilute to 200 ml with distilled water.

Working ammonium cerium(IV) sulphate solution. Dilute 25 ml of stock ammonium cerium(IV) sulphate solution to 100 ml with distilled water.

Buffer solution. Dissolve, in the following order, 8 g of sodium hydroxide, 2 g of EDTA (disodium salt) and 8 g of boric acid in 200 ml of distilled water. Dilute the solution to 250 ml.

Sodium hydroxide solution, 1 N. Dissolve 40 g of sodium hydroxide in 800 ml of distilled water. Dilute the solution to 1 l.

Sodium hydroxide solution, 0·01 N. Dilute 2 ml of 1 N sodium hydroxide solution to 200 ml with distilled water.

Distillation solution, 10 per cent. sulphuric acid. Add 100 ml of concentrated sulphuric acid slowly to 800 ml of distilled water. Cool the solution and dilute it to 1 l.

Sulphuric acid, approximately 1 per cent. Add 1 ml of concentrated sulphuric acid to 100 ml of distilled water.

Wash water. Add 20 ml of 1 N sodium hydroxide solution to 20 l of distilled water.

Procedure

Set up the manifold as shown in Fig. 1. Fill the wash receptacle by syphoning, using Kel-F tubing and a fast flow (1 l h^{-1}). Use polyethylene tubing for the sample line. Pump 0·01 N

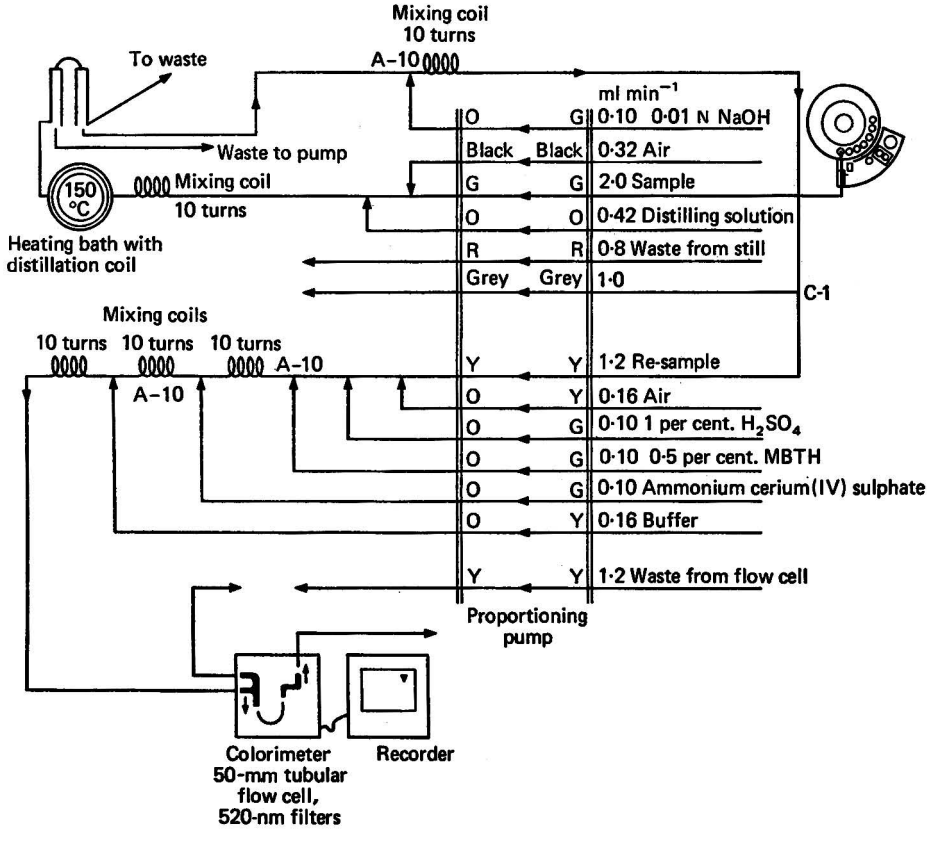

Fig. 1. AutoAnalyzer II circuit for the determination of phenols. Sample rate, 20 per hour. Colour code: O = orange, G = green, R = red and Y = yellow.

TABLE I

COMPARISON OF RESULTS OBTAINED FOR PHENOLS IN DISTILLED WATER BY THE MANUAL MBTH AND 4AP METHODS

Compound	Concentration added (calculated as phenol)/ $\mu g\ l^{-1}$	Concentration found (calculated as phenol)/ $\mu g\ l^{-1}$	
		4AP	MBTH
o-Cresol	140	100	130
m-Cresol	100	70	95
p-Cresol	100	<1	65
o-Chlorophenol	100	97	95
m-Chlorophenol	100	92	97
2,3-Dimethylphenol	100	45	45
3,4-Dimethylphenol	100	12	50
3,5-Dimethylphenol	100	23	60
2,6-Dimethylphenol	100	32	53
p-n-Butoxyphenol	100	19	45
2-Naphthol	100	<1	50

sodium hydroxide solution through new tubing for 30 min in order to remove phenolic material from the walls. Allow the colorimeter and recorder to warm up for 30 min. Next, run a baseline with all of the reagents present, feeding distilled water through the sample line. Place appropriate standards in the sampler tray in order of decreasing concentration, then complete the loading of the sampler tray with unknown samples, using glass tubes. If samples have not been preserved add 0·1 g of copper sulphate and 2 drops of concentrated sulphuric acid to 100 ml of sample.

Discussion

Friestad *et al.*'s automated method, with a detection level of 10 $\mu g\ l^{-1}$, is not sufficiently sensitive for most surface waters. However, by increasing the sampling rate before distillation from 0·8 to 2 ml min^{-1} and the sampling rate after distillation from 0·42 to 1·2 ml min^{-1}, the detection limit was extended to 1 $\mu g\ l^{-1}$. The concentration of the MBTH solution was not changed, but its flow-rate was reduced from 0·42 to 0·1 ml min^{-1}. The concentration of the ammonium cerium(IV) sulphate solution was reduced from 1 to 0·25 per cent. The flow-rate of the buffer solution was also reduced from 1·2 to 0·16 ml min^{-1}.

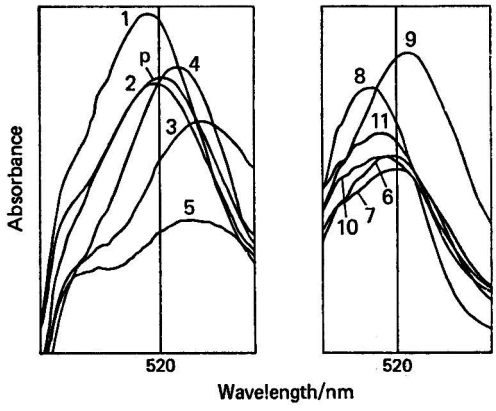

Fig. 2. Absorbance curves of 11 phenols analysed by use of the 3-methyl-2-benzothiazolinone hydrazone method. p, Phenol; 1, *o*-cresol; 2, *m*-cresol; 3, *p*-cresol; 4, *o*-chlorophenol; 5, 3,4-dimethylphenol; 6, 2-naphthol; 7, *p*-n-butoxyphenol; 8, 2,6-dimethylphenol; 9, *m*-chlorophenol; 10, 2,3-dimethylphenol; 11, 3,5-dimethylphenol.

TABLE II

COMPARISON OF RESULTS OBTAINED FOR PHENOLS IN DISTILLED WATER BY THE AUTOMATED MBTH AND 4AP METHODS

Compound	Concentration added (calculated as phenol)/ $\mu g\ l^{-1}$	Concentration found (calculated as phenol)/ $\mu g\ l^{-1}$	
		4AP	MBTH
o-Cresol	87	65	84
m-Cresol	87	69	86
p-Cresol	87	2	52
o-Chlorophenol	73	60	44
p-Chlorophenol	73	60	53
2-Naphthol	65	6	22
3,4-Dimethylphenol	77	4	30
p-n-Butoxyphenol	81	23	31

Results

Results obtained by the MBTH and 4AP methods with and without distillation were compared. Table I lists the results obtained from a series of phenolic compounds added to distilled water and analysed by the manual MBTH and 4AP methods (distillation omitted). Except for *o*-chlorophenol, *m*-chlorophenol and 2,3-dimethylphenol, which gave comparable results, higher results were obtained by use of the MBTH method. Fig. 2 shows the absorbance curves of these compounds with MBTH. The maxima for these compounds appear between 495 and 550 nm and a mixture of these 11 compounds showed a maximum absorbance at 520 nm.

TABLE III

COMPARISON OF RESULTS OBTAINED FOR PHENOLS IN OHIO RIVER WATER BY THE AUTOMATED MBTH AND 4AP METHODS

Compound	Concentration found (calculated as phenol)/ $\mu g\ l^{-1}$	
	4AP	MBTH
o-Chlorophenol + phenol	158	128
o-Chlorophenol + *p*-cresol	89	80
o-Cresol + *m*-cresol	61	87
p-Chlorophenol	51	47
2-Naphthol	10	20
3,4-Dimethylphenol	9	31
p-n-Butoxyphenol	21	32

Table II lists the results obtained from eight phenols added to distilled water and analysed by the automated MBTH and 4AP methods with distillation. Higher results were obtained by the MBTH method for all but two of the compounds, *o*-chlorophenol and *p*-chlorophenol. Subsequently, samples of Ohio River water were spiked with a series of phenols. As shown in Table III, these results confirmed the findings obtained with the distilled water samples.

The recovery of *o*-chlorophenol was determined by analysing a series of concentrations of this compound by the automated and manual MBTH methods. As shown in Table IV, the

TABLE IV

RECOVERY OF *o*-CHLOROPHENOL BY THE AUTOMATED MBTH METHOD

Concentration added (calculated as phenol)/ $\mu g\ l^{-1}$	Concentration found (calculated as phenol)/ $\mu g\ l^{-1}$	Recovery, per cent.
15	14	93
37	28	76
73	52	71
143	99	67

TABLE V

RECOVERY OF *o*-CHLOROPHENOL BY THE MANUAL 4AP AND MBTH METHODS WITH SOLVENT EXTRACTION

Sample	Concentration added (calculated as phenol)/ $\mu g\ l^{-1}$	Concentration found (calculated as phenol)/ $\mu g\ l^{-1}$		Recovery, per cent.	
		4AP	MBTH	4AP	MBTH
Sewage	29	25	33	86	114
Sewage	58	51	57	88	98
Sewage	146	151	146	103	100
Sewage	292	259	254	89	87
Distilled water	29	34	28	117	96
Distilled water	58	46	47	79	81
Distilled water	146	141	129	96	88
Distilled water	292	309	296	106	101
Mean	—	—	—	**96**	**96**

recovery of *o*-chlorophenol with the automated method decreased as the concentration increased. Table V gives the recovery of *o*-chlorophenol by the manual 4AP and MBTH methods from distilled water and sewage; unlike the results obtained by the automated method, the results obtained by the two manual methods were comparable.

The two manual methods were compared by analysing a petroleum waste and a raw sewage sample after spiking them with *p*-cresol. Significantly higher results were obtained for both samples with the MBTH method. The recovery of *p*-cresol with the MBTH method was 95 per cent. for the petroleum waste and 99 per cent. for the raw sewage, and less than 1 per cent. for both samples with the 4AP method.

In earlier studies on the determination of phenol by using the 4AP method, results obtained with the automated method were equal to those obtained with the manual method. Therefore, automated 4AP and MBTH methods were used to determine the reliability of the MBTH methods. Table VI shows the results obtained on oil refinery and lumber mill wastes by the two automated methods. Table VII lists the results obtained by use of the manual 4AP and MBTH methods on industrial wastes. In all instances except one the MBTH method gave higher results.

The precision of the automated MBTH method was determined at four separate concentration levels over two working ranges (2–42 $\mu g\ l^{-1}$ and 20–134 $\mu g\ l^{-1}$). They included a concentration near to the detection limit of the method, two concentrations at intermediate levels and one near the upper limit. Seven replicate determinations were made for each concentration tested. For phenol concentrations of 2·1, 5·7, 20 and 42 $\mu g\ l^{-1}$, standard deviations were $\pm 0{\cdot}7$, $\pm 0{\cdot}5$, $\pm 1{\cdot}2$ and $\pm 0{\cdot}7\ \mu g\ l^{-1}$, respectively. At concentrations of 2, 11, 53 and 134 $\mu g\ l^{-1}$, standard deviations were $\pm 0{\cdot}9$, $\pm 0{\cdot}4$, $\pm 1{\cdot}1$ and $\pm 1{\cdot}3\ \mu g\ l^{-1}$, respectively. The

TABLE VI

COMPARISON OF RESULTS OBTAINED FOR OIL REFINERY AND LUMBER MILL WASTE BY THE AUTOMATED MBTH AND 4AP METHODS

Type of waste	Sample dilution ratio	Concentration found (calculated as phenol)/ $\mu g\ l^{-1}$	
		4AP	MBTH
Oil refinery waste	1:100	24	24
Oil refinery waste	1:50	47	48
Oil refinery waste	3:100	71	70
Oil refinery waste	1:20	120	124
Lumber mill waste			
5 Plywood	1:20	104	98
5 Log pond	1:20	9	10
0·5 Hardwood	1:200	66	76

percentage recovery with the automated method was determined at two levels by spiking Little Miami River water with *o*-cresol. At concentrations of 2·7 and 65 $\mu g\ l^{-1}$, the recoveries were 83 and 98 per cent., respectively.

TABLE VII

COMPARISON OF RESULTS OBTAINED BY THE MANUAL 4AP AND MBTH METHODS ON VARIOUS INDUSTRIAL WASTES

	Concentration found (calculated as phenol)/$\mu g\ l^{-1}$	
Sample	4AP	MBTH
1	10	20
2	12	24
3	16	27
4	14	17
5	33	35
6	29	16
7	2060	2900
8	253	396

Interferences

MBTH is also used to determine aliphatic aldehydes[6] and aromatic amines.[7] Thus these materials, if present, could cause interference. According to the literature,[4] however, distillation removes aromatic amines from the samples, whereas aliphatic aldehydes remain in the distilled sample. The effect of the presence of these compounds was determined by analysing a sample containing n-butyraldehyde, glyoxal and formaldehyde at concentrations of 100 $\mu g\ l^{-1}$. With this procedure a green dye was formed when these compounds were made to react with MBTH; however, the dye was destroyed when the buffer solution was added. n-Butyraldehyde did give a response equivalent to 3 $\mu g\ l^{-1}$ of phenol at this level, but glyoxal and formaldehyde gave no response.

Conclusion

The results obtained with the MBTH method are higher than those obtained by use of the 4AP method when cresol, naphthol and *para*-substituted phenols are the dominant phenols in the sample. As with the 4AP method, the MBTH method will not give 100 per cent. recovery of all phenolic compounds when phenol is the basis for the standard curve. The detection limit and precision of the method are satisfactory for the determination of phenol in surface waters, domestic wastes and industrial wastes. The 4AP method results in a highly coloured blank, making it difficult to detect 5 $\mu g\ l^{-1}$ but the MBTH method, with extraction, has a very low blank and 1 $\mu g\ l^{-1}$ can easily be detected.

Although the MBTH method appears to offer certain advantages, the usefulness and applicability of the method can be determined only after exhaustive analyses on a large variety of sample types.

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A Rapid Method for the Simultaneous Determination of Paraquat and Diquat in Pond and River Waters by Pyrolysis and Gas Chromatography

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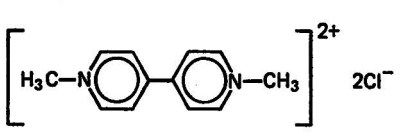
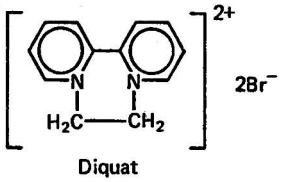
A rapid method is described for the determination, in aqueous systems, of two widely used herbicides known commercially as paraquat and diquat. Pyrolysis of these herbicides under carefully controlled conditions, followed by gas-chromatographic analysis of the pyrolysate, allows detection of the herbicides down to 0·01 p.p.m. At the lowest concentration levels (0·01–0·1 p.p.m.), there is some loss of linearity of response, possibly as a result of adsorption of the herbicides on the glass surfaces of the vessels used. This effect may have resulted in substantial errors in previously reported low levels of paraquat and diquat and may also occur in the determination of other ionic herbicides.

In recent years, the commercial production and use of herbicides has increased rapidly. Of these, 1,1′-dimethyl-4,4′-bipyridylium chloride (paraquat) and to a lesser degree 1,1′-ethylene-2,2′-bipyridylium bromide (diquat) are of particular importance.[1] Both are quaternary ammonium salts and their structural formulae are given below.



Paraquat



Diquat

As a result of their inherent stability and toxicity there has been increasing interest in them, not least from analytical chemists, with regard to environmental pollution. Previous methods of analysis have depended either on the reduction of the herbicide with sodium dithionite[2] to form a free radical that absorbs in the ultraviolet region or on reduction to the corresponding piperidine followed by gas-chromatographic analysis.[3] Additionally, a bioassay technique has been reported[4] but this procedure, although extremely sensitive, is very time consuming and is of little use if a rapid result is required. A method for the analysis of soils,[5] involving catalytic hydrogenation of the herbicide followed by gas chromatography, has recently been reported, and also a pyrolytic method,[6] but the latter method incorporates a preliminary ion-exchange procedure.

The gas-chromatographic procedure described here requires no sample pre-treatment and can normally be completed in 15–20 min. The method has been developed with regard to the affinity of the herbicides for glass surfaces, a factor not previously reported and apparently not taken into consideration in current analytical procedures.

Experimental

Reagents

Paraquat. Methyl viologen hydrate, obtainable from Aldrich Chemical Co. Ltd.

Diquat. Obtained by crystallisation from Reglone A (Plant Protection Ltd., ICI).

Preparation of standard solutions

Standard solutions of the herbicides were prepared in de-ionised water. In order to mitigate errors due to possible differential adsorption of paraquat and diquat on glass, a single calibrated flask was used for the initial calibration. After containing herbicide solutions the flask was thoroughly cleaned by repeated washing with de-ionised water. It was then

filled with de-ionised water and allowed to stand for 1 h before checking the contents for the absence of herbicide by the proposed gas-chromatographic method.

In order to examine the affinity of the herbicides for glass, standard solutions were prepared with added glass beads (1 g of BDH 100-mesh glass beads for gas - liquid chromatography) having a surface area of about 0·04 m^2, *i.e.*, about ten times that of the vessel used. Determinations of the herbicide content were made before and after addition of the beads.

Apparatus

A Perkin-Elmer F30 gas chromatograph having a standard injection-port modification for a Chemical Data Systems (CDS) Pyroprobe 190 was used throughout. Silica pyrolysis tubes were as supplied with the Pyroprobe. Studies were carried out in order to establish optimum conditions for the pyrolysis, *viz.*, pyrolysis temperature and time, heating rate (ramp) and probe insertion distance. The data (Table I, Figs. 1 and 2) show that best results were obtained under the following conditions: temperature, 1000 °C; pyrolysis time, 5 s; ramp, 2·0 °C ms^{-1}; and probe insertion distance, maximum. The significance of these values is discussed in detail below.

TABLE I

EFFECT OF PROBE INSERTION DISTANCE ON PEAK AREA AND RETENTION TIME FOR 2,2′-BIPYRIDYL

Withdrawal from maximum insertion distance/mm	2,2′-Bipyridyl peak area/counts × 10^{-3}	Retention time/s
0	99·9	264
3	96·5	264
8	99·6	265
14	94·4	266
19	99·8	268
23	97·5	268
29	94·2	270
38	95·6	270
43	98·7	272
48	94·2	273
58	92·0	278
65	85·9	282

Gas-chromatographic conditions were as follows: column, 10 per cent. Carbowax 20M and 2 per cent. potassium hydroxide on Celite (80–100 mesh) in a 600 × 3·5 mm i.d. glass column; column temperature, 190 °C; injection-port temperature, 110 °C; carrier gas, nitrogen at a flow-rate of 40 cm^3 min^{-1}. A flame-ionisation detector was used.

Measurements of peak area were made electronically by using an Infotronics CRS 208 digital integrator fitted with an angular base-line corrector.

It was necessary to allow for slight day-to-day variations in detector response. In the

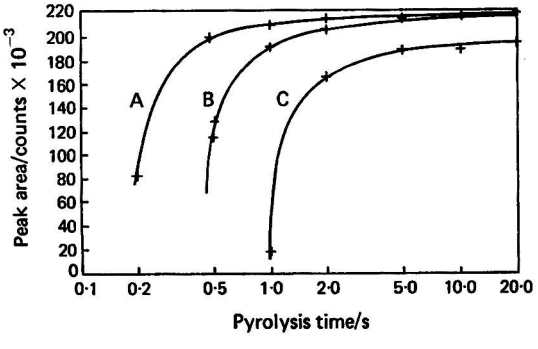


Fig. 1. Variation of yield of bipyridyls with pyrolysis time at different final pyrolysis temperatures: A, 1000; B, 800; and C, 600 °C.

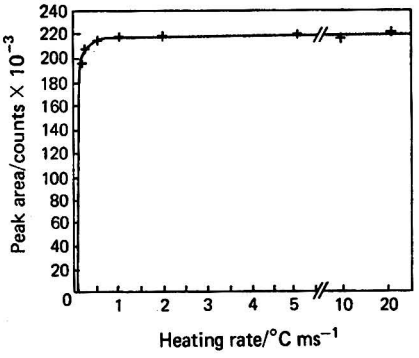


Fig. 2. Variation of yield of bipyridyls with probe heating rate (ramp).

absence of an internal standard, a standard herbicide solution (100 μl, 1 p.p.m.) was run twice daily in order to obtain a response factor.

Procedure

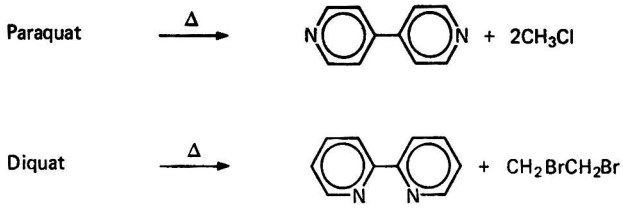
Standard volumes of herbicide solution (up to 100 μl) were injected carefully from a 100-μl syringe [Scientific Glass Engineering Pty. Ltd. (Australia)] into the centre of a silica tube and the tube was heated continuously at 100–110 °C in an air stream from a hot-air blower. The best results were obtained when the solution was injected continuously so that it was not allowed to evaporate to dryness until all of the solution had been introduced into the tube. Care should also be taken not to allow the solution to fill completely the bore of the tube.

The tube was then inserted into the coil probe of the CDS Pyroprobe 190 and the sample pyrolysed under optimum conditions (see below). Analysis of the pyrolysate was carried out by use of the Perkin-Elmer F30 gas chromatograph.

The silica tubes used in this procedure were permanently kept in a furnace at 800 °C and were always handled with stainless-steel forceps.

Results and Discussion

The method described in this paper for the determination of paraquat and diquat is based on the following reactions.



Although other reactions occur that give smaller fragments, it will be apparent [Fig. 3 (*b*) and (*c*)] that the pyrolysis of both compounds produces few products with relative molecular masses comparable to those of the free bases, a feature which renders the method particularly suitable for both quantitative and qualitative analysis. Soderquist and Crosby[3] referred to the thermal breakdown of paraquat during direct injection at high injection-port temperatures, but did not attempt to develop an analytical procedure based on this effect.

One of the main problems encountered in pyrolysis studies has been lack of reproducibility but use of the CDS Pyroprobe 190 minimises errors of this type and successive pyrolyses now give an acceptably reproducible pattern (Table II). However, for best results, the procedure described in this paper must be strictly adhered to. In particular, pyrolysis of samples applied directly to the coil probe or ribbon probe gave extremely poor sensitivity, owing to

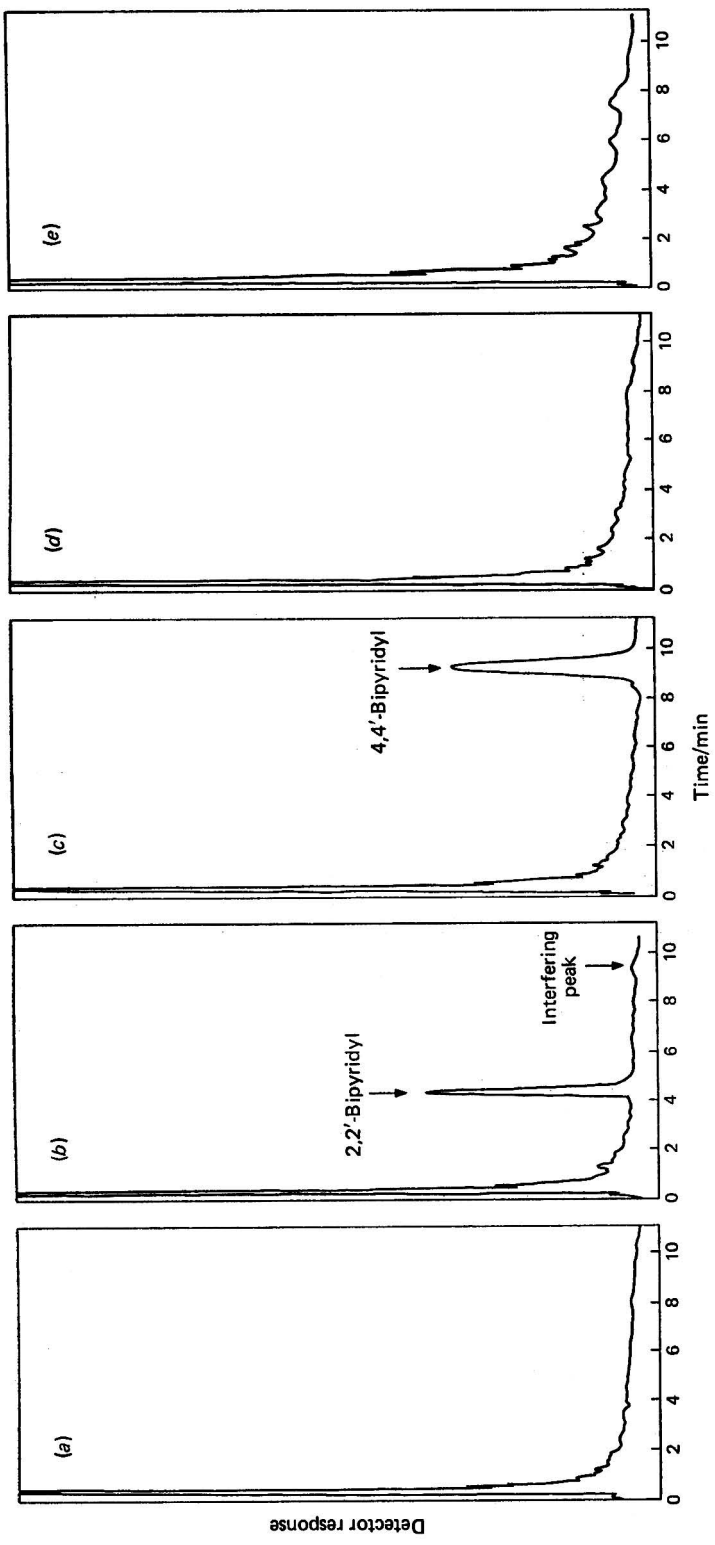


Fig. 3. Pyrolysis patterns obtained from 100-μl samples of various aqueous systems: (*a*), de-ionised water; (*b*), diquat solution, 0·3 p.p.m.; (*c*), paraquat solution, 0·5 p.p.m.; (*d*), River Gwynnedd; and (*e*), River Ebbw.

low yields of the bipyridyls, and the results obtained were not satisfactorily reproducible. A possible explanation is that the rapid rise in the temperature of the probe surface gives a thermal shock to the sample and solid material is thus ejected from the surface, which would result at most in partial pyrolysis of the sample with resulting low yields of base. Reproducibility would thus be a function of sample film thickness, a parameter virtually impossible to control at this level of sample size. These problems do not arise when the pyrolyses occur in the silica tube. The confined nature of the system allows time for completion of the pyrolysis before the volatile compounds emerge from the tube, as unpyrolysed or partially pyrolysed material cannot easily escape from the tube. Other advantages of the method are the ease of sample application and the fact that several samples can be prepared prior to pyrolysis, which would not be possible if the probe alone were used.

TABLE II

VARIATION OF BIPYRIDYL PEAK AREA WITH CONCENTRATION OF PARAQUAT OR DIQUAT

Concentration, p.p.m.	Bipyridyl peak area/ counts × 10^{-3}	Relative standard deviation, per cent.*
0·01	0·6	20·0
0·02	1·5	13·0
0·05	4·4	11·4
0·08	7·5	10·2
0·10	9·3	9·9
0·20	19·4	7·2
0·50	51·3 (12·4)†	3·8
0·80	83·3	2·6
1·00	104·4 (12·9)†	2·1
1·00	125·0	2·0
1·40	146·5	2·0
1·60	167·6	2·0
1·80	188·8	2·0
2·00	210·0	2·0

* Values calculated on the basis of a minimum of five determinations.
† Values for the bipyridyl peak area obtained in the presence of added glass beads.

The effects of the various pyrolysis parameters are shown in Table I and in Figs. 1 and 2. The greatest sensitivity is obtained with the highest available working temperature, *i.e.*, 1000 °C, but increasing the pyrolysis time above 5 s does not significantly improve the method. In fact, it is desirable to keep the pyrolysis time to a minimum in order to reduce the ageing effect on the column produced by the pulse of hot carrier gas, which results in stationary-phase material being stripped from the column immediately beyond the probe [Fig. 3 (*a*)]. This effect can result in adsorption of the bases on the exposed support material, giving low results. Further, it is essential that glass columns be used, as stainless-steel columns give substantially reduced yields of the bipyridyls, particularly at low concentrations of herbicide.

The heating rate (ramp) of the probe is critical. Fig. 2 shows that ramps not less than 2·0 °C ms^{-1} are necessary for highest sensitivity, but for maximum column life this value should not be significantly exceeded. The position of the probe in the injection port is not critical to the sensitivity except at withdrawal distances near to the maximum (Table I), but there is a steady increase in retention time as the probe is withdrawn. This increase is accompanied by a substantial loss in resolution (probably owing to an increase in the injection-port dead volume), which would be undesirable if pyrolysis products with similar retention characteristics to that of the bipyridyls were present.

The detection limits for the method as applied to pond and river waters are governed by two main factors: the size of sample that can conveniently be introduced into the pyrolysis tube; and the ability of the column to resolve the bipyridyl peaks from those due to other pyrolysis products. Of a total of nine samples of local pond and river waters most gave a simple pyrolysis pattern [Fig. 3 (*d*)]. The most complex pattern so far obtained [Fig. 3 (*e*)] shows that no interference with paraquat will occur, and that only slight interference with diquat is likely. However, a small diquat pyrolysis peak [Fig. 3 (*b*)] can interfere to a slight extent with the 4,4′-bipyridyl peak derived from paraquat but the value for paraquat may be simply corrected when appropriate, as the size of the interfering peak is proportional to the size of the 2,2′-bipyridyl peak derived from diquat.

While many references have been made to the adsorption of herbicides on various materials,[1] none appears to have been made to the adsorption of herbicides on glass surfaces. It will be apparent from the work described above, in which solutions were prepared with added glass beads (Table II) that significant errors can occur when herbicide solutions are transferred from one vessel to another before analytical determinations are carried out. This observation is particularly important when solutions that have low herbicide concentrations are being studied. It is recommended, therefore, that field sampling be carried out in vessels that are subsequently used for the analytical determination and the samples should be treated identically with standard solutions used for calibration purposes.

The authors thank the National Environmental Research Council for financial support and Dr. J. D. R. Thomas for helpful discussions.

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Determination of Dimetridazole in Feedstuffs and Pre-mixes by High-speed Liquid Chromatography

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A method is described for the determination of dimetridazole (1,2-dimethyl-5-nitroimidazole) in feedstuffs and pre-mixes by high-speed liquid chromatography. Dimetridazole is extracted from the sample with methanol - water (1 + 2). After liquid - liquid extraction into dichloromethane, an aliquot of the solution is injected into a high-speed liquid chromatograph.

Dimetridazole (1,2-dimethyl-5-nitroimidazole) is a feed additive used for the control of blackhead in turkeys and for the treatment of haemorrhagic dysentery in pigs. The usual content in feedstuffs is 125–150 mg kg^{-1}. Dimetridazole can be assayed by a number of methods.[1–5] We normally use a thin-layer chromatographic method and a modification of the polarographic method published by the Analytical Methods Committee.[5] Liquid chromatography is an increasingly applied technique, and it was of interest to ascertain whether this technique could be applied in feedstuffs analysis.

Experimental

Reagents

All reagents should be of analytical-reagent grade.
Methanol - water (1 + 2 V/V).
Hydrochloric acid, 5 mol l^{-1}.
Dichloromethane.
Sodium sulphate, anhydrous.
Eluting solvent: chloroform - methanol (99·5 + 0·5 V/V).*
Dimetridazole reference standard.

Apparatus

The high-speed liquid chromatographic apparatus was assembled from the following parts: a high-pressure pump, Model 6000 (Waters Associates Inc., Milford, Mass., USA), and a universal injection system, Model U6K, from the same suppliers.

A stainless-steel column (600 × 3 mm), packed with Lichrosorb SI 60 slurry of particle size 10 μm, was used. A smaller column (250 × 3 mm), packed with alumina (Merck, Darmstadt, 1097) sieved to give a fraction greater than 200 mesh, was used as a pre-cleaning column directly coupled to the injector. These columns have been in use for almost 1 year. So far, there has been no necessity to change packings. Eluted components were measured with a spectrophotometer (Beckmann, Model 25, Fullerton, Calif., USA) equipped with a flow-through cell of path length 18 μm specially designed for this model (available from Waters Associates Inc.).

Measurements were recorded on the recorder of the spectrophotometer.

The operating conditions used were as follows: flow-rate, 0·5 ml min^{-1}; monochromator set at 308 nm (measurements were made against eluting solvent in the reference cell); paper speed, 0·1 in min^{-1}; and measuring range, 0–1 or 0–2 absorbance full scale.

Other equipment included a rotary evaporator and normal laboratory glassware.

Procedure

Weigh accurately 2–20 g of sample containing 0·2–40 mg of dimetridazole into a 200-ml glass-stoppered flask. (For feeds containing 125 mg kg^{-1} of dimetridazole, a 10-g sample is usually taken.) Add 100·0 ml of methanol - water and agitate the mixture mechanically for 30 min. (For feeds containing 125 mg kg^{-1} of dimetridazole, 50·0 ml of methanol - water

* The chloroform used contained about 1 per cent. of ethanol.

is usually added.) Centrifuge it for 5 min at 3000 rev min^{-1} and filter it on a filter-paper or on cotton-wool if necessary. From this filtrate, prepare a solution in methanol - water containing 2–40 mg l^{-1} of dimetridazole (dilution factor F = final volume divided by the initial volume).

Transfer 25·0 ml of this solution into a 100-ml separating funnel and adjust the pH to about 1·5 with hydrochloric acid (5 mol l^{-1}), checking with test paper so as to ensure that the pH is in the range 0–6. Add 25 ml of dichloromethane and invert the funnel ten times.

After the phases have separated, run off the dichloromethane layer through a funnel fitted with a cotton-wool plug, and containing about 5 g of anhydrous sodium sulphate, into a 250-ml evaporating flask. Extract the aqueous layer three more times with 25-ml portions of dichloromethane, shaking the funnel well for 1 min each time. Collect all of the dichloromethane extracts in the same evaporating flask. Finally, rinse the funnel containing anhydrous sodium sulphate twice with 5-ml portions of dichloromethane.

Evaporate the combined extracts just to dryness at room temperature, ensuring that the temperature does not rise above 30 °C, and dissolve the residue in 2·0 ml of eluting solvent. Operate the chromatograph under the conditions described above and inject 0·02 ml of the solution.

A 0·02-ml aliquot of a standard solution of dimetridazole (500 mg l^{-1}) should be injected with each run of samples.

Calculation

Compare the peak height of the sample with the peak height of a standard.

When 50·0 ml of extraction solvent have been used, the content of dimetridazole is given by:

$$w = 20 \times 10^4 F m_A / m_S$$

and when 100·0 ml of extraction solvent have been used, by

$$w = 40 \times 10^4 F m_A / m_S$$

where w mg kg^{-1} is the mass fraction of dimetridazole in the sample, F is the dilution factor as defined above, m_A mg is the mass of dimetridazole injected on to the column and m_S g is the mass of sample.

Results and Discussion

When developing an assay method with high-speed liquid chromatography, one can work by analogy with experience gained with thin-layer chromatography. Our experience with the latter method with dimetridazole proved to be a good starting point. In our thin-layer chromatographic method for the determination of dimetridazole, chloroform - methanol (95 + 5) was used as eluting solvent and silica gel as the adsorbent. The R_F value for dimetridazole with this system is about 0·6, and with alumina as adsorbent 1·0. For our purpose, a less polar solvent than that required for thin-layer chromatography should be used, and the solvent chloroform - methanol (99·5 + 0·5) was found to be suitable. With this solvent, the chromatograms shown in Fig. 1 were obtained.

No interference was found from: ipronidazole, amprolium, ethopabate, dinitolmide, buquinolate, decoquinate, methyl benzoquate, acetyl enheptin, nitrofurazone, furnicozone, nicarbazin, nitrovin, carbadox, robenidine, pyrimethamine, ronidazole, monensin, sulphaquinoxaline, sulphamezathine, sulphacetamide, tetracycline, oxytetracycline, penicillin, streptomycin, zinc bacitracin, tylosin, oleandomycin, virginiamycin and spiramycin.

Meticlorpindol, when present in the usual concentration (125 mg kg^{-1}), gave a "dimetridazole recovery" of 3 mg kg^{-1}. Furazolidone also interfered, when present in the usual concentration (50 mg kg^{-1}), and in this instance a "dimetridazole recovery" of 12 mg kg^{-1} was found. This interference could be eliminated by choosing a different eluting solvent: n-hexane - methanol - ethanol (65 + 15 + 20 V/V). The retention time of dimetridazole was substantially increased by the use of this solvent. In practice, however, combinations of dimetridazole with meticlorpindol or furazolidone seldom occur, and we were therefore able to continue with our original eluting solvent.

Concentrations of dimetridazole down to 10 mg kg^{-1} can be determined by the above

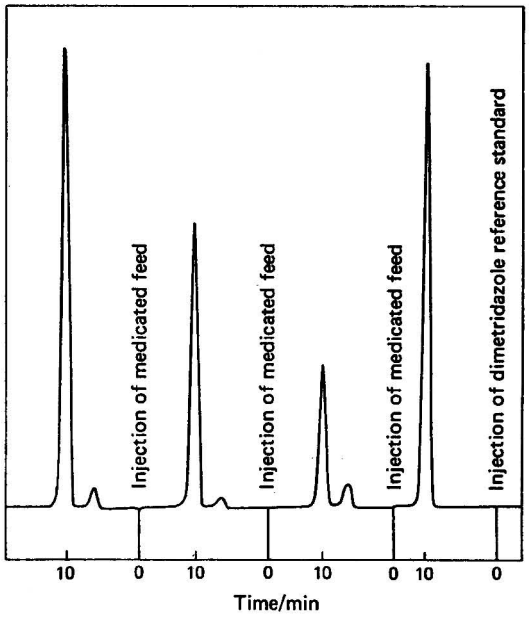

Fig. 1. Typical chromatogram obtained by high-speed liquid chromatography of feedstuffs medicated with dimetridazole.

method, and a lower level of detection can be achieved by making a few modifications to the method.

Dimetridazole reference standard taken through the method in different amounts was recovered completely by this method, with a standard deviation of 2 per cent. A satisfactorily linear relationship between peak height and mass of dimetridazole injected was found, and no increase in the width of the band was observed when injecting volumes of up to 0·1 ml.

Dimetridazole was incorporated, at a concentration of 150 mg kg^{-1}, into a poultry feed containing 2 per cent. of grass meal and 5 per cent. of fish meal and into a pig feed containing 4 per cent. of grass meal and 2 per cent. of fish meal. The blank value for both feeds was less than 1 mg kg^{-1}. The dimetridazole was recovered completely from both feeds, with a standard deviation of 7 mg kg^{-1}. The limit of error for a 95 per cent. probability level was 4 mg kg^{-1} for the poultry feed and 5 mg kg^{-1} for the pig feed. The results of 15 determinations for the recovery of 150 mg kg^{-1} of dimetridazole in the poultry feed were 162, 153, 142, 150, 159, 147, 155, 147, 147, 154, 145, 154, 143, 160 and 139 mg kg^{-1}; and of nine determinations in the pig feed were 139, 144, 156, 145, 155, 157, 142, 152 and 155 mg kg^{-1}.

Results by this method have been continuously compared with those obtained by our current assay methods; no significant differences between the results of the three methods were found.

The presence of bentonite, sometimes used as an aid in the pelleting of feeds, caused low recoveries of dimetridazole. When 2 per cent. of bentonite was added to a feed medicated with dimetridazole about 80 per cent. of the dimetridazole was recovered.

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A Rapid Method for Monitoring Low Levels of Di-(2-ethylhexyl) Phthalate in Solutions

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A simple gas - liquid chromatographic method using a nickel-63 electron-capture detector for the determination of di-(2-ethylhexyl) phthalate (DEHP) in blood and aqueous solutions is described. The DEHP is extracted into n-hexane in a single-step extraction and is injected directly, using 5 per cent. SE-30 as liquid phase. The detection limit was 2 ng and a linear detector response was found in the range 2–20 ng. The method was used to monitor DEHP in blood and aqueous or lipophilic solutions.

The widespread use of plastic materials in medicine has been accompanied by a rapid increase in the use of phthalate esters as plasticisers. In particular, the ester di-(2-ethylhexyl) phthalate (DEHP) is added in high concentration (20–40 per cent.) to poly(vinyl chloride) in order to increase the softness and flexibility of the plastic material.

For many years phthalate esters were assumed to be of low toxicity. However, recent reports have described toxic and teratogenic effects of phthalate esters in laboratory animals.[1,2] DEHP is also known to be an environmental pollutant[3] and a contaminant of many products that are packed in plastic[4]; the attention of investigators has therefore been focused on this new potential health hazard.[5] Residues of DEHP have been reported in human blood stored in poly(vinyl chloride) bags,[6] in patients receiving such blood,[7] in milk,[8] in soya oil,[9] in water[10] and in the mitochondria of the heart muscle of different animals.[11]

Analytical procedures have been developed for the micro-determination of DEHP; sensitive and selective techniques are based on gas chromatography using a flame-ionisation detector[12] or combined gas chromatography - mass spectrometry.[13] The latter method is time consuming, requires sophisticated instruments and is therefore not suitable for screening large numbers of samples. A simple and rapid method that possesses high sensitivity, specificity and reproducibility is required, so that the levels of DEHP accumulating in drugs, foodstuffs, blood, tissues and the environment can easily be monitored. The most practical methods for monitoring programmes are based on gas - liquid chromatography using electron-capture detectors. Lee *et al.*[14] employed gas - liquid chromatography with an electron-capture detector equipped with a tritium foil to detect low concentrations of phthalate esters extracted into n-hexane. This method was subsequently applied to the quantitative determination of phthalate esters in water[15]; a graph of peak area *versus* concentration was found to be linear over the range 100–1000 ng, the lower limit of detection being 10 ng.

This paper describes a sensitive and simple method for the quantitative determination of DEHP using a gas chromatograph equipped with a nickel-63 electron-capture detector. The method was used to monitor DEHP residues in solutions (especially aqueous solutions and blood) that had been packed in poly(vinyl chloride) bags.

Materials and Methods

Reagents

n-Hexane. This solvent was purified by distillation or by the method of Williams.[16]

Di-(2-ethylhexyl) phthalate, 100·5 per cent. This material was supplied by Travenol, Ashdod, Israel.

Acetonitrile.

All other reagents were of analytical-reagent grade and were tested for freedom from DEHP.

Apparatus

A Packard, Model 7400, dual-column gas chromatograph, equipped with a nickel-63 electron-capture detector and a coiled glass column, 6 ft × $\frac{1}{8}$ in i.d., was packed with 5 per cent. SE-30 on Gas-Chrom Q, 80–100 mesh. Nitrogen was used as the carrier gas at a flow-rate of

20 ml min^{-1}, and the sensitivity of the electrometer was 1×10^{-9} A. The temperatures of the injection port, detector and column were set at 270, 260 and 240 °C, respectively. A 10-μl Hamilton syringe was used to inject the solutions.

Method

Aqueous solutions

To 50 ml of the aqueous solution in a 100-ml glass-stoppered graduated cylinder, 6 ml of acetonitrile and 10 ml of n-hexane were added. The mixture was agitated for 90 min on a wrist-action shaker. After complete separation between the two phases had been obtained on standing the mixture, the n-hexane phase was collected. A series of aliquots (2–8 μl) of the n-hexane phase were then injected into the gas chromatograph. The heights of the peaks were measured and compared with those obtained from standard dilutions of DEHP run under the same conditions.

Blood

Absorbent cotton-wool (25 mg) was introduced into the bottom of a threaded test-tube. Acetonitrile (0·2 ml) and plasma (0·5 ml) were added. After the fluids were completely absorbed into the cotton-wool, 5 ml of n-hexane were added. The test-tube was next tightly stoppered with an ordinary cork (not with a screw cap) and agitated in a wrist-action shaker for 45 min. Then the n-hexane phase was separated and 2–8-μl aliquots were injected into the gas chromatograph.

Results

A nickel-63 radioactive source, rather than a tritium foil, was used as the detector because better resolution was obtained with the former. This results from the ability of the nickel detector to withstand high temperatures, which serve to keep the detector clean, a prerequisite for obtaining good analytical results with electron-capture detection. Different stationary phases have been recommended for the determination of DEHP by use of gas chromatography. SE-30, which was used by other investigators for the determination of DEHP by gas chromatography with a flame-ionisation[9] or electron-capture detector,[17] was employed in this study. Our method, using electron-capture detection, was 100 times more sensitive than that employing flame-ionisation detection. This high sensitivity can be explained by the type of so-called electrophores present in the molecules. The electrophore, CO–CH:CH–CO, present in phthalate esters has a high electron absorption similar to that of polychloro derivatives.[18] It has been observed[19] that the electron affinity of the phthalate esters decreases from the low to the high homologues. Dimethyl, diethyl and dibutyl phthalates can be determined at levels of 100–200 pg, whereas DEHP can be determined only in the nanogram range.

A typical chromatograph of a mixture of DEHP and aldrin, used as internal standard, is shown in Fig. 1. The peak is symmetrical and Gaussian in shape and can be used for quantitative analysis in the nanogram range (2–20 ng). Detector responses could, therefore, be measured by reference to the height of the peak and not to its area; the latter measurement is time consuming or requires an expensive electronic integrator (Table I). However, the day-to-day

TABLE I

CORRELATION BETWEEN PEAK HEIGHT AND PEAK AREA OF DEHP AND RATIO OF ALDRIN (INTERNAL STANDARD) TO DEHP

Amount		Peak height			
Aldrin/ pg	DEHP/ ng	Aldrin/ cm	DEHP*/ cm	Ratio, aldrin: DEHP	Peak area for DEHP*/cm^2
55	4	2·0	1·70	1·18	0·9
110	8	3·7	3·20 ± 0·14 (10)	1·16	1·54 ± 0·06 (10)
165	12	5·4	4·7	1·15	—
220	16	7·4	6·2 ± 0·34 (10)	1·18	2·97 ± 0·17 (10)

* Values in parentheses denote the number of determinations.

variations in the sensitivity of the electron-capture detector must be taken into consideration and therefore daily standardisation with DEHP is recommended. Under the conditions described above, the minimum amount measured was 2 ng. Smaller amounts of DEHP could be detected at a lower electrometer range although this is limited by the background and baseline noise.

In order to study the linearity of the detector response, a series of standard solutions of DEHP in n-hexane were prepared. Each dilution was mixed with an equal volume of standard aldrin solution and injected into the chromatograph. From the results shown in Fig. 2 it can be seen that a linear relationship was found between the response of the electron-capture detector and the concentration of DEHP in the 4–18-ng range, with a straight correlation ratio to the aldrin. Good reproducibility of results was established by repeated determinations of DEHP that contained aldrin as a comparative standard. The response of the electron-capture detector was found to be independent of the volume injected; identical responses were obtained when the same amount of DEHP was introduced in volumes of 2–10 μl of n-hexane.

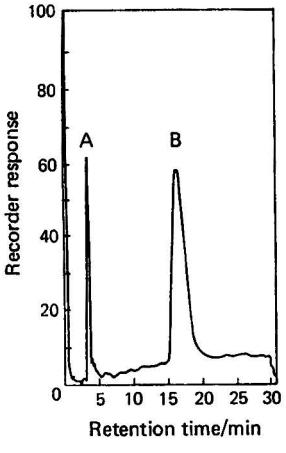

Fig. 1. Chromatograms of aldrin (A) (internal standard) and DEHP (B). For instrument parameters, see text.

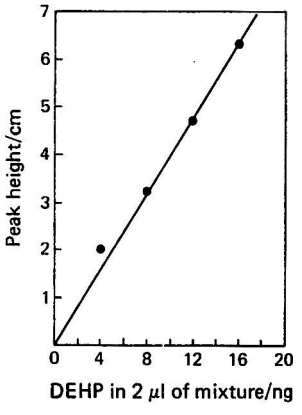

Fig. 2. Calibration graph for DEHP.

The efficiency of this simple extraction system was verified by running blanks consisting of water fortified with DEHP. n-Hexane was found to be an effective solvent and the addition of acetonitrile optimised the extraction of the DEHP (Table II). n-Hexane extracted more than 90 per cent. of the DEHP from the aqueous solution on the first extraction and less than 5 per cent. on the second. We therefore carried out only one extraction, which we considered to be sufficiently accurate for a rapid method.

TABLE II

RESULTS OF REPLICATE ANALYSES OF PURIFIED WATER CONTAINING ADDED DEHP

Three samples were analysed at each concentration.

Amount of DEHP added/mg	DEHP found/mg	Recovery, per cent.
12·5	11·8	91·2 ± 5·2
25·0	22·7	90·8 ± 4·1
31·25	29·9	95·7 ± 2·9

A series of samples of water and saline and glucose solutions packed in poly(vinyl chloride) were tested. The solutions were found to be free from DEHP [the sensitivity limit was 50 p.p.b. (parts per 10^9)]. By increasing the volumes of the solutions used a sensitivity of 10 p.p.b. could be obtained. The results obtained for aqueous solutions stored in plastic bags show that the above residues of DEHP fall within a satisfactory margin of safety (Table III).

TABLE III

DEHP RESIDUES IN AQUEOUS SOLUTIONS

Source	DEHP found, p.p.m.
Distilled water (glass bottle)	None
Saline solution (glass bottle)	None
Saline solution - glucose solution (in plastic bags)	0·05–0·08 (0·13)*
Saline solution (glass bottle with rubber stopper, equipped with plastic tube and stored for 10 years)	0·07–0·8

* Sample of saline kept for 6 months at 37 °C.

A sample of normal saline in poly(vinyl chloride) bags, kept in our laboratory for 6 months at 37 °C, was found to contain 80 p.p.b. of DEHP. Higher concentrations were found in saline solutions that had been stored for 10 years in glass bottles that were stoppered with a rubber cork fitted with a plastic tube. It is therefore worthwhile to test for DEHP contamination of similar preparations used in infusions, such as protein hydrolysates, or solutions containing lipophilic substances. When using gas - liquid chromatography with flame-ionisation detection, Rubin[20] was unable to detect DEHP in a solution stored in a Travenol Viaflex container (formula PL 146) for more than 1 year over a wide range of ambient temperatures. In his experiments the blanks gave high values (0·24 p.p.m.) for DEHP; these high values can be related to the fact that chemicals are usually stored in plastic containers and can consequently be contaminated with DEHP. We therefore stress the importance not only of examining all chemicals for the presence of DEHP but also of checking all possible sources of contamination, such as water, rubber stoppers, plastic tubing and even the septum used in the chromatograph. This aspect, the appearance of interfering peaks from materials and chemicals, has recently been discussed by Levi and Nowick[21] with reference to the determination of organochlorine pesticides.

The migration of DEHP into blood stored in plastic containers presents a very serious problem. Blood stored in plastic at 4 °C can extract 0·25 mg of DEHP per 100 ml per day; after 21 days the concentration of DEHP in whole blood was found to be within the limit of 50 p.p.m. Our method is suitable for the rapid determination of DEHP in large numbers of plasma samples; it is not time consuming and requires a minimum of blood, glassware and reagents. We have established that more than 85 per cent. of the DEHP can be extracted from the plasma with reproducible results (Table IV). We used plasma rather than whole blood as many investigators have established that all of the DEHP extracted by blood is concentrated in the plasma.[22]

TABLE IV

RESULTS OF REPLICATE GAS - LIQUID CHROMATOGRAPHIC ANALYSES OF HUMAN PLASMA

Each sample was analysed five times.

Sample	DEHP, p.p.m.
I	70 ± 4·4
II	82 ± 4·7
III	86 ± 4·3

The above procedure has also been used to extract pesticides. However, under the experimental conditions employed, no interfering peaks appeared near the retention time

of DEHP. The peaks of the pesticides would interfere in the determination of phthalate esters of low relative molecular mass. Our method has also been applied to the rapid determination of DEHP in milk, the results of which will be published separately.

Conclusion

We are confident that our simple, rapid procedure will be of practical value for monitoring not only the migration of DEHP from plastic containers into blood and aqueous or lipophilic solutions but also the pollution produced by phthalate esters in the environment.

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The Determination of Oxygen-18 to Oxygen-16 Ratios in Inorganic Phosphates by Gas - Liquid Chromatographic - Mass Spectrometric Examination of the Tri-n-butyl Derivative

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Aqueous solutions containing inorganic orthophosphates from biological materials were purified on ion-exchange columns and the phosphate was precipitated as its silver salt. The dried silver phosphate was made to react with 1-bromobutane in dimethylformamide, yielding tri-n-butyl phosphate. This solution was suitable for injection directly into a combined gas chromatograph - mass spectrometer for analysis, enabling the oxygen-18 to oxygen-16 ratio of the original inorganic orthophosphate to be determined.

Previous methods for the determination of oxygen-18 in enriched phosphates have involved its conversion into gaseous carbon dioxide or oxygen for examination by mass spectrometry.[1] These methods require specialised apparatus and handling techniques. The conversion of inorganic anions into their trimethylsilyl derivatives,[2] which have volatilities suitable for gas - liquid chromatography, and the use of these derivatives for determinations by mass spectrometry of oxygen-18 labelled phosphate[3] have been described. In this paper an alternative method, which has been found convenient for the determination of inorganic phosphates in low concentrations in solutions derived from biological sources, is described. This procedure was developed for the determination of inorganic phosphate tracer in homogenates of milk and faeces derived from metabolic balance studies in the newly born.

Experimental

Reagents

These were of analytical-reagent grade unless otherwise stated.
Dowex 50W-X8 ion-exchange resin, 200–400 mesh.
Dowex 2-X8 ion-exchange resin, 200–400 mesh.
Nitric acid, 0·1 and 1 N.
Sodium hydroxide solutions, 0·2, 0·3, 0·4 and 0·5 N.
Silver nitrate solution, 0·05 g ml^{-1}.
1-Bromobutane.
Dimethylformamide.
Potassium dihydrogen orthophosphate (oxygen-18, 85 per cent. abundance) solution, 1 mg ml^{-1}.

Apparatus

The apparatus consisted of a Varian Aerograph 1700 gas chromatograph coupled via a helium separator to a Varian CH5 mass spectrometer. The chromatographic column was a 1·54 m long × 7 mm o.d. glass column packed with 3 per cent. silicone OV-101 on Celite AW-DMCS, 100–120 mesh (obtained from Phase Separations Ltd.). The operating conditions were as follows: carrier gas, helium at a flow-rate of 30 ml min^{-1}; injection temperature, 210 °C; column temperature, 170 °C; source temperature, 150 °C; ionisation voltage, 70 eV; and ionisation current, 300 μA.

Procedure

Acidify samples (2–20 ml) that contain at least 1 mg of phosphorus as inorganic phosphate with 1 N nitric acid in order to ensure the complete dissolution of the inorganic phosphate, and then ultracentrifuge at 90 000 *g* to obtain clear solutions. Determine the total free inorganic phosphate concentrations by use of the colorimetric method of Delsal and Manhouri[4] on 0·1-ml aliquots.

Purify the solutions by passing them through two ion-exchange columns. The first column removes cations that are likely to cause precipitation of phosphate under the alkaline conditions used in the second column, which is required to remove interfering anions. The first column is 1 cm in diameter by 4 cm long and is packed with Dowex 50W-X8 in the H^+ form. Elute the column with 0·1 N nitric acid, then collect the first fraction, which is equivalent to the volume applied plus 5 ml, neutralise it with 5 N sodium hydroxide solution and add further hydroxide solution to give a final concentration of 0·2 N. Apply this alkaline fraction to the second column, which is 1 cm in diameter and 12 cm in length and is packed with Dowex 2-X8 in the OH^- form. Elute this column with successive 50-ml amounts of 0·2, 0·3 and 0·4 N solutions of sodium hydroxide prepared in carbon dioxide free water. Collect 2-ml fractions of the eluate and locate the phosphate-containing fractions by carrying out colorimetric determinations on 0·1-ml aliquots.

Next neutralise the combined phosphate fractions with nitric acid and precipitate the phosphate by the addition of 5 per cent. silver nitrate solution. Collect the yellow - green precipitate by centrifugation and wash it three times with 2 ml of distilled water, then dry it at 105 °C, weigh and store it in a desiccator over phosphorus(V) oxide.

Convert the inorganic silver phosphate into tri-n-butyl phosphate by reaction with 1-bromobutane, using a modification of the method of Baldwin and Higgins.[5] In the original method silver phosphate and a two-fold excess of 1-bromobutane were refluxed at 103 °C for 8 h and were reported to give a 60 per cent. yield of tri-n-butyl phosphate.

$$Ag_3PO_4 + 3C_4H_9Br \longrightarrow 3AgBr + (C_4H_9)_3PO_4$$

Increased yields and a less viscous reaction medium are obtained by the addition of dimethylformamide in a volume twice that of the volume of 1-bromobutane used. In this method, heat the reaction mixture under reflux in an oil-bath at 100–110 °C for 2 h to give 90–100 per cent. yields of tri-n-butyl phosphate from silver phosphate.

Finally, dilute a portion of the supernatant liquid approximately 100-fold with dimethylformamide and inject 2 μl of this solution directly into the combined gas - liquid chromatograph - mass spectrometer.

Results

Tri-n-butyl phosphate gave the fragmentation pattern illustrated in Fig. 1, which shows the relative abundances of the fragments with their probable identities. Fig. 2 shows a sample enriched with 20 per cent. of oxygen-18. The $P^{18}O_4$ to $P^{16}O_4$ ratios were thus determined by comparison of the 99 and corresponding 107, 105, 103 and 101 mass peaks. As different batches of labelled phosphate might vary in the proportions of the various phosphate species $P^{16}O_3{}^{18}O$, $P^{16}O_2{}^{18}O_2$, $P^{16}O^{18}O_3$ and $P^{18}O_4$, the relative abundance of the oxygen-18 was determined by calculating the ratio of the sum of the intensities of the 101–107 ions to that of the 99–107 ions. Relating this ratio to the total phosphate concentrations that had been determined colorimetrically allowed the concentration of oxygen-18 labelled phosphate to be ascertained.

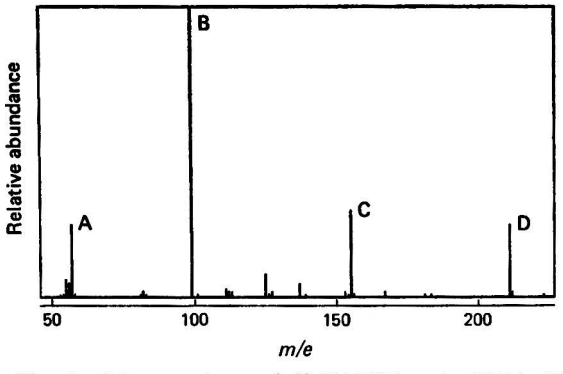

Fig. 1. Mass spectrum of $(C_4H_9)_3P^{16}O_4$. A, $C_4H_9^+$; B, $P^{16}O_4H_4$; C, $C_4H_9P^{16}O_4H_3^+$; and D, $(C_4H_9)_2P^{16}O_4H_2^+$.

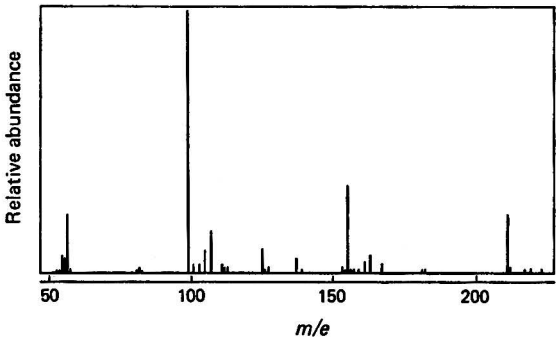

Fig. 2. Mass spectrum of $(C_4H_9)_3P^{16}O_4$ - $(C_4H_9)_3P^{18}O_4$.

The precision and accuracy of the method are indicated in Table I, in which labelled potassium dihydrogen orthophosphate solution was added in different amounts to unlabelled inorganic phosphate, milk and faeces.

TABLE I

RECOVERY OF ADDED PHOSPHATE LABELLED WITH OXYGEN-18

Sample	Relative abundance of ^{18}O, per cent. Added	Observed*	Observed minus blank	Standard deviation	Coefficient of variation, per cent.
Aqueous orthophosphate solution	0·0	0·91	0·00	0·07	8·1
	0·085	1·46	0·55	0·27	48·6
	0·425	1·55	0·64	0·12	18·6
	0·85	1·90	0·99	0·06	5·9
	4·25	5·23	4·32	0·35	8·0
	8·5	9·74	8·83	0·28	3·2
Milk	8·84	9·57	8·66	0·72	9·5
Faeces	19·7	21·25	20·35	1·02	4·8

* Mean of ten results.

Although concentrations of 1 per cent. of oxygen-18 in oxygen-16 could be detected, for the accurate determination of their ratios a level of at least 5 per cent. was desirable for the instrument described. The possibility of oxygen-18 enrichment in the helium separator did not appear to have any significant effect on the results.

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Procedures for the Deoxygenation of Liquids

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Procedures are described for the deoxygenation of pure liquids, liquid mixtures and solutions of solids in liquids using tris(2,2′-bipyridyl)cobalt(II) perchlorate {$[Co(bpy)_3](ClO_4)_2$} and sodium tetrahydroborate ($NaBH_4$) in a colour-indicating reaction. The efficiency of the procedures is assessed by reference to the ^1H spin - lattice relaxation times of a variety of materials. The values obtained show that the procedures are efficient, reproducible and time saving.

It has been suggested[1] that a mixture of tris(2,2′-bipyridyl)cobalt(II) perchlorate {$[Co(bpy)_3](ClO_4)_2$} and sodium tetrahydroborate ($NaBH_4$) is effective for the removal of oxygen from samples when its presence is detrimental, *e.g.*, when basic experiments on nuclear magnetic resonance are to be carried out. This paper reports a more definitive examination of the procedure, and additional techniques developed recently for use with the procedure are described. The methods developed permit the deoxygenation of several types of samples, *viz.*, pure liquids, liquid mixtures and solutions of solids in liquids.

Comments on the Detection of Oxygen

While commercial equipment specifically designed for the determination of dissolved oxygen, particularly in aqueous media, is readily available, it is well known that the nuclear magnetic resonance spin - lattice relaxation time (T_1) is extremely sensitive to the presence of oxygen and so this parameter can be used as a more extensive guide to the concentration of oxygen in solutions. For this reason, and because of the importance of obtaining T_1 data for their intrinsic value, this parameter has been studied for a representative selection of samples in order to confirm the validity of the procedures used to deoxygenate them.

Fundamentally, nuclear magnetic resonance spectra derive from the quantised energy changes of individual nuclei when subject to a strong homogeneous static magnetic field (B_0) and a weaker rotating field (B_1) produced by a radiofrequency signal. However, the detection of the spectra depends on changes in the macroscopic magnetisation vector (M_z) in the direction of B_0. Away from resonance and at thermal equilibrium the magnetisation vector has the value M_0. After perturbation due to nuclear resonance the transient value M_z returns to M_0 in the characteristic spin - lattice relaxation time. The mechanism of the relaxation process can be simply considered, at a nuclear level, to be due to a net transfer of energy from the nuclei to their environment, *i.e.*, to the lattice, which transfer is brought about by the time-dependent magnetic fields produced at the nuclei by various effects of the atoms and molecules constituting the lattice. Paramagnetic entities such as oxygen produce large fields that enhance the relaxation process and significantly reduce the value of T_1.

For two nuclei A and B the rate equations can be written[2] as

$$\frac{dM_z^A}{dt} = \frac{(M_0^A - M_z^A)}{T_1^{AA}} + \frac{(M_0^B - M_z^B)}{T_1^{BA}} \quad \ldots \quad (1)$$

$$\frac{dM_z^B}{dt} = \frac{(M_0^B - M_z^B)}{T_1^{BB}} + \frac{(M_0^A - M_z^A)}{T_1^{AB}} \quad \ldots \quad (2)$$

where T_1^{AA} is the total of the inter- and intramolecular contributions made by nuclei of type A to the relaxation time of A and T_1^{BA} is the specific contribution to the relaxation time of A arising from the interaction of A with B. Inspection of either equation (1) or (2) reveals that for one nucleus alone, say A, if M_0 is changed by a factor a at time $t = 0$ with a strong radiofrequency field, the instantaneous values of M_z, detected by using a low value of B_1, follow an exponential decay from aM_0 to M_0.

$$\ln (M_0 - M_z) = \frac{-t}{T_1} + \ln (M_0 - aM_0) \quad \ldots \quad (3)$$

Consequently, values of T_1 can be deduced from this so-called adiabatic rapid passage with repetitive sampling (ARPS)[3] technique by plotting ln $(M_0 - M_z)$ *versus t*. A similar simple exponential decay situation can be achieved for two nuclei provided that saturation of one nucleus [*e.g.*, $M_z^B = 0$ in equation (1)] is achieved by double irradiation. When the resonance of one nucleus is saturated in this way an additional effect occurs. For example, when $M_z^B = 0$ and the A system regains equilibrium, $dM_z^A/dt = 0$, and equation (1) reduces to

$$M_z^A = M_0^A + \frac{T_1^{AA}}{T_1^{BA}} \times M_0^B \quad .. \quad .. \quad .. \quad .. \quad (4)$$

so that the intensity of the A signal increases, owing to what is called the nuclear Overhauser effect (NOE).[4,5] Because the NOE enhancement is related to the sixth power of the distance between A and B it has obvious immense potential in the determination of molecular structures. However, it can be seen from equation (4) that the NOE depends on T_1 values, which are sensitive to the presence of oxygen, and before the effect can be used definitively all of the dissolved oxygen must be removed from the samples studied.

Principles of Deoxygenation

In solution $[Co(bpy)_3](ClO_4)_2$ and sodium tetrahydroborate yield a deep blue colour when all of the free oxygen has been removed. Should this solution be exposed to additional oxygen it reverts to its original brown colour. The presence of excess of sodium tetrahydroborate may induce the original blue colour, but the cycle cannot be repeated indefinitely. The detailed mechanism of the deoxygenation reaction has not been elucidated fully but evidence exists that points to two likely alternatives. The first depends on the fact that it is possible to isolate from the blue solution tris(2,2′-bipyridyl)cobalt(I) perchlorate[6] and an outer-sphere reduction of oxygen by this complex may occur to give a cobalt(III) complex. The latter, in the presence of excess of tetrahydroborate, can produce a cobalt(I) complex, which is characterised by a blue colour due to the strong charge-transfer band of the 2,2′-bipyridyl-cobalt(I) complex. The second possible mechanism depends on the fact that in the presence of phosphine ligands $[Co(bpy)H_2(PR_3)_2]ClO_4$ can be isolated.[7] This suggests that the tris complex dissociates to give a monobipyridyl complex that, by analogy with work on the related rhodium system,[8] may take up hydrogen reversibly or oxygen irreversibly such that cobalt(III) complexes can be formed. In the presence of excess of tetrahydroborate the cobalt(III) complexes yield a cobalt(I) complex with its characteristic blue colour.

Despite the fact that the detailed mechanism of the above chemical method for deoxygenating liquids is uncertain, there can be little doubt that it is most efficient. Nevertheless, it is necessary to compare this method with more conventional procedures such as those based on freeze - thawing or displacement of oxygen by inert gases. The T_1 data for benzene given in Table I facilitate this comparison. These data show that while each procedure results in the removal of oxygen the chemical method is by far the most efficient and reproducible. An estimate of the extent of deoxygenation achieved can be made by reference to T_1 data for water. Because a saturated solution of sodium sulphite in water is commonly used as a zero point for the calibration of commercial oxygen detectors, the T_1 value of water distilled from such a solution under vacuum serves as a useful standard. The value of 4·0 s so obtained is higher than that of 3·4 s for air-saturated water but less than that of 4·4 s obtained for a chemically deoxygenated sample. Although these values are very similar the determinations were repeated many times and in no instance was the relative order of these values different, or was there any overlap in the small range of values obtained for each system. The small differences in these data thus indicate a very slight residual oxygen content in the sodium sulphite treated sample, which would give rise to a small but significant zero error.

Experimental Procedures

A. General

The recognised method of preparation[12] of $[Co(bpy)_3](ClO_4)_2$ has been superseded by the following simpler method of Bhuyat.[13] Add a solution of hydrated cobalt(II) perchlorate (0·01 mol, 3·67 g, in 10 ml of ethanol) to a solution of 2,2′-bipyridyl (0·03 mol, 4·60 g, in 20 ml of ethanol). Recrystallise the resulting precipitate from ethanol - water (1 + 1), wash

with ice-cold ethanol and dry over phosphorus(V) oxide so as to obtain golden brown crystals of the cobalt complex.

The amounts of the two compounds used for deoxygenation should be kept as small as possible in order to minimise reduction of the sample. It is possible to deoxygenate successfully samples of total volume 20 ml by using only 1 mg of the cobalt complex; with smaller amounts, the colour change is indistinct. The amount of tetrahydroborate used depends upon the compounds being deoxygenated. If the compounds are inert to tetrahydroborate, then about 15–20 mg of it will suffice, but if any of them react in some way 50–60 mg may be required. It should be noted that care must be exercised when handling the compounds firstly because little is known about the physical and toxicological properties of the cobalt compound and secondly because of possible side-reactions.

B. Basic Deoxygenating Procedures

The prime prerequisite for deoxygenation is that the cobalt complex and the sodium tetrahydroborate should both come into intimate contact with the material being deoxygenated. This is not possible directly in all instances and an intermediate solvent may be required in order to form a link between the deoxygenating compounds and the sample. It may be miscible with the sample, thus forming a homogeneous mixture, or it may be immiscible, forming a heterogeneous mixture. In the latter instance prolonged vigorous stirring is necessary to produce a coarse emulsion so that the oxygen can diffuse into the intermediate solvent from which it is removed.

The main requirements of the intermediate solvent are that it must dissolve the cobalt compound and the tetrahydroborate, preferably without reacting with either of them, and that it should have a low vapour pressure at the working temperature so that subject materials can easily be removed from it by distillation. Some suitable solvents for the active compounds are water, acetone, dichloromethane, dimethyl sulphoxide and dimethylformamide.

C. Deoxygenation of Pure Liquids and Liquid Mixtures Using Distillation for Sample Transfer

Deoxygenation is most simply carried out when the material under study dissolves the deoxygenating compounds. In this instance, and when the sample forms the top layer of a heterogeneous mixture, the following procedure should be adopted.

Introduce about 10 ml of sample into a suitable flask together with a magnetic follower and the appropriate amounts of the deoxygenating compounds; when an intermediate solvent is used it should be added first together with the active compounds. Rapidly attach the flask to a vacuum line (at about 10^{-3} torr) and freeze the contents of the flask. Remove air via the vacuum system and allow a good vacuum to develop. Isolate the flask from the vacuum system, permit it to warm up and then stir the contents of the flask in order to allow the reaction to proceed to completion. The reaction results in the evolution of gas (presumably mainly hydrogen) and therefore distillation of the sample into the nuclear magnetic resonance tube can be hindered. When the production of hydrogen has slowed down, freeze the contents of the flask again and open the flask to the vacuum line in order to remove hydrogen. Isolate the flask and allow the contents to melt prior to distilling the required amount of sample into a pre-cooled nuclear magnetic resonance tube. After isolating the flask, freeze the contents of the resonance tube and re-open the vacuum line before sealing the tube under vacuum.

In certain instances these techniques must be modified in order to avoid violent side-reactions, because, particularly with some chlorinated samples such as tri- and tetrachloromethane, when the deoxygenating compounds are added to a heterogeneous mixture of the sample and, for example, water, an exothermic reaction occurs to give solutions with colours ranging from orange to green. The required orderly deoxygenation should be obtained by first deoxygenating the intermediate solvent layer, then freezing it, and subsequently adding the sample to it, thus freezing the latter. Finally, the trapped air should be removed and the mixture melted so as to allow the two liquids to come into contact.

D. Deoxygenation of Liquid Mixtures Using Syphoning for Sample Transfer

An apparatus which is suitable for the transfer of deoxygenated samples that occupy the

lower layer of a heterogeneous mixture to nuclear magnetic resonance tubes, and which is particularly useful for transferring samples of known composition, is shown in Fig. 1.

Attach the apparatus to a vacuum line and allow a vacuum to develop. Rotate the three-way tap and close the Rotaflo taps in order to isolate the flask from the remainder of the apparatus. Add to the flask about 5 ml of a heterogeneous intermediate solvent together with the appropriate amounts of the deoxygenating compounds, place a magnetic follower in the flask and replace the stopper. Open the flask to the isolated manifold and draw off the trapped air plus evolved hydrogen. Isolate the flask, re-evacuate the manifold and repeat the process so that any remaining hydrogen is removed from the heterogeneous intermediate solvent.

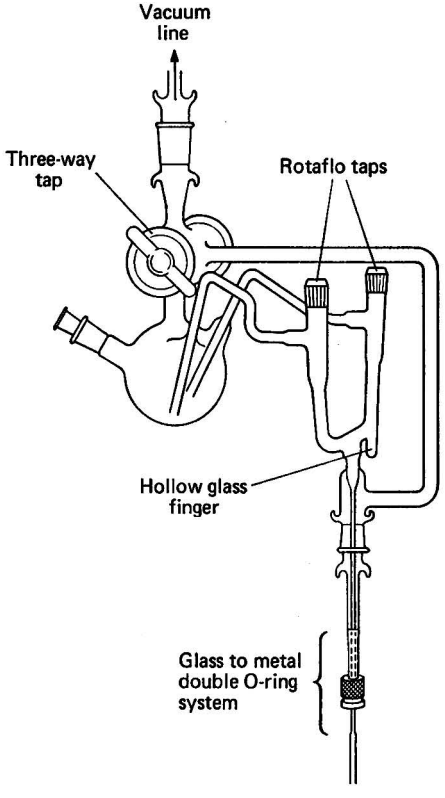

Fig. 1. Apparatus for deoxygenation using a syphoning process to transfer the sample.

Freeze the solvent and add the sample material in order to freeze it. It should be noted that the volumes of each solvent must be such that the capillary tube by which the transfer of sample is made has its end well away from the unwanted layer, in order to prevent the introduction of unwanted material while stirring the contents of the flask. Evacuate and isolate the flask. At this stage the region between the flask and the Rotaflo taps must also be evacuated. Allow the contents of the flask to warm up while the mixture is stirred vigorously. The sample should now be drawn up the appropriate capillary tube by cooling the region near the Rotaflo tap (continued production of hydrogen in the main body of the flask facilitates this step). This capillary tube should be washed out by repeatedly cooling and gently warming the region of the tube near the Rotaflo tap, the liquid pushed back into the flask being allowed to equilibrate before sucking more liquid back up the tube. On the satisfactory completion of this operation set the three-way tap to the all-isolated position and very slowly open the pertinent Rotaflo tap so as to allow the sample to pass through to the nuclear magnetic resonance tube. At first it may distil but when the saturated vapour

pressure is reached the sample will flow down the side of the glass tube into the resonance tube. Having collected sufficient sample, shut the Rotaflo tap, freeze the sample and evacuate and seal the resonance tube.

E. Deoxygenation of Solutions of Solids in Liquids

The procedure devised for deoxygenation of solutions of solids in liquids is basically the same as for liquid mixtures although there are additional problems, *viz.*, (*i*) cooling may induce precipitation of the solid, which may not all be redissolved, (*ii*) initially, after passing through the Rotaflo tap, the solvent may evaporate, leaving a deposit of solid on the walls of the tube. This deposit may be taken up by the solution after equilibrium vapour pressure has been reached. The first problem should be minimised by avoiding saturated solutions, keeping careful control of the magnetic stirrer and allowing the flask to warm to room temperature after each cooling. The second problem can be remedied by the addition of a small glass-finger tube on the side of the Rotaflo taps near to the nuclear magnetic resonance tube.

Prepare sufficient sample to cover both of the capillary tube ends in the flask. When preparing to draw off the sample, open the tap nearest to the finger and allow a flow of liquid through it. The design of the apparatus is such that the liquid will run down into the finger and not into the resonance tube. When the saturated vapour pressure of the solvent is reached in the vicinity of the resonance tube, open the other Rotaflo tap and allow the sample liquid to flow down into the resonance tube. The sample in this tube should then be carefully frozen and the tube evacuated and sealed.

Results and Discussion

The procedures for deoxygenating pure liquids and liquid mixtures were tested using compounds for which accepted literature values of T_1 exist.

TABLE I

SPIN - LATTICE RELAXATION TIMES OF BENZENE MEASURED BY THE ARPS TECHNIQUE AFTER DE-GASSING BY CONVENTIONAL AND CHEMICAL METHODS

Values other than the literature values were measured at about 309 K.

De-gassing technique	T_1/s
No de-gassing	4·8*
Freeze - thaw procedure:	
at 10^{-3} torr, 4 cycles	5·5*
at 10^{-5} torr, 1 cycle†	23·6
2 cycles	21·8
3 cycles	21·4
4 cycles	20·8
5 cycles	17·7
6 cycles	22·1
Bubbling oxygen-free nitrogen for:	
15 min	20·9
30 min	20·9
60 min	13·1
Chemical de-gassing:	
homogeneous mixture containing dimethylformamide	22·7 22·8
homogeneous mixture containing dimethyl sulphoxide	23·1 23·4
heterogeneous mixture with water	23·0 24·8
Reported in reference 9 (temperature, 298 K)	19·3‡
Reported in reference 10 (temperature, 303 K)	22·0‡
Reported in reference 11 (temperature, 305·1 K)	18·4‡

* Average of four measurements.

† The freeze - thaw operations were continuous; samples were removed after each cycle into nuclear magnetic resonance tubes on a specially made all-glass manifold.

‡ Highest reported values.

Nuclear magnetic resonance tubes were attached to the vacuum manifold via a double O-ring glass to metal connection. Before use each tube was heated under vacuum to dispel oxygen from the walls and the deoxygenated sample was transferred into the resonance tube by distillation or syphoning. In preliminary experiments it was found in some instances that, despite critical inspection, the seal of the resonance tube was defective in some way, for example, during 1 d the T_1 value for a sample of benzene would fall from about 20 s to about 6 s. It is thought that with the thin-walled resonance tubes normally used, cracks occurred on cooling after sealing. The initial method of sealing was to heat the tube under vacuum at about six places around its circumference with a pencil flame, allowing the tube to collapse gradually. As this proved inadequate, each resonance tube was subsequently heated by an all-encompassing flame near the open end, so that the tube was restricted and thickened prior to installation on the vacuum system. Under vacuum it was then possible to obtain a good seal by using a pencil flame.

Values for the spin - lattice relaxation times were obtained, using the ARPS method,[3] on a Varian HA100D nuclear magnetic resonance spectrometer connected to a Hellige He-lt fast-response recorder. An Airmec 422 signal generator was used for double resonance in order to produce effective single-spin systems where necessary. The relaxation times were calculated by using a computer analysis of ln $(M_0 - M_z)$ *versus* t data.

Some typical values obtained for single compounds are given in Tables I and II. It should particularly be noted that the T_1 values obtained for both the ring and the methyl protons in samples of the methylbenzenes are significantly higher than the literature values, even after taking into account the slight difference in experimental temperatures. The relaxation times of benzene (discussed earlier) and cyclohexane, which were obtained by using both the distillation and syphoning procedures, are also higher than the literature values.

TABLE II

COMPARISON OF THE ^{1}H-T_1 VALUES OF PHYSICALLY AND CHEMICALLY DE-GASSED COMPOUNDS

	T_1/s			
Sample material	No de-gassing	After freeze - thaw procedure	After chemical de-gassing*	Literature values
Toluene†				
(ring protons)	3·76	4·18	21·3‡	16·0[9]
(methyl protons) ..	3·29	3·50	11·6‡	9·0[9]
p-Xylene†				
(ring protons)	3·43	4·36	16·7‡	14·0[9]
(methyl protons) ..	3·32	3·88	7·3‡	7·5[9]
Mesitylene†				
(ring protons)	—	—	13·1§	10·0[9]
(methyl protons) ..	—	—	6·1§	5·0[9]
Acetone	—	—	17·6‡¶	15·8[14]
Dichloromethane ..	—	—	36·1‡	28·5[3]
Cyclohexane	—	—	7·9**‡	7·2[15,16]

* Values obtained (except for cyclohexane) after distillation from the pure liquid containing the deoxygenating compounds.

† Ring and methyl ^{1}H-T_1 values were measured separately by saturating out the unwanted resonance.

‡ Values reported are the average of several measurements at about 309 K.

§ Values reported are the average of several measurements at about 306·4 K.

¶ The estimated error for 13 determinations is ±0·2 s.

** Average of values obtained after distillation from a heterogeneous mixture containing dimethyl sulphoxide, and after syphoning from heterogeneous mixtures with water.

The procedure for the deoxygenation of liquid mixtures was assessed, using benzene and carbon tetrachloride, as described earlier in section D. This procedure was used to obtain deoxygenated samples of benzene - carbon tetrachloride mixtures over the whole range of benzene molar fractions. The molar fractions of the components of each sample before they were deoxygenated were calculated by mass. As a check, when samples had been used in

the nuclear magnetic resonance experiments, the tubes were opened and the molar fractions found by measuring the refractive indices and referring to a calibration graph. For 24 prepared samples the difference between the molar fractions before and after deoxygenation was less than 0·02 and in 19 instances was below 0·005, which is within the experimental error for the refractive index measurements. The graphs of T_1 against the molar fraction of benzene (Fig. 2) are nearly identical in shape with those obtained by Mitchell and Eisner.[15,16] except that the relaxation times are slightly longer, indicating an increase in the efficiency of removing oxygen in the present work.

The syphoning principle was also applied to solutions of solids in liquids. However, values of the spin - lattice relaxation times for these solutions could not be found in the literature and thus no reference was available with which to assess the merit of this procedure. Nevertheless, it was decided to investigate the dependence of the spin - lattice relaxation time of the protons of 1,2,4,5-tetramethylbenzene on the concentration of this compound in carbon tetrachloride. Fig. 3 shows the values obtained. The deviations of the experimental points from the lines drawn are within experimental error, thus indicating that the required consistency in the experimental procedures has been achieved.

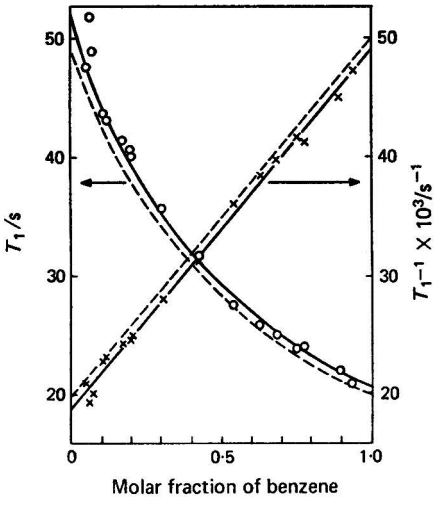

Fig. 2. A comparison between the ^{1}H-T_1 values of physically and chemically degassed samples of benzene - carbon tetrachloride: points with full lines, this work; broken lines, references 15 and 16.

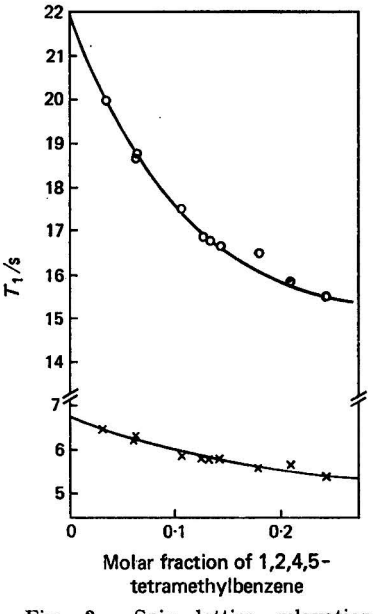

Fig. 3. Spin - lattice relaxation times of 1,2,4,5-tetramethylbenzene in carbon tetrachloride as a function of concentration, ○, T_1 for ring protons; X, T_1 for methyl protons.

It has to be stressed that the benefits of the above procedures for removing free oxygen, in a colour-indicating method, from samples in which its presence is not wanted, lie in the efficiency, ease and speed with which they can be carried out. While the procedures have been used to remove oxygen from samples that were then analysed by using nuclear magnetic resonance spectroscopy, their use is not restricted to this field. For example, it is possible that they may find applications in fluorescence spectroscopy, electron spin resonance spectroscopy and in other physical analytical techniques.

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A Rapid Method for the Assay of Ascorbic Acid Tablets

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A method is described for the rapid routine assay of ascorbic acid tablets by using a subtractive enthalpimetric technique. The ascorbic acid is oxidised by hexacyanoferrate(III).

The effects of excipients have been studied. A comparison of the results obtained by using this method and the BP method shows that there is no significant difference in the accuracy of the two techniques. The main advantages of the proposed method are the time taken, the potential for automation of the process and the ability to carry out the assay of single dosage forms.

Changes in attitudes towards tolerances in the amounts of active constituents in tablets have led to an interest in methods that are capable of assaying single dosage forms. The high labour cost of analysis has made it feasible to use automatic methods for multiple routine assays when heretofore the capital outlay often precluded the installation of automatic methods of assay. Among the various methods that have been proposed for the rapid assay of materials, enthalpimetric analysis[1] commends itself for the routine assay of pharmaceuticals that contain one active constituent and several inactive components, *viz.*, the matrix or the excipients.

The titrimetric determination of milligram amounts of ascorbic acid has been reviewed previously.[2] The methods described are generally oxidimetric and the presence of excipients often necessitates the separation of the active ingredient prior to its determination. One of the main advantages of enthalpimetric and thermometric methods is that matrix effects can be regarded as being relatively insignificant provided that the reaction is chosen to be selective towards the active ingredient and that the matrix is thermally neutral.[3] It has been shown[4] that the presence of the commonly used excipients in ascorbic acid tablets has no deleterious effect on the iodimetric determination of ascorbic acid in tablets when using a thermometric method of assay. The enthalpimetric methods have advantages over the thermometric method for some routine assays.

The use of hexacyanoferrate(III) for the oxidimetric determination of ascorbic acid has several advantages, the greatest of which is that it can be used at various pH levels without any significant alteration in its oxidative powers.[5] The main disadvantage is that solutions of hexacyanoferrate(III) have relatively large heats of dilution, and to overcome this it was necessary to devise a double-injection system which automatically subtracted the heat of dilution of an aliquot of the hexacyanoferrate(III) solution from the sum of the heat of dilution and the heat of reaction of a portion of the oxidant. This system is described below, and is designated a subtractive system. Although systems have been described previously for continuous thermometric titration, no apparatus has been described previously for use with an enthalpimetric technique.

Experimental

Enthalpimetric System

A subtractive, direct-injection, enthalpimetric system was devised that involved the use of a simple d.c. Wheatstone bridge circuit with matched thermistors (10-kΩ nominal resistance at 25 °C) and a double-injection system capable of simultaneous injection of the reagent into a sample and a blank (see Fig. 1). The off-balance voltage of the bridge was monitored either by feeding it directly to a potentiometric recorder which had various ranges for

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full-scale deflection of 1–100 mV, or by amplifying it using a simple d.c. operational amplifier of 10 times gain, the amplified voltage then being fed to a digital voltmeter of 200-mV range.

The syringes used were simple hypodermic syringes with a capacity of 5 cm³. The pipettes were designed so as to facilitate adequate stirring in a slurry and the rapid attainment of thermal equilibrium throughout the reaction cell.[6]

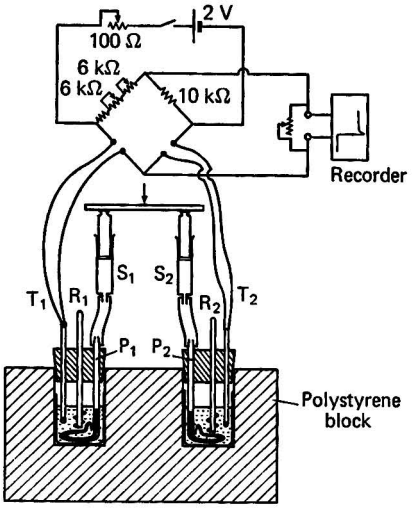

Fig. 1. The enthalpimetric system. S_1 and S_2, 2-ml syringes; T_1 and T_2, 10-kΩ thermistors; R_1 and R_2, rotary stirrers; P_1 and P_2, 1-ml submersible pipettes.

Reagents

Potassium hexacyanoferrate(III) solution. Approximately 1·0 M aqueous solutions of potassium hexacyanoferrate(III), $K_3[Fe(CN)_6]$, were prepared by dissolution of the appropriate amount of solid in water. (It has been previously reported that the solution deteriorates on standing; however, assay of the above solutions indicated less than 1–2 per cent. variation after standing for 48 h in an amber-glass bottle with an air atmosphere above the solution.)

Assay of Analytical-reagent Grade Ascorbic Acid

Known amounts of ascorbic acid were dissolved in distilled water (15 cm³) and fixed amounts (nominally about 1·0 cm³) of the potassium hexacyanoferrate(III) solution were injected into each solution after thermal equilibrium had been attained. In order to eliminate dilution effects, the same amount (about 1·0 cm³) of the hexacyanoferrate(III) solution was simultaneously injected into a further volume (15 cm³) of distilled water.

The recorder pulse indicated the heat produced in the reaction between the ascorbic acid and the hexacyanoferrate(III); the dilution effects were automatically subtracted as a result of the bridge circuit design. The results are given in Table I and Fig. 2.

TABLE I

VALUES FOR CALIBRATION GRAPH OF PURE ASCORBIC ACID

Amount of ascorbic acid/g ..	0·0193	0·0184	0·0152	0·0124	0·0069	0·0028
Heat pulse (arbitrary units) ..	68·8	66·6	54·0	45·8	24·1	10·0

Ascorbic Acid Tablets BP

Thirty Ascorbic Acid Tablets BP, nominally containing 50 mg of ascorbic acid per 200-mg tablet, were crushed and weighed.

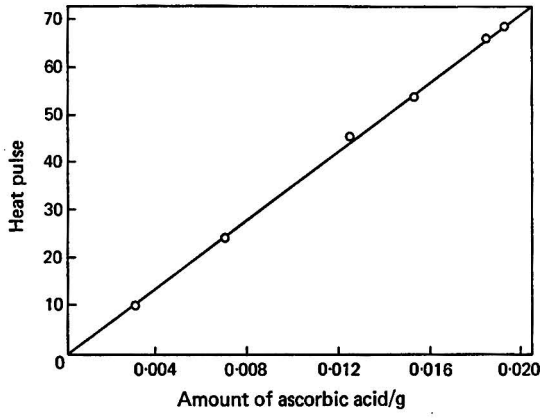

Fig. 2. Calibration graph for pure ascorbic acid. The heat pulse scale is in arbitrary units.

(i) *BP method of assay*

A known amount of the powdered tablets was dissolved in water and aliquots of the solution were assayed by the recommended BP method[7] using previously standardised ammonium cerium(IV) sulphate as the oxidising titrant and tris(1,10-phenanthroline)iron(II) sulphate (ferroin) as indicator.

TABLE II

DETERMINATION OF ASCORBIC ACID IN ASCORBIC ACID TABLETS

Mass of tablet/g ..	0·0805	0·0696	0·0600	0·0498	0·0385	0·0266	0·0175
Heat pulse.. .. (arbitrary units)	71·2	61·1	54·3	45·6	34·3	24·5	15·6

(ii) *Enthalpimetric method of assay*

A known amount of the powdered tablets was stirred with a fixed volume (15 cm^3) of distilled water and assayed by the method used for analytical-reagent grade ascorbic acid. The results are given in Table II and Fig. 3.

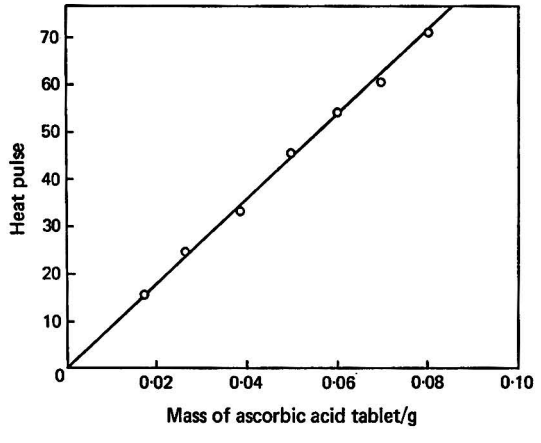

Fig. 3. Determination of ascorbic acid in ascorbic acid tablets. The heat pulse scale is in arbitrary units.

Test of reproducibility

By using the method described above, 15 identical samples (0·0142 g each) of ascorbic acid were assayed. The results are given in Table III.

TABLE III

REPRODUCIBILITY OF RESULTS: ASSAY OF PURE ASCORBIC ACID BY THE PROPOSED METHOD

Initial amount of ascorbic acid, 14·0 mg.

Run number ..	1	2	3	4	5	6	7	
Amount found/mg ..	14·5	14·3	14·3	14·0	13·8	14·2	14·5	
Run number	8	9	10	11	12	13	14	15
Amount found/mg ..	13·7	13·6	14·4	14·4	14·1	14·4	13·8	13·8

Average reading, 14·12 mg; standard deviation, 0·3 mg.

Results

(i) *BP method of assay*

Portions of the sample equivalent to 0·6018 g of Ascorbic Acid Tablets BP required 17·52 cm³ of 0·0974 M ammonium cerium(IV) sulphate solution. The average mass of a tablet was 0·2000 g. The average ascorbic acid content is therefore 49·8 mg.

(ii) *Enthalpimetric method of assay*

The slope of the graph for ascorbic acid given in Fig. 2 is $35{\cdot}8 \times 10^2$ unit g^{-1}, while the slope of the graph for ascorbic acid in tablets given in Fig. 3 is $8{\cdot}89 \times 10^2$ unit g^{-1}.

The conversion factor is thus 35·8/8·89 = 4·03. Therefore, the amount of ascorbic acid in a 0·2000-g tablet is 0·2000/4·03 g = 49·6 mg.

Test of reproducibility

It can be seen from Table III that the average value for the assay of 14·0 mg of ascorbic acid is 14·12 mg, with a standard deviation of 0·3 mg, which is acceptable for the purposes of a routine method.

Discussion

The fact that both graphs (Figs. 2 and 3) pass through the origin indicates that the thermistors are matched, because with a zero amount of active material the heats of dilution, etc., cancel each other. Similarly, because the graphs pass through the origin there can be no heat from the tablet matrix. This conclusion was verified by taking up to 1·000 g of starch, calcium lactate and talc and suspending or dissolving them in 15 cm³ of solution. Addition of accepted food colours to the solution also had no effect.

As the Ascorbic Acid BP tablets normally contain 50 mg of active ingredient per tablet, and in order to ensure that a sufficiently large excess of hexacyanoferrate(III) is present, it is necessary to replace the 1-cm³ pipettes with pipettes of nominally 2-cm³ capacity. A calibration run, carried out as previously described, but using a lower sensitivity for the recorder, gives the same range of heat pulse heights.

It is possible to adjust the sensitivity of the recorder system such that the heat pulse from 5 mg of active ingredient in one series can be made as large as the heat pulse from 50 mg in a second series (and *vice versa*). Thus, one is able to obtain the same precision for these diverse masses using separate calibration runs.

By suitable shunting of the digital voltmeter, it is possible to obtain a read-out of 100 units for 50 mg of ascorbic acid per sample. Hence, for comparison of individual tablets it is only necessary to crush the tablet in the reaction cell, stir until thermal equilibrium is obtained and then assay by the proposed method. The reading obtained is a direct percentage evaluation of the content of the tablet, *e.g.*, a reading of 96 units indicates that the tablet contains 96 per cent. of the active ingredient of a standard tablet. The potential of the method for rapid and routine assay of single dosage forms is therefore apparent. It is easily possible to assay one tablet every 3–4 min by use of this method.

The advantages of enthalpimetric methods for such determinations are firstly, that it is not necessary to prepare the hexacyanoferrate(III) solution accurately as it is present in such a large excess that any reasonable variation in the concentration of the solution has no effect, secondly, that it is not necessary to inject exactly known volumes of the solution provided that the same volume is injected into each cell, this being an obvious advantage in automation, thirdly, that variations in the components of the matrix have no effect, whether or not they are coloured, provided that these components do not react with the hexacyanoferrate(III) solution, and fourthly, that the method is rapid; for single tablet assay the tablet is dispersed by stirring in 1–2 min, and the result can be obtained in digital form or printed within 3 min of selecting the tablet.

For the assay of materials that are not in tablet or capsule form, for example, vitamin-enriched fruit cordials, it is necessary either to use a calibration graph or to dispense a known mass or volume of the cordial.

The method compares favourably in accuracy with the standard BP method and is much more advantageous with respect to time of operation, potential for automation and the assay of single tablets or dosage forms; the method is flexible with regard to the amounts to be assayed, and will give acceptable results over the range 10–50 mg of active ingredient with appropriate alterations to the concentration of the titrant and sensitivity of the recording system.

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An Improved Field Test for Barbiturates and Hydantoins with a Modified Cobalt(II) Thiocyanate Reagent

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The classical Dille - Koppanyi cobalt(II) acetate reagent has been rendered more sensitive and stable so as to provide a convenient single-fluid reagent for field testing for barbiturates. The test will also detect hydantoins and other hypnotics. Reagents based on cobalt(II) thiocyanate are essentially stable and are able to detect sub-microgram amounts of barbiturates in the keto form. The colour developed with barbiturates can be preserved indefinitely.

The traditional colour test for barbiturates consists of the Dille - Koppanyi modification[1] of the Zwikker test,[2] a colour being developed in an anhydrous cobalt(II) acetate solution of a barbiturate by the addition of an amine. Numerous field and laboratory test kits based on this test have been devised, essentially without further modification of the reagents.

The reagents are stored separately and are often sealed into ampoules for the convenience of the user. A portion of the sample tablet is mixed with the first reagent, usually a 0·1 per cent. *m/V* solution of cobalt(II) acetate in anhydrous methanol containing 0·2 per cent. *V/V* of acetic acid. After allowing a few seconds for the sample to dissolve, the second reagent, a 5 per cent. *V/V* solution of an amine, usually isopropylamine, in anhydrous methanol, is added, a violet coloration being produced with some barbiturates. The volume of each reagent taken varies according to the kit in use and is usually between 0·5 and 2 ml. Even with 0·5 ml of the reagents, amounts of barbiturate in excess of 100 μg are frequently required to yield a distinct coloration.

In the search for an alternative field test for use by non-technical personnel, it was obvious that the Dille - Koppanyi test had received general acceptance, even though it lacked sensitivity, specificity and permanence. There was also a disposal problem after the test had been completed; for instance, in some kits the reaction vessels (plastic tubes) tended to disintegrate after exposure to the reagent mixture. Attempts to produce single-fluid reagents for application on test papers, comparable with tests devised for lysergic acid diethylamide (LSD)[3,4] and cocaine,[5] have been successful. In addition to being easier to apply and to keep, the increase in sensitivity with the modified reagents was most marked, particularly with barbiturates in the keto form. Some of these non-ionised barbiturates will not respond to the Dille - Koppanyi reagents with the amounts specified in test kits. The proposed tests also produce a permanent record on the test paper and obviate disposal problems.

Method

Reagents

Cobalt(II) thiocyanate. Commercial grade solid.

Methanol. Analytical-reagent grade.

2,6-*Dimethylmorpholine.* Commercial grade.

Test solution. Dissolve 10 g of cobalt(II) thiocyanate plus 50 ml of 2,6-dimethylmorpholine in 1 l of methanol.

Apparatus

Test papers. Whatman grade 1 filter-paper circles, 5·5 or 7 cm in diameter, or equivalent grade.

Procedure

Place 1–2 mg of the suspected substance or fragments of a tablet in the middle of a test

paper. Add one drop of the modified cobalt(II) thiocyanate test solution and observe any violet to purple colour that develops in the time taken for the drop to evaporate to dryness. The appearance of a shade of blue colour is presumptive evidence for the presence of another hypnotic. If necessary, preserve the test paper in a sealable container, such as a polythene bag.

Results and Discussion

The essential conditions for the satisfactory functioning of the test are stated to be the absence of water and the later addition of a base[6]; some of the variables have been studied.

Variation of the base

It was observed that a wide range of amines could be added to non-aqueous cobalt(II) solutions without causing immediate precipitation. In some instances precipitation did not commence until a period of some months had elapsed, and even then the supernatant liquid remained effective for barbiturate detection. It was also found that the amine concentration was relatively unimportant. Attention was directed to those amines which were free from objectionable odours and which evaporated quickly.

Lithium hydroxide is stated to provide a more specific test for barbiturates.[7] It was, however, not found possible to add solid lithium hydroxide to non-aqueous solutions of cobalt(II) without precipitation occurring. Addition of a solution of the alkali sometimes gave rise to a deep orange-coloured fluid that was capable of responding to barbiturates, but the technique required to achieve this orange solution was not sufficiently reproducible to render this method practical as the basis of a field test. As with sodium and barium hydroxides,[7] precipitate formation normally occurred immediately. Further work with inorganic alkalis and quaternary amines, which behaved in a similar way, was discontinued. Table I summarises the storage and response characteristics of typical reagents examined.

TABLE I

STORAGE AND RESPONSE CHARACTERISTICS OF ALKALINE COBALT(II) THIOCYANATE SOLUTIONS

Reagent solutions: 5 per cent. *V*/*V* of base added to a 1 per cent. *m*/*V* solution of cobalt(II) thiocyanate in methanol.

	Fresh solution		Solution after storage for 1 week	
Typical base	Precipitate	Response	Precipitate	Response of supernatant liquid
Lithium hydroxide	Yes	On drying	Yes	None
Tetramethyl-ammonium hydroxide	Yes	On drying	Yes	None
Diethylamine	No	Rapid and strong	Heavy	Rapid
Di-n-propylamine	No	Rapid and strong	Heavy	Rapid
Trimethylamine	No	Immediate	Slight*	Rapid
Piperidine	No	Immediate	Heavy	Rapid†
2-Picoline	No	No response	—	—
Morpholine	No	Rapid	Yes	Slow but strong on drying
N-Methylmorpholine	No	Rapid†	None*	Rapid†
N-Ethylmorpholine	No	Rapid†	None*	Rapid†
2,6-Dimethylmorpholine	No	Immediate	None*	Immediate

* Storage for 1 month or longer produced the same result.

† At the concentration used in this comparison, the background colour on drying would obscure a weak response. A lower concentration of amine would be used in practice.

Variation of the cobalt(II) salt

Only the acetate salt of cobalt(II) is commonly used in a field test for barbiturates. Shellard and Osisiogu[8] used the nitrate as a chromogen in adapting the test for use in thin-layer chromatography. Direct addition of a methanolic solution of an amine of either salt will yield a reagent for detecting barbiturates, although the response is not always immediate, and a precipitate may form relatively rapidly when the nitrate is used (see *Availability of water* below).

Initial experiments indicated that of the common salts, the thiocyanate was less prone to form precipitates on standing and yielded more definite reactions. Direct comparison of reagents prepared by adding 10 per cent. *V*/*V* of typical classes of amines to a 1 per cent. *m*/*V* solution of cobalt(II) salt in methanol was carried out, and the results are summarised in

Table II. The test solutions were added to approximately 50 μg of amylobarbitone derived from approximately 0·3-mg portions of a Drinamyl tablet. These results confirmed the selection of the thiocyanate as the preferred salt. The thiocyanate complex also has its own intense natural colour, which enhances the final colour, and it is readily soluble in non-aqueous media, which enhances reaction rates.

TABLE II

RELATIVE EFFICIENCIES OF ALKALINE COBALT(II) SALT SOLUTIONS IN DETECTING AMYLOBARBITONE

Reagents prepared by the addition of a high concentration of amine at 10 per cent. *V/V* to a 1 per cent. *m/V* solution of cobalt(II) salt in methanol.

	Response of cobalt(II) salt		
Amine added	Acetate	Nitrate	Thiocyanate
Isopropylamine	Strong, immediate colour; grey background on drying	Precipitate	Strong, immediate colour; blue background on drying
Diethylamine	As above	As above	As above
Trimethylamine	Slow reaction	As above	Immediate response
2-Picoline	Weak pink colour against pink background	Weak pink colour against brown background	No response
Morpholine	Strong, immediate colour	Precipitate	Strong, immediate background colour not obtrusive
2,6-Dimethylmorpholine	As for morpholine	Precipitate	As for morpholine

Variation of the solvent

Methanol combines high volatility with high purity and is readily available. No advantage appeared to be offered by other solvents, some of which caused inactivation due to precipitation. Acetone is typical of the other solvents studied and a comparison is made in columns D and E of Table III.

TABLE III

COMPARISON OF BARBITURATE REAGENT EFFICIENCIES WITH THAT FOR 30 μg OF BARBITURATE DISPERSED IN A POWDERED TABLET

	Reagent solution					
Substance	A	B	C	D	E	F
Amylobarbitone	—	++*	−	++	+	++
Amylobarbitone sodium	−	++*	++	++	++	++
Phenobarbitone	−	++*	−	++	++	++
Cyclobarbitone calcium	−	−	−	+	+	++
Hexobarbitone	−	++*	−	++	++	++
Hydantoin	−	++	−	+	−	++
Phenytoin sodium	−	−	−	++	+	++
Glutethimide	−	−	++†	+	−	++
Caffeine	−	−	−	+†	−	+†

Key: ++, responds in less than 5 s; +, responds between 5 s and 1 min; −, no response; * fades rapidly; † green with excess of test substance.

Known two-component reagent solutions[9]:

A, B. Dille - Koppanyi, using 2 and 0·5 ml of a solution containing 0·1 per cent. *m/V* of cobalt(II) acetate and 0·2 per cent. *V/V* of acetic acid in anhydrous methanol, respectively, as solution first applied and 5 per cent. *V/V* isopropylamine in anhydrous methanol as second solution.

C. Zwikker test: 0·5 ml of 0·5 per cent. *m/V* aqueous solution of copper(II) acetate as solution first applied and an excess added of second solution of 5 per cent. *V/V* pyridine in chloroform.

Modified one-component reagent solutions:

D. 1 per cent. *m/V* of cobalt(II) thiocyanate plus 0·5 per cent. *V/V* of morpholine in methanol (low amine example).

E. 1 per cent. *m/V* of cobalt(II) thiocyanate plus 1 per cent. *V/V* of morpholine in acetone (intermediate amine example).

F. 1 per cent. *m/V* of cobalt(II) thiocyanate plus 5 per cent. *V/V* of 2,6-dimethylmorpholine in methanol (high amine example).

Availability of water

The water content had already been shown to be an important factor in the cocaine test that is based on the use of acidified cobalt(II) thiocyanate solutions.[5] Addition of a base under essentially non-aqueous conditions was found to accelerate precipitation of basic cobalt salts unless the cobalt ion was already firmly complexed. This finding is illustrated in Table II where the relatively small amount of water of crystallisation that is freed from the 1 per cent. *m/V* nitrate salt is sufficient to initiate rapid precipitation. The effectiveness of the thiocyanate salt has been attributed to the direct attachment of the thiocyanate ligand to the cobalt ion, thus allowing water molecules free mobility prior to barbiturate chelation. Various numbers of water molecules have been attributed to the thiocyanate salt[9] in support of this contention of the importance of the mobility of water, and further evidence is supplied by the use of this salt in atmospheric humidity testing.[10] Commercial grade solvents can be used without paying special attention to further dehydration. The results presented in Tables I to IV are derived from the use of solutions in normal analytical-reagent grade solvents.

Variation of the metal ion

The Zwikker reagents[2] included copper(II) - pyridine complexes, and a range of single liquid reagents for detecting barbiturates can be prepared on this principle[11] and will be reported separately. A standardised version of the copper(II) Zwikker test is cited by Butler,[12] the relative effectiveness of which is compared as reagent C in Table III. The cobalt(II) tests reported here are recommended as a new standard field test as they are based on widely accepted chemical reactions and have the advantage in a field test of yielding the same (now familiar) colour for all barbiturates.

Limits of detection

These are set by the barbiturate under test and the user's notion of what constitutes one drop of reagent. Most field test kits direct the use of at least one quarter of a tablet, and under these conditions all of them function effectively.

In Table III the relative detection efficiencies of conventional field test reagents are compared with examples of those of the modified single-liquid reagents for approximately 30-μg drug portions. It was found that the standardised Dille - Koppanyi test had a detection limit of about 30 μg for most barbiturates when 0·5-ml reagent portions were used, but this limit was somewhat subjective as the developed colour faded within a few seconds. A nil response to all barbiturates was recorded at this level with 2-ml reagent portions (column A). The table also includes examples of drugs likely to be encountered in practice as substitutes or adulterants for barbiturates. Glutethimide has a hypnotic action that is similar to those of the barbiturates and hydantoins and can be detected, whereas caffeine, a potential adulterant, does not respond.

In Table IV are listed the responses of common hypnotics as pure drugs and preparations encountered as potential items for seizure. With tablets and capsules, about 250-μg portions were taken, being approximately 1 per cent. of the amount recommended with other field test kits. For most tablets and capsules the detection limit is one or two orders smaller still, giving a practical barbiturate detection level in the 0·2–0·5-μg range. Several hundred evaluation tests were completed on a portion of a Drinamyl tablet, which contains approximately 15 per cent. of amylobarbitone.

Conclusions

The simple addition of various amines to anhydrous methanolic solutions of cobalt(II) thiocyanate produces a range of reagents for the detection of barbiturates. For immediate use within a laboratory, the proportion of amine added is in no way critical. The limiting proportions required for a field test are, however, more exacting, and are restricted by the need for the absence of any appreciable precipitate on prolonged storage. Although no mixture was found to be totally free from this defect, a number remained fully efficient after many months. Tertiary amines, such as trimethylamine and morpholine and its derivatives, were found to be the most satisfactory: use of 2,6-dimethylmorpholine is recommended at a concentration of between 1 and 5 per cent. *V/V* in a 1 per cent. *m/V* solution of cobalt(II) thiocyanate in methanol. At higher concentrations, precipitation, although incipient, was insignificant after 2 months.

TABLE IV

RESPONSE OF A MODIFIED COBALT(II) THIOCYANATE REAGENT TO COMMON HYPNOTIC DRUGS

Reagent solution: 1·6 per cent. *m/V* of cobalt(II) thiocyanate plus 4 per cent. *m/V* of morpholine in methanol, a typical solution that produces little precipitation on storage; not optimum.

Trade name	Hypnotic ingredient	Response
Barbiturates	Amylobarbitone B.P.	++
	Barbitone B.P.C.	++
	Barbitone Sodium B.P.	++
	Butobarbitone B.P.	++
	Cyclobarbitone Calcium B.P.	++
	Hexobarbitone B.P.C. (1959)	++
	Pentobarbitone	++
	Phenobarbitone B.P.	++
	Quinalbarbitone	++
Hydantoins	5,5-Dimethylhydantoin	++ (blue)
	Hydantoin	+ (blue; fades)
	Phenytoin I.P.	++ (blue)
	Phenytoin Sodium B.P.	++ (blue; fades)
Glutarimide	Glutethimide B.P.	+ (blue; weak and fades)
Tablets and capsules—		
Bustaid*	Pentobarbitone	+
Butobarbitone 100	Butobarbitone	++ (intense)
Censedal	Nealbarbitone	++
Cyclobarbitone 200	Cyclobarbitone	+
Drinamyl	Amylobarbitone	++
Gardenal	Phenobarbitone	++
Gardenal sodium	Phenobarbitone sodium	++
Garoin	Phenytoin sodium plus phenobarbitone sodium	++
Halabar	Butobarbitone	+
Lum calcio*	Phenobarbitone calcium	+
Medomina*	Heptabarbitone	++
Nactisol	Secbutobarbitone	+
Natisedine*	Quinidine phenobarbitone	++
Nembutal	Pentobarbitone	++
Neutrodonna	Amylobarbitone	− (very low content)
Optanox*	Butylvinal	++
Peritrate	Phenobarbitone	++
Quinalbarbitone Sodium B.P., 100 mg	Quinalbarbitone Sodium	++ (intense)
Rutonyl	Phenylmethylbarbitone	++
Sodium amytal	Amylobarbitone sodium	++ (intense)
Somnytic	Barbitone	++
Sonalgin	Butobarbitone	++ (purple tablet)

Key: ++, responds in less than 5 s; +, responds between 5 s and 1 min; −, no response; * not manufactured in the U.K.

In practice, a sequence of field tests[11,13] is advised as a screening procedure for misused drugs. The first test should be for hallucinogens[4] followed by the Marquis test[7] and then by a modified (acidified) cobalt(II) thiocyanate test,[5] finishing with this (alkaline) cobalt(II) thiocyanate test for barbiturates. A field test kit has been devised that is based on these conclusions.[14] Whether the modified reagent is used as part of a sequence or in a broad-spectrum sorting procedure, its use is intended to limit the number of occasions when professional analysis becomes necessary. A negative response to all four tests eliminates the substance from the need for further examination and avoids unnecessary detention of persons or goods.

I thank Miss B. Savage of BDH Chemicals Ltd. for carrying out the necessary confirmatory storage trials. I also thank the Government Chemist for permission to publish this paper.

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Determination of Aluminium in Dental Enamel by the Carbon Cup Atomic-absorption Method*

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The possibility of using the carbon cup atomisation technique for the atomic-absorption determination of aluminium in dental enamel has been investigated. Various parameters that influence the sensitivity and accuracy of the measurements were studied by use of a standard electronic detection system (Varian AA-5; chart recorder f.s.d., 1 s) and oscilloscopic (Tektronix) recording of the absorption signal. The latter system enabled the atomic- and non-specific absorption signals to be studied as a function of the time of atomisation. Thus, increasing the rate of heating of the carbon cup in the atomisation step resulted in a substantial enhancement of the aluminium peak absorption signal. The presence of calcium in the solution (7·2 mg ml^{-1}, in the form of the phosphate) produced a two-fold increase in sensitivity for aluminium.

For routine determinations of aluminium in tooth material (*in vitro*) 10 mg of finely ground and homogenised sample are dissolved in 6 N nitric acid and the solution is diluted to 0·5 ml; 5-μl aliquots are sampled for a single determination. A single calibration graph obtained by the standard addition method is adequate for one set of samples. No correction due to non-specific absorption is necessary, but the blank value (nitric acid) should be checked from time to time.

The absolute sensitivity of the method ($3·5 \times 10^{-11}$ g of aluminium) permits the determination of aluminium in tooth material both *in vitro* and *in vivo* at microgram per gram concentration levels with a general precision of 8 per cent.

In vivo analysis can be carried out on 300–600 μg of sample obtained by a chemical biopsy method. The main problem encountered in *in vivo* analysis lies in obtaining an aluminium-free support material for etching purposes. In addition, "clean room" facilities are recommended for improving the accuracy.

It has been recognised that the occurrence of dental caries might be correlated with the concentration and distribution of certain trace elements in dental enamel and in dentine. This assumption has stimulated research into the determination of the distribution of trace elements in normal human dental enamel. Söremark and his collaborators[1–3] determined 11 major and minor constituents of normal human dentine and dental enamel. Losee, Cutress and Brown[4] found that about 35 trace elements were present in human enamel at concentration levels of not less than 0·01 $\mu g\ g^{-1}$. Retief *et al.*[5] investigated the normal distribution of 16 major and trace elements in normal human dentine and dental enamel.

Among the trace elements studied, fluorine, aluminium,[6] strontium[7] and vanadium[8] seem to be the most important for increasing resistance to caries. However, further clinical studies are necessary before the physiological role of trace elements will be fully understood. One of the basic requirements for carrying out research in this field is the selection of a suitable analytical method. The need for high sensitivity, selectivity and precision is reinforced by the limited amount of sample available (about 0·5 mg), particularly in *in vivo* analysis.

Neutron activation,[1,3,5,9] X-ray fluorescence[2] and spark-source mass spectrometry[4,10] have been used most extensively for these purposes. However, these techniques require rather expensive instrumentation and the first of them is generally associated with a chemical separation, which is tedious and time consuming. In addition, aluminium, one of the most interesting elements, cannot easily be determined by neutron-activation analysis owing to the rather short half-life of the ^{28}Al isotope produced by the reaction $^{27}Al(n,\gamma)^{28}Al$, and interference from the phosphate matrix by the fast-neutron reaction $^{31}P(n,\alpha)^{28}Al$.

A standard addition method has been proposed[7] for the accurate determination of strontium in dental enamel using an air - acetylene flame. Development of flameless atomisation

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enabled atomic absorption to be employed more efficiently in this field for the determination of metals. Parker[11] demonstrated the use of a carbon rod for the determination of lithium, potassium, strontium and lead, and a flame for the determination of calcium and magnesium *in vivo*. Langmyhr, Sundli and Jonsen,[12] using an inductively heated graphite furnace, determined cadmium and lead in dental material by direct atomisation of the solid sample.

The object of the present paper was to extend the use of the carbon cup atomisation technique to the determination of aluminium in dental enamel, and thus enable both *in vivo* and *in vitro* analyses to be carried out.

Experimental

Equipment

The atomic-absorption instrument used was a Varian AA-5 model in which the burner was replaced by a locally made carbon cup atomiser. Usually a Hitachi Perkin-Elmer 165 chart recorder (10 mV, 1-s full-scale deflection) was employed for recording the signals. In some experiments, however, oscilloscopic recording of the absorption signals with a Tektronix 547 oscilloscope was employed.

The power unit supplied a maximum of 600 A at 10 V to the carbon cup atomiser. In order to obtain temperature-controlled atomisation, the current passing through the carbon cup was varied by an automatic regulating system. The details of the carbon cup attachment, power unit and current regulating system have been given elsewhere.[13]

The following atomisation programme was selected for the determination of aluminium in dental enamel solutions: drying at 100 °C for 15 s; ashing at 350 °C for 10 s; and atomisation at 2500 °C for 4 s.

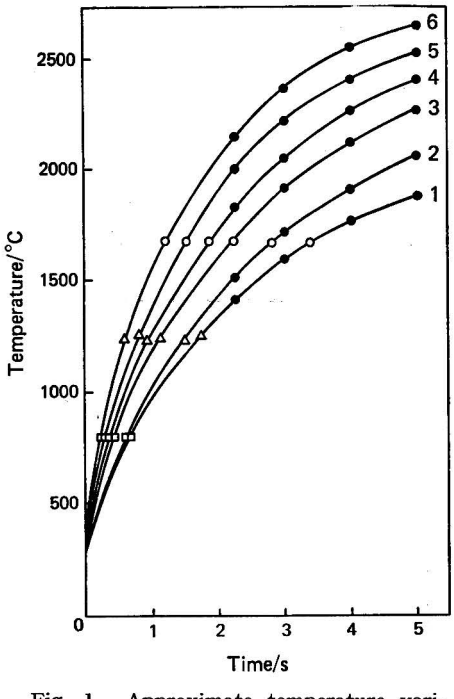

Fig. 1. Approximate temperature variation of the carbon cup during the atomisation cycle at different selected voltages. ●, Temperature measured with an optical pyrometer; and ○, △ and □, temperatures at which a measurable degree of atomisation is recorded with Al, Cu and Cd, respectively. Curves 1–6 relate, in order, to the average secondary voltages of the step-down transformer: 4·7; 5·0; 5·5; 6·0; 6·3; and 6·6 V.

Five microlitres of sample solution were taken with Oxford micropipettes for single measurements.

Measurement of Aluminium Absorption: Selection of Optimum Parameters

In order to select the optimum conditions for measurement of aluminium in the presence of large amounts of calcium phosphate, the absorption of aluminium was studied as a function of the following two parameters: (*i*) rate of temperature increase in the carbon cup; and (*ii*) concentration of calcium (as calcium phosphate) in the solution.

(*i*) The rate of temperature increase of the carbon cup during the atomisation cycle can be varied considerably by appropriate selection of the voltage on the step-down transformer.

Curves 1–6 in Fig. 1, corresponding to six different pre-selected atomisation voltages, illustrate the approximate increase in temperature of the carbon cup (inner walls) within the 5-s period of heating. On completion of the ashing cycle (zero time) the temperature of the carbon cup was estimated to be about 350 °C. The temperatures at the second, third, fourth and fifth seconds of the atomisation cycle were measured at the bottom of the cup with an optical pyrometer. However, measurement of temperature variation within the first 2 s was not possible by this method. Therefore, the additional points in the diagram were obtained by atomisation of aluminium chloride, copper chloride and cadmium chloride in the carbon cup. Variation of the ground-state atom concentration in the cup was monitored during the atomisation cycle by means of a storage oscilloscope.

It was assumed that an element will begin to atomise at one particular temperature regardless of the rate of increase in the temperature of the carbon cup. Therefore data from the oscillograms that show the absorption - time variation for the above elements were used to draw the initial course of the curves in Fig. 1. Absorption - time variation curves of aluminium corresponding to different rates of heating of the cup are presented in Fig. 2.

It is apparent that the height of the absorption peaks increases substantially when the carbon cup is heated more rapidly. On the other hand, the integrated absorption signals (area under the curves) show a similar tendency, although the effect is much less pronounced. It was assumed that at a slower rate of heating there is a greater chance for aluminium species to diffuse out of the carbon cup before appreciable atomisation occurs.

(*ii*) As dental enamel consists essentially of $Ca_{10}(PO_4)_6(OH)_2$ the influence of calcium (as calcium phosphate) upon aluminium absorbance was investigated. The diagram in Fig. 3 illustrates the effect of varying the calcium concentration in the solution upon the peak

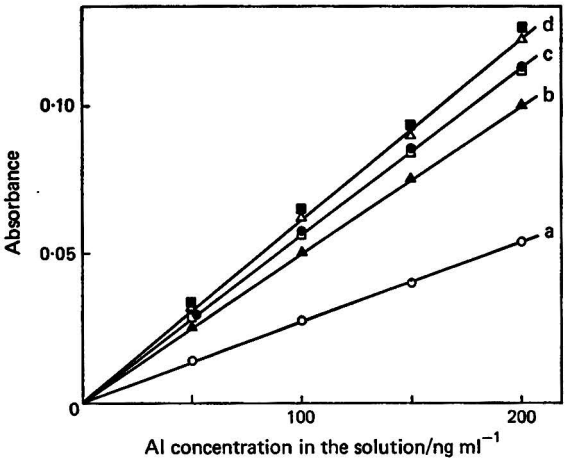

Fig. 3. Calibration graphs of aluminium; variation of sensitivity with calcium concentration in the solution (calcium present as phosphate in 1·2 N nitric acid solution). Calcium present: ○, 0; ▲, 0·65; □, 1·6; ●, 3·2; △, 6·5; and ■, 7·2 mg ml^{-1}. Graphs a–d are explained in the text.

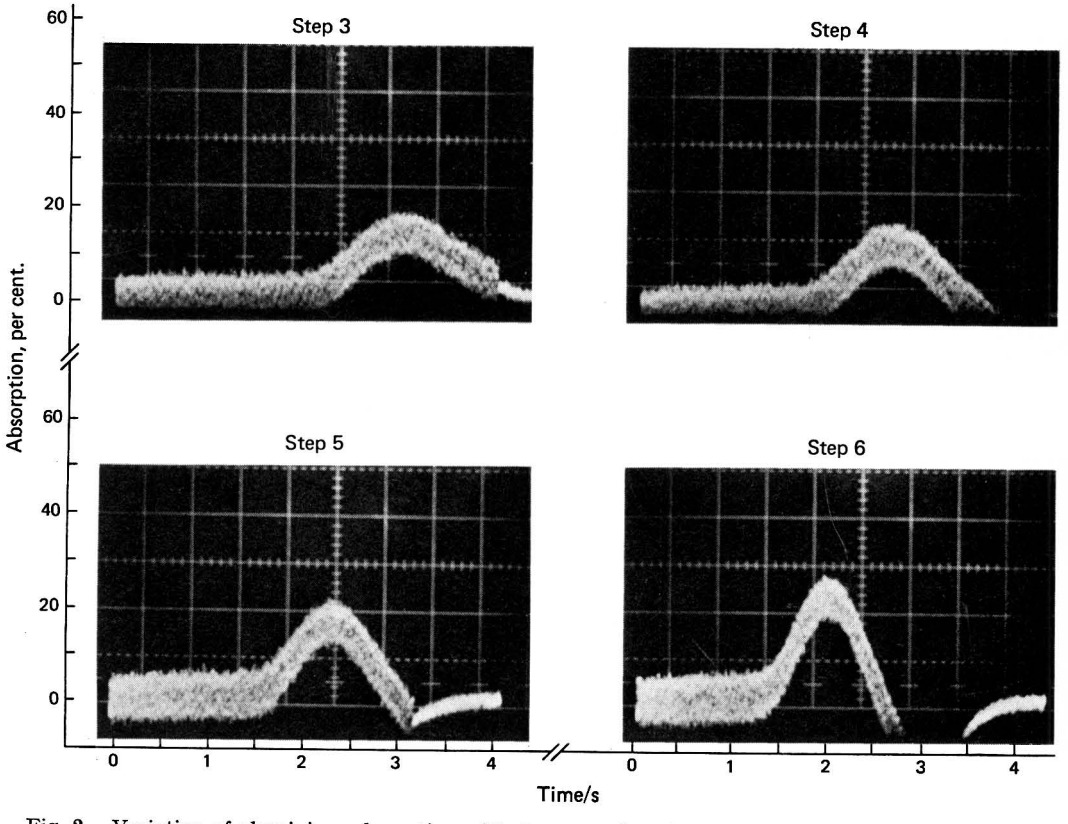

Fig. 2. Variation of aluminium absorption with time as a function of the rate of temperature increase during the atomisation cycle (steps 3–6 refer to the diagram in Fig. 1).

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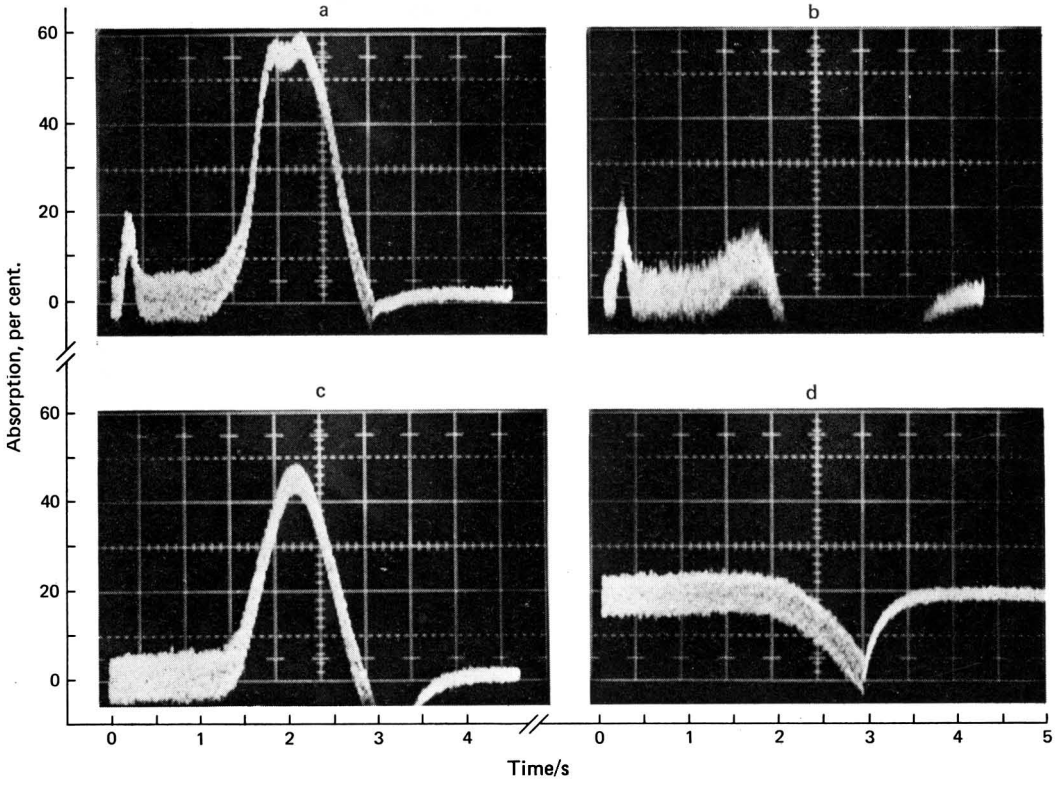

Fig. 4. Oscilloscope trace of the absorption signal: a, 110 ng ml^{-1} of Al + 7·2 mg ml^{-1} of Ca; c, 300 ng ml^{-1} of Al, no Ca present; b, as for a, hydrogen background corrector; and d, aluminium 309·3-nm base-line signal, no Al added in the cup.

absorbance of aluminium. Graph (a) corresponds to aqueous solutions of aluminium in the form of sulphate, chloride or nitrate. Graphs (b), (c) and (d) relate to an increased concentration of calcium in the solution (0·65–7·2 mg ml^{-1} of calcium). It is shown clearly that the enhancing effect of calcium upon aluminium sensitivity is not additive but approaches a constant value. A maximum two-fold increase in sensitivity to aluminium was obtained in the presence of 7·2 mg ml^{-1} of calcium, which corresponds to 20 mg of dental enamel per millilitre of the solution chosen for *in vitro* analysis. We have also observed an enhancing effect of calcium chloride by measurement of the lead and cadmium absorption.[13] Thompson, Godden and Thomerson[14] have recently reported the similar behaviour of calcium nitrate in enhancing markedly the absorption signals of aluminium, barium, beryllium, silicon and tin.

It seems unlikely that calcium would interfere with the atomisation of aluminium in the vapour phase by shifting the dissociation equilibria as suggested by the above authors. We attribute the enhancing effect of calcium to variation in the rates of vaporisation - diffusion processes.

The oscilloscope traces of the absorption signals presented in Fig. 4 (a and c) support the latter assumptions. By comparison of the absorption *versus* time functions, it can be noted that in the presence of calcium the aluminium atom concentration is built up much faster and persists longer in the optical path [note the difference in the shape of curves (a) and (c) in Fig. 4]. However, some additional evidence is necessary in order to identify the exact mechanism of this interference. Curve (b) in Fig. 4 illustrates the variation of the non-specific absorption signal during the atomisation cycle measured with a hydrogen background corrector. It is evident that the non-specific absorption component is essentially zero at the point of maximum atomic absorption of aluminium. Curve (d) in Fig. 4 shows that the variation of the aluminium base-line signal due to a change of aperture is practically negligible at the point of maximum atomic absorption of aluminium. No correction of the absorption signal is therefore necessary when carrying out the analysis of an enamel sample solution.

Determination of Aluminium in Dental Enamel *In Vitro*

The determination of aluminium in samples requires the dissolution of dental material in an appropriate acid and dilution of the solution to give the optimum concentration; nitric acid (6 N) and a concentration of 20 mg ml^{-1} of dental enamel were chosen for practical reasons.

In vitro analyses were performed as follows: 10 mg of finely ground and homogenised dental enamel were dissolved in 0·1 ml of 6 N nitric acid (Merck, Suprapure grade); 0·4 ml of distilled water was added and the solution left overnight in a sealed 1·5-ml plastic vial. The analyses were carried out by making six separate measurements on 5-μl aliquots and the results obtained averaged. A single calibration graph, prepared by the standard addition method, was adequate for a number of samples analysed at the same time. Recorder traces for a typical dental enamel analysis in which the standard addition method was used are shown in Fig. 5.

Special care was taken to prevent contamination of the samples during their preparation for analysis. The extracted teeth were carefully washed in order to remove trace amounts of various organic and inorganic material from the surface. The enamel was separated from the dentine mechanically either by abrading the surface with a diamond disc or by chipping it and grinding the chips in an agate mortar. In the latter instance only larger pieces of dental enamel were separated from the dentine and these were further homogenised before being weighed. The whole sample preparation including the dissolution step was made in a room the conditions in which approached "clean-room" conditions.

The 6 N nitric acid used for the dissolution of the samples and the distilled water used for the dilutions were checked for aluminium content; no measurable amounts of this metal were detected.

Some typical results for the determination of aluminium in human dental enamel are presented in Table I.

Sample No. 9 consists of different layers of the enamel from the same tooth. It is evident that the surface enamel sample No. 9A contains substantially more aluminium than the inside of the tooth. The approximate depth of the layers analysed is indicated in Table I. The samples were taken from the buccal side of the tooth.

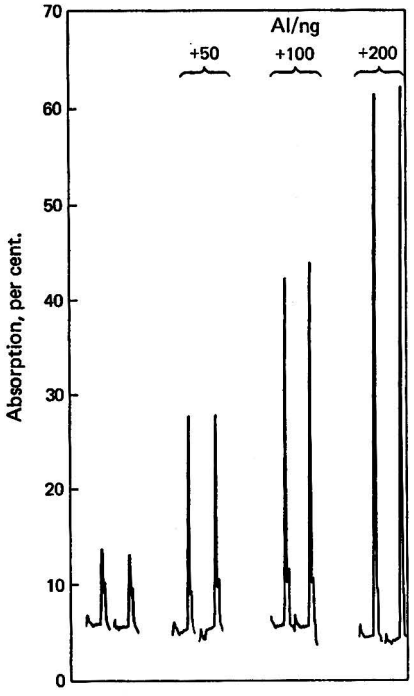

Fig. 5. Recorder traces of a typical dental enamel analysis employing the standard addition method (scale expansion × 2).

Sensitivity, Precision and Detection Limit

The sensitivity (1 per cent. absorption) and detection limit were calculated from the average slope of the calibration graph and standard deviation of the blank. For 5 μl of sample solution containing 20 mg ml^{-1} of dental enamel and a scale expansion factor of 2, the calculated values were 340 ng g^{-1} and 150 ng g^{-1}, respectively.

Repetitive measurements of the same sample solution (see Table I, samples Nos. 6, 7 and 8) showed that the precision of the atomic-absorption measurements is about $\pm$ 8 per cent. There is a distinct difference in precision between homogenised (Table I, samples Nos. 4 and 5) and non-homogenised samples (Table I, samples Nos. 1, 2 and 3).

TABLE I

ALUMINIUM CONTENT OF HUMAN DENTAL ENAMEL

Sample No.		Amount of sample/mg	Mean aluminium content/μg g^{-1}	Coefficient of variation, per cent.	Number of determinations
1		10	3·9	±14	14*
2		10	5·3	±14	8*
3		10	9·9	±13	5*
4		10	11·5	±9·0	6*
5		10	4·2	±7·5	5*
6		10	4·6	±5·4	16†
7		10	5·5	±10	16†
8		10	5·0	±5·2	16†
9	A	4·4 (0–120 μm)‡	39	—	8†
	B	20·5 (120–750 μm)	12	—	8†
	C	9·9 (750–1000 μm)	24	—	8†
	D	13·9 (1000–1300 μm)	18	—	8†

* Number of separate analyses.
† Number of separate measurements of a single solution.
‡ Depth of layer.

The accuracy of the method was not tested owing to the lack of a suitable dental enamel standard or of an appropriate comparative analytical method. The results obtained fell in the range of values (1–10 $\mu g\ g^{-1}$ of aluminium) found by Losee *et al.*,[4] who employed spark-source mass spectrometry, while neutron activation analysis[5] gives somewhat higher values [about 86 $\mu g\ g^{-1}$ of aluminium (mean of eight analyses)].

Studies on Aluminium Determination in Dental Enamel *In Vivo*

In clinical work in which only a small amount (about 0·5 mg) of dental enamel can be obtained from the patient, analyses must be carried out *in vivo*. Biopsy of the enamel can be performed mechanically, by polishing, or chemically, by etching with nitric acid. We chose the latter method for our experiments. Accordingly, we prepared from various plastic foam materials, discs, 4 mm in diameter, which were impregnated with 5 μl of 6 N nitric acid and placed upon the patient's tooth for approximately 60 s. The plastic disc was inserted immediately into a 1·5-ml polyethylene vial, 200 μl of distilled water were added and the vial sealed; 100 μl of this solution were diluted to 10 ml with a solution containing 2 mg ml^{-1} of potassium ions and the calcium content was measured in a nitrous oxide - acetylene flame. The mass of the sample was determined from the calcium concentration, assuming 36 per cent. of calcium in dental enamel. Experiments showed that 300–600 μg of sample are generally removed from the patient's tooth, corresponding to a surface layer of approximately 12 μm in thickness. It was assumed that the tooth enamel and aluminium present were dissolved in 6 N nitric acid in the same proportions. However, any deviation from this assumption may lead to an error in the aluminium determination.

Aluminium was determined in the remaining solution, a 10-μl aliquot being taken for each single measurement. A single calibration graph (curve b in Fig. 3) was employed with adequate accuracy for sample masses ranging between 300 and 600 μg. The error arising from the different sensitivities that resulted from variations in the concentration of calcium in the sample solution was estimated to be less than 5 per cent. in the extreme instance and could therefore be neglected.

One of the main problems associated with *in vivo* analysis is the preparation of aluminium-free discs for sampling purposes. Various plastic foam materials proved rather unsatisfactory, the blank values being of the same order of magnitude as samples and showing an extremely high standard deviation. Therefore, routine analysis *in vivo*, although theoretically possible, could not be carried out accurately by use of these materials. Various filter-paper media of adequate thickness and extreme purity are being investigated for this purpose.

Contamination of samples with saliva, dental plaque and various other materials, which might come into contact with the nitric acid impregnated discs during the sampling, could also be possible. However, isolation of the enamel, for example, by rubber dam, could equally well give rise to contamination problems. In dealing with nanogram amounts of a relatively abundant element such as aluminium, contamination cannot be completely prevented. "Clean room" facilities are certainly to be recommended if highly accurate analyses are required.

Conclusions

Atomic absorption employing the carbon cup atomisation technique provides a simple, sensitive and rapid method for the determination of aluminium in dental enamel at microgram per gram levels. Sample masses from 10–0·5 mg are adequate for replicate determinations, permitting both *in vitro* and *in vivo* analyses to be carried out.

The precision of the method of about $\pm$ 8 per cent. (*in vitro* analysis) is satisfactory for most applications in dental caries research.

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Effects Due to the Type of Glass Used in the Discharge Tubes on the Calibration of Emission Spectrometers for the Measurement of Nitrogen-15

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The influence of the type of glass used in the discharge tubes on the calibration of a spectrometer that is used for the measurement of nitrogen-15 by emission spectrometry has been investigated. Discrepancies in the values obtained for the same experimental samples measured with a Statron, Model NOI-5, emission spectrometer and with a Model NOI-4 emission spectrometer were traced to the use, with the latter instrument, of sealed glass calibration standards supplied by the manufacturer and prepared with glass different from that supplied for the preparation of experimental samples.

Three consignments each of Pyrex and Rasotherm glass were used to prepare discharge tubes containing nitrogen gas at six different levels of known nitrogen-15 abundance. Both the type and the consignment of glass had a considerable effect on the values obtained for apparent nitrogen-15 abundance. By applying a simple calibration expression appropriate to each batch of glass, corrected values in good agreement were obtained.

Results for experimental samples measured with the NOI-5 and NOI-4 emission spectrometers were in good agreement when each instrument was calibrated with standards prepared from the same consignment of glass as was used for the experimental samples.

Since the early reports[1,2] of the use of the technique of emission spectrometry to measure the abundance of the stable isotope nitrogen-15 the method has received only slow acceptance. Recently, with the introduction of commercial instruments specifically designed for nitrogen-15 determination, the method has found wider application[3–5] and with further improvements in technique[6] its use is likely to become more widespread.

Samples are prepared in expendable discharge tubes, and it is advantageous if Pyrex glass can be used because it is cheaper and easier to work with than silica. Pyrex absorbs light strongly at 300 nm, where its transmission falls rapidly with decreasing wavelength. It has been reported, however, that reproducibility suffers if Pyrex is used.[7] This was attributed to variations in the thickness of the capillary portion of the tube and to differences between batches of Pyrex.

The technique depends upon the principle that when nitrogen gas at low pressure is excited by radiofrequency energy, the wavelength of the light emitted is related to the isotopic form of nitrogen. Thus in the 2–0 band the nitrogen molecules $^{14}N^{14}N$ emit light at wavelength 297·7 nm, $^{14}N^{15}N$ at 298·3 nm and $^{15}N^{15}N$ at 298·9 nm. In the Statron spectrometers, Models NOI-5 and NOI-4, used in the studies reported here a rock salt prism monochromator is fitted with an automatic scanning device that traverses these wavelengths repeatedly at 1-min intervals. The monochromator output is detected by a photomultiplier and after appropriate amplification the signal is fed to a chart recorder. The nitrogen-15 enrichments are calculated from measurements of the relative intensities of the $^{14}N^{14}N$ and $^{14}N^{15}N$ peaks. The limited resolution of these instruments renders calibration with known standards essential, correction for background emission being a major problem at low nitrogen-15 abundances.

Modifications to a Statron, Model NOI-5, spectrometer and a new sample preparation procedure have been described previously.[6] The calibration of this instrument was carried out by using discharge tubes prepared by Dumas combustion of [^{15}N]ammonium chloride standards that were supplied by CZ Scientific Ltd. A Statron, Model NOI-4, spectrometer at

the National Institute for Research in Dairying had been calibrated by using standards, also supplied by CZ Scientific Ltd., consisting of sealed glass tubes containing nitrogen gas of stated enrichment. Results obtained with the NOI-4 for samples of natural abundance prepared from analytical-reagent grade ammonium chloride were high when this manufacturer's gas standards were used for calibration.

When samples from an experiment using nitrogen-15 were measured at the Long Ashton Research Station with the NOI-5 and at the National Institute for Research in Dairying with the NOI-4, the percentage values obtained for nitrogen-15 were found to be in poor agreement. Subsequent investigations have shown that this poor agreement was due to discrepancies in calibration arising from the use of different glass for calibration and sample discharge tubes. It will be shown below that an instrument must be calibrated not only for the type of glass used for the discharge tubes but for each consignment of glass. With appropriate calibration the type of glass used has no effect on the percentage values obtained for nitrogen-15. Results are presented that show the excellent agreement between the percentage values for nitrogen-15 thus obtained on samples measured both at the National Institute for Research in Dairying with an NOI-4 and at Long Ashton Research Station with an NOI-5.

Apparatus, Reagents and Methods

The Statron NOI-4 emission spectrometer is similar in many respects to the Model NOI-5, which has been described previously.[6] The two instruments have identical optics but differ mainly in that the NOI-5 has improved electronics. The associated apparatus, reagents, sample preparation and measurement techniques were as described previously,[6] except that the test samples listed in Table III were prepared by a semi-micro Kjeldahl digestion. Samples (containing 1–2 mg of nitrogen) of various fractions of calf rumen contents were digested with 3 ml of concentrated sulphuric acid and one tablet of mercury(II) oxide catalyst (Kjeltabs F. M., Thompson & Capper Ltd., Manufacturing Chemists, Liverpool). The ammonia released from portions of diluted digests (containing about 500 μg of nitrogen) by addition of an excess of 10 M sodium hydroxide solution containing 25 g l^{-1} of sodium thiosulphate was distilled in a Markham's apparatus and collected in 10 ml of 0·025 M hydrochloric acid. The resulting ammonium chloride solution was evaporated to dryness at 70 °C and the solid redissolved in distilled water to give a solution containing 1 μg μl^{-1} of nitrogen. A 10-μl volume of this solution was introduced into a capillary tube and the subsequent preparation of nitrogen-15 emission tubes was as reported previously.[6]

The discharge tubes were made from Pyrex glass supplied on three different occasions, designated consignments A, B and C. Discharge tubes of Rasotherm glass were obtained from CZ Scientific Ltd. on three different occasions and are designated consignments D, E and F.

Results

Calibration and Calculation

Discharge tubes were prepared with samples of ammonium chloride of known isotopic abundance and nitrogen gas was produced by Dumas combustion. After analysis in the spectrometer the heights of the nitrogen-28 and -29 peaks on the chart were measured using the interpolative method. From these measurements the ratio $R = \text{height}_{28}/\text{height}_{29}$ was obtained for insertion in the expression:

$$\text{Apparent nitrogen-15, per cent.} = 100/(2R + 1)$$

The percentage values for apparent nitrogen-15 at six levels of known abundance obtained using discharge tubes made from six different glasses are given in Table I. It is apparent that with both Pyrex and Rasotherm glass the actual consignment used to make the discharge tubes has an effect on the value obtained for apparent enrichment. However, apparent enrichment values are corrected to give true nitrogen-15 values by a simple calibration expression[6] in the following form:

$$\text{Actual nitrogen-15, per cent.} = [\text{First constant } (b) \times \text{apparent nitrogen-15, per cent.}] - \text{second constant } (c)$$

The appropriate values for these constants for each of the six glasses are also given in Table I.

Application of these expressions to the data given in Table I gives the percentage values for nitrogen-15 (Table II). There is very good agreement between these values, the differences introduced by the variations between glassware having been eliminated by the calibration.

TABLE I

APPARENT PERCENTAGE OF NITROGEN-15

Comparison of results obtained using three consignments of two types of glass for discharge tubes.

Glass	Actual nitrogen-15 content, per cent. 0·366	0·718	1·36	2·11	4·15	6·11	Calibration equation constants* *b*	*c*
	Apparent nitrogen-15 content (mean of three replicates), per cent.							
Pyrex A	0·347	0·649	1·21	1·88	3·66	5·39	1·142	0·030
B	0·352	0·673	1·24	1·92	3·78	5·50	1·115	0·026
C	0·358	0·680	1·27	1·96	3·86	5·66	1·083	0·022
Rasotherm D	0·354	0·657	1·23	1·89	3·73	5·45	1·121	0·030
E	0·371	0·687	1·26	1·94	3·81	5·61	1·107	0·044
F	0·379	0·713	1·30	1·99	3·85	5·61	1·099	0·054
Standard error as percentage of replicate mean	1·4	1·1	0·6	0·5	0·4	0·6	0.4†	

* Correlation coefficients for regression lines all greater than 0·9999.
† Maximum standard error.

Sample preparation and the spectrophotometric analysis were randomised and completed over a period of 3 weeks. The long-term stability of the instrument has been demonstrated previously.[6]

TABLE II

PERCENTAGE VALUES FOR NITROGEN-15 BY USING APPROPRIATE CALIBRATION EQUATION

Glass	Actual nitrogen-15 content, per cent. 0·366	0·718	1·36	2·11	4·15	6·11
	Corrected nitrogen-15 content (mean of three replicates), per cent.					
Pyrex A	0·366	0·711	1·35	2·12	4·15	6·13
B	0·367	0·724	1·36	2·11	4·19	6·11
C	0·366	0·714	1·35	2·10	4·16	6·11
Rasotherm D	0·367	•0·707	1·35	2·09	4·15	6·08
E	0·367	0·717	1·35	2·10	4·17	6·17
F	0·363	0·730	1·37	2·13	4·18	6·11
Standard error as percentage of replicate mean	1·4	1·1	0·6	0·5	0·4	0·6

Comparison of Results Obtained in Two Different Laboratories

In order to test the efficacy of the above calibration procedure, nitrogen was prepared from several biological samples containing nitrogen-15. Rasotherm consignment D discharge tubes were used for both the samples and the similarly prepared calibration standards and were analysed by using the Statron NOI-4 at the National Institute for Research in Dairying and the Statron NOI-5 at Long Ashton Research Station. The percentage values for nitrogen-15 obtained on these samples are compared with the values obtained with the NOI-4 when calibrated with the manufacturer's gas standards (Table III). For all but one of the experimental samples the values obtained from the two instruments agreed to within 0·01 atom per cent. of nitrogen-15; for the remaining sample, values differed by 0·03 atom per cent., corresponding to 1·3 per cent. of the value. These results illustrate the reproducibility that can be achieved in the measurement of nitrogen-15 by emission spectrometers in a given set of discharge tubes in two different laboratories.

We have not been able to detect any consistent variations between tubes made from one consignment of glass. Müller[8] compared the absorptivities of three types of glass (silica, Uviol and Rasotherm) at the wavelengths of the emission bands. He showed that the absorptivity was much higher for Rasotherm, which is similar to Pyrex, than for silica, while that for Uviol was intermediate between the two. Further, the transmission varied with wavelength, so that the effect on the intensities of the two nitrogen band heads measured was slightly different. Variation in the wall thickness of the glass might therefore be expected to cause some variation in the observed results. However, although the discharge tubes are made manually and the wall thickness of the constricted part of the tube (from which the light to be analysed is emitted) varies, there was no evidence to relate this variation to variations in the observed apparent percentage of nitrogen-15 values. This lack of evidence suggests that the effect of variations in wall thickness was small within the batches of tubes tested in the present experiment.

TABLE III

COMPARISON OF VALUES FOR NITROGEN-15 OBTAINED ON SAMPLES MEASURED AT THE NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING (NIRD) AND AT LONG ASHTON RESEARCH STATION (LARS)

	Nitrogen-15 (mean of two replicates), per cent.		
	Samples and calibration standards in same consignment of Rasotherm D glass		Samples in Rasotherm D. Calibration by manufacturer's gas standards in glass of unspecified type.
Code of sample	LARS using NOI-5 spectrometer	NIRD using NOI-4 spectrometer	NIRD using NOI-4 spectrometer
A 8	1·91	1·92	1·98
A 9	1·83	1·82	1·88
B 2	0·446	0·436	0·60
B 3	0·800	0·796	0·91
B 4	0·840	0·836	0·95
B 7	0·407	0·416	0·55
B 8	0·386	0·386	0·53
B 9	2·20	2·21	2·23
B 10	2·23	2·26	2·25
C 1 (NH_4Cl)	0·368	0·373	0·47
C 2 (NH_4Cl)	0·364	0·373	0·47

The authors are indebted to Dr. F. C. Sedgwick of J. A. Jobling & Co. Ltd. for the suggestion that the variation in the optical properties in the 300-nm wavelength region that is found in tubing purchased at different times may well be due to changes in the state of the iron (present at about 0·04 per cent. concentration) in the glass.

Conclusion

The results given in the tables show that with appropriate calibration the type of glass used for the discharge tubes has no effect on the value obtained for percentage of nitrogen-15 and that Pyrex or Rasotherm glass give perfectly satisfactory discharge tubes. Excellent agreement has been found between values for nitrogen-15 obtained on samples measured both at the National Institute for Research in Dairying with an NOI-4 and at Long Ashton Research Station with an NOI-5. It must be concluded that discharge tubes of the same type and consignment of glass must be used for experimental samples and for calibration standards, whether the latter are prepared by Dumas combustion of ammonium chloride or directly prepared from nitrogen gas.

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PTFE Apparatus for Vapour-phase Decomposition of High-purity Materials

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The determination of ultra-trace impurities in high-purity inorganic materials is often limited by the "blank" value of the solvent acids used for the decomposition of the samples. A device is described with which the problem is overcome by means of decomposition of the material in the vapour phase of acid mixtures produced in a sealed vessel heated to temperatures of up to 250 °C. It is shown that no contamination of the sample occurs when using relatively impure acid mixtures.

The ever increasing demands upon the analyst for the accurate determination of low levels of impurity elements in high-purity materials create many problems with general technique, sample dissolution and detection. Instrumental techniques such as atomic-absorption spectroscopy, especially when using flameless atomisation, enable the determination of many elements to be made at the concentrations of interest, and working conditions can be improved by using laminar-flow work benches in clean rooms, which reduces the risk of atmospheric contamination.

The process of sample decomposition and dissolution is still, however, the major source of high blank values and many attempts have been made to overcome this problem. Kuehner *et al.*[1] used a sub-boiling distillation technique to produce various acids of extremely high purity. The commercially available grades of high-purity acids and solvents are well known and are suitable for many purposes. In our experience, however, there still remains the problem of storage and of dispensing these high-purity solvents once they have been produced.

Ideally the level of trace impurities introduced into a sample during decomposition, prior to analysis, should be zero. The ideal is not easily attained but considerable reduction of blank values can be achieved if the sample material can be decomposed in the solvent vapour phase rather than in the liquid phase. Thomas and Smythe[2] have described a technique for the vapour-phase oxidation of plant material with nitric acid. The technique gave zero or very low blank values, but it is not easily applied to inorganic materials that require vigorous attack with acid, *e.g.*, siliceous materials. Zil'bershtein *et al.*[3] and more recently Mitchell and Nash[4] have described techniques for vapour-phase decomposition of siliceous materials prior to the determination of trace elements in these materials; decomposition of various materials has readily been achieved by the pressure-vessel or bomb technique as described, for example, by Bernas.[5]

The device described in this paper has been designed so as to incorporate the technique of pressure-vessel and vapour-phase decomposition into a single unit. It has the advantage of being easier to construct than the apparatus described by Mitchell and Nash, and it requires considerably smaller volumes of solvent acids. Heating can be carried out in an ordinary laboratory oven.

Experimental

Apparatus

A low-temperature (up to 110 °C) and high-temperature (up to 250 °C) version of the apparatus are shown in Fig. 1. Both devices consist of a sealable vessel machined from PTFE. Two concentric chambers are provided, an outer chamber, and an inner chamber that holds the sample cup. The low-temperature version is sealed by a PTFE lid and an aluminium cap; and the high-temperature version is completely encased in a stainless-steel container and cap. The essential dimensions, in millimetres, are given in Fig. 1(*a*).

Method

The finely ground sample (typically 100–200 mg) is weighed into the PTFE sample cup,

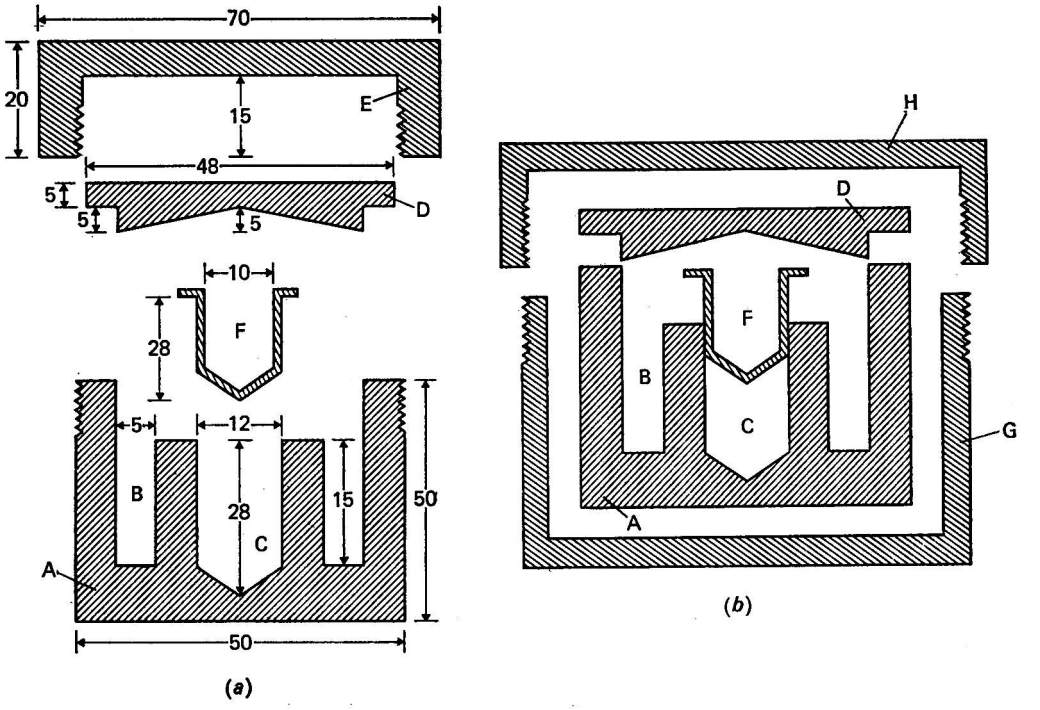

Fig. 1. Low-temperature (*a*) and high-temperature (*b*) vapour-phase decomposition vessel: A, PTFE vessel; B, outer chamber; C, inner chamber; D, PTFE lid; E, aluminium cap; F, sample cup; G, stainless-steel container; and H, stainless-steel cap. All dimensions are in millimetres.

which is then inserted in the central chamber of the vessel. About 5 ml of the solvent acid are introduced into the outer concentric chamber and the lid is fitted into position and tightly sealed by means of the screw cap. The complete vessel is then heated in an oven at 105–110 °C for several hours, usually overnight, and is then cooled to room temperature. Although the lid of the vessel has been designed to promote drainage of condensate back into the acid chamber, it is an advantage to rotate the vessel about its vertical axis before removing the lid, in order to centrifuge droplets into the outer chamber.

The device described was designed in the first instance for the decomposition of high-purity glasses prior to the determination of various impurity elements by means of atomic-absorption spectroscopy using the flameless-atomisation technique. The effectiveness of the decomposition technique was therefore assessed using samples of this type. A bulk sample of sodium silicate - calcium silicate glass of high purity was reduced to a fine powder by the electro-hydraulic method so as to reduce risk of contamination. Suitable equipment for this technique is available from the Analytical R & D Unit, AERE, Harwell, Oxfordshire.

Sample masses of 200 mg were used in all the experiments and decomposition was carried out using a mixture of 6 ml of concentrated nitric acid (sp. gr. 1·4) and 4 ml of 50 per cent. hydrofluoric acid (both Carlo Erba high-purity grades). The effect of increased impurity of the solvent acid was determined by adding relatively high concentrations of iron and copper to the acid mixture prior to decomposition of the glass sample. After vapour-phase attack the powdered sample of glass remains as a dry powder in the sample cup. It is virtually free of fluoride and silicon and consists of the nitrates of the other elements. Silicon tetrafluoride formed during decomposition is absorbed into the acid mixture when the vessel is cooled and is thus separated from the sample matrix.

In order to carry out duplicate sample and blank determinations simultaneously, a vessel having a four-sample inner chamber was used. Two of the four sample cups were left empty and were used in the final stages of the determination to provide the blank values. The dry residues in the sample cups were dissolved in 2 ml of silica-distilled water and the solutions

transferred to silica tubes (50 × 12 mm i.d.), the ends of which were tapered to 2–3 mm i.d., and the tubes were stoppered with PTFE stoppers. The sample cups were rinsed with two 1-ml portions of 0·2 per cent. *m/V* ammonium tetramethylenedithiocarbamate that had been buffered at pH 6 with sodium acetate and purified by extraction with chloroform. The rinsings were transferred to the silica tubes and 0·2 ml of chloroform, redistilled from silica apparatus, was added to the solutions and the mixtures were shaken for 1 min. The layers were separated by centrifuging at 2000 rev min^{-1} for 1 min and the aqueous layer was removed by suction through a thin silica tube.

Calibration graphs for the ranges 0·05–0·5 $\mu g\ g^{-1}$ of iron and 0·02–0·1 $\mu g\ g^{-1}$ of copper were prepared by carrying out the extraction procedure on dilute solutions of copper and iron that had been added to 2 ml of silica-distilled water in the silica tubes.

The concentrations of copper and iron in the chloroform extracts were determined by flameless atomic-absorption spectrophotometry using the 110 Digital Flameless Atomiser (S. & J. Juniper & Co.) in conjunction with the Pye Unicam SP1900 atomic-absorption spectrophotometer. For the determination of copper, 20-μl aliquots of the extract were used at 324·7 nm and for iron 10-μl aliquots at 372·0 nm.

Results and Discussion

The results given in Table I show that no contamination of the sample occurs during decomposition even when the acid mixture contains relatively high concentrations of impurity elements.

TABLE I

EFFECT OF ADDITION OF COPPER AND IRON TO DECOMPOSITION ACID

Added copper and iron/$\mu g\ ml^{-1}$	Concentration of copper/$\mu g\ g^{-1}$		Concentration of iron/$\mu g\ g^{-1}$	
	Blank	Sample	Blank	Sample
0	< 0·005	0·07	0·1	0·31
5	< 0·005	0·07	<0·01	0·30
250	< 0·005	0·07	0·01	0·31

These results, and in particular the blank values, may be compared with those obtained when a liquid-phase decomposition technique is used. The method of Fuller and Whitehead[6] was used for this comparison and blank values of 0·08 and 0·15 $\mu g\ g^{-1}$ for iron and copper, respectively, were obtained. While the mean values for the samples were similar to those obtained by use of the vapour-phase decomposition method the precision was worse, particularly for copper, for which the level is closer to the limit of detection.

In addition to the obvious advantages of lower blank values, the vapour-phase decomposition method, when used for the decomposition of siliceous materials such as glasses, has the advantage of producing a decomposition product that is soluble in water. By increasing the ratio of nitric acid to hydrofluoric acid, glasses other than soda-lime glasses, *e.g.*, lead glasses, have been readily decomposed.

The device described can be used for the decomposition of high-purity inorganic materials using relatively impure solvent acids. Ordinary reagent-grade acids are used in these laboratories for the analysis of high-purity glasses and application of the technique to other sample types is under consideration.

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Analytical Methods Committee

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

Determination of Small Amounts of Lead in Organic Matter by Atomic-absorption Spectrometry

The Analytical Methods Committee has received and approved for publication the following Report from its Metallic Impurities in Organic Matter Sub-Committee.

Report

The constitution of the Sub-Committee responsible for the preparation of this Report was: Dr. L. E. Coles (Chairman), Mr. J. R. Bishop (resigned May, 1973), Mr. W. Cassidy, Mr. S. Greenfield (appointed July, 1973), Mr. W. H. Hill (appointed May, 1973), Dr. R. A. Hoodless, Mr. E. E. J. King, Mr. D. A. Lambie, Dr. H. Liebmann (resigned June, 1972), Dr. R. F. Milton, Mr. W. L. Sheppard and Mr. C. A. Watson, with Mr. P. W. Shallis as Secretary and Mr. J. J. Wilson as Assistant Secretary.

Introduction

Many methods have been described for the determination of lead over a wide range of concentrations and in many different substances. With the increasing interest in toxicological problems presented by the widespread occurrence of lead there has been increasing pressure on analytical chemists to determine the element in a wide range of substances at ever lower levels. In the work discussed in this Report the Sub-Committee concentrated on the determination of lead below the parts per million level by use of atomic-absorption spectrometry. The organic matter was destroyed by wet oxidation in the presence of sulphuric acid and the lead was recovered from the resulting aqueous solution by complexing it with an organic reagent and extracting the complex into an organic solvent that could be aspirated into the flame.

Experimental

It is usually convenient to destroy the organic matter with hydrogen peroxide solution in the presence of hot concentrated sulphuric acid,[1,2] and this procedure was adopted by the Sub-Committee. 1,5-Diphenylthiocarbazone (dithizone) and ammonium pyrrolidine dithiocarbamate (APDC) were examined as complexing agents for the recovery of the lead from the resulting aqueous solution.[3,4]

Initially the members of the Sub-Committee carried out determinations on lead solutions that they had prepared, in order to gain experience with the techniques and to establish optimum operating conditions for their instruments before the first collaborative exercise was arranged. Two solutions, one containing 0·3 and the other 0·8 p.p.m. of lead, were pre-

TABLE I

DETERMINATION OF LEAD IN SYNTHETIC SOLUTIONS

Sample A contained 0·3 p.p.m. of added lead; sample B contained 0·8 p.p.m. of added lead.

			Lead found, p.p.m., after extraction with—			
			Dithizone		APDC	
Laboratory	Instrument	Wavelength/nm	A	B	A	B
A	Single beam, grating	217·0	0·20	0·80	0·36	0·86
B	Single beam, grating	217·0	0·28	0·84	0·27	0·81
C	Double beam, grating	283·3	—	—	0·31	0·73
D	Double beam, grating	283·3	0·20	1·0	0·20	1·0
E	Single beam, prism	217·0	0·28	0·57	0·23	0·62

pared in one laboratory by adding a solution of a lead compound to sucrose, digesting it with nitric acid - sulphuric acid and diluting with sulphuric acid; portions of these solutions were distributed to the participating members. Three standard solutions containing 0·2, 0·5 and 1·0 p.p.m. of lead were also prepared and distributed for calibration purposes. The extraction of lead from the solutions was carried out with dithizone and APDC in parallel experiments and the results are given in Table I. It was noticed in many of these sets of results for both the unknown and the standard solutions that when three runs had been made on one solution two figures were in good agreement and the third was low.

Although the results in Table I do not prove that either extractant is better than the other it was decided to adopt APDC for extraction of lead from the aqueous medium and members again carried out preliminary tests on solutions of lead prepared in their own laboratories before starting on the next collaborative exercise. For this purpose lead nitrate was added to lime juice, giving an added lead content of 2·0 p.p.m., and portions of this and of the untreated lime juice were distributed to all members. The method used was that given in the Appendix, except that amyl acetate and 4-methylpentan-2-one were compared as extraction solvents. The results obtained are given in Table II. It was decided to standardise on 4-methylpentan-2-one; amyl acetate was reported as being easier to use but giving less sensitivity and more erratic results.

TABLE II

DETERMINATION OF LEAD IN LIME JUICE

Laboratory	Instrument	Wavelength/nm	Lead found, p.p.m., in— Sample	Sample + 2·0 p.p.m. of added lead
A	Single beam, grating	217·0	0·06, 0·08, 0·04	2·16, 2·08, 2·02
B	Single beam, grating	217·0	0·03, 0·03, 0·04	1·92, 2·00, 2·08
C	Double beam, grating	283·3	—	2·08
D	Double beam, grating	283·3	<0·05, <0·05, <0·05	1·92, 1·86, 1·84
E	Double beam, grating	283·3	<0·05, <0·05, 0·0	2·00, 2·05, 1·76

The organic matter in lime juice was relatively easy to destroy by wet oxidation; in consequence a baby food consisting of a strained cheese savoury was selected for the next test as it was known to be a difficult material to oxidise. The cheese savoury was divided into two portions and lead equivalent to 0·5 p.p.m. was added to one of them. The cans used for containing the cheese savoury were fully lacquered and the seams sealed with a pure tin solder, *i.e.*, a solder that contained no lead. Portions of these two mixtures were then sealed in the cans for distribution to the participating laboratories. The results obtained are given in Table III. In addition, one collaborator took four extra cans of the food with added lead and carried out three determinations on the contents of each can; these results are given in Table IV. Another collaborator added an extra 1 p.p.m. of lead to both portions and calculated the recovery of this added lead (Table V).

TABLE III

DETERMINATION OF LEAD IN CHEESE SAVOURY

Laboratory	Lead found in sample, p.p.m.	Mean, p.p.m.	Lead found in sample + 0·5 p.p.m. of added lead, p.p.m.	Mean, p.p.m.
A	0·02, 0·04, 0·02, 0·04	0·03	0·59, 0·54, 0·49, 0·46	0·52
B	0·01, 0·01	0·01	0·37, 0·33	0·35
	0·06, 0·05	0·06	0·48, 0·50	0·49
D	—	0·07	0·65, 0·48, 0·58, 0·53, 0·48, 0·49, 0·51, 0·51, 0·48, 0·49	0·52
G	0·10, 0·00, 0·10, 0·00	0·05	0·39, 0·91, 0·35, 0·74	0·60
	0·00, 0·00, 0·00, 0·00	0·00	0·49, 0·40, 0·42, 0·40	0·43

A further test was arranged on a sample of tomato soup to which lead and cadmium had been added before canning. The same procedure was followed as for the lime juice and the cheese savoury and the results are given in Table VI. This sample had previously been used

TABLE IV

ADDITIONAL RESULTS FROM LABORATORY D

Can	Lead found in sample + 0·5 p.p.m. of added lead, p.p.m.	Mean, p.p.m.	Standard deviation, p.p.m.
1	0·52, 0·56, 0·50	0·56	0·036
2	0·58, 0·56, 0·57		
3	0·54, 0·54, 0·52		
4	0·59, 0·57, 0·63		

for a collaborative exercise on a method for the determination of cadmium, and the satisfactory agreement between results obtained[5] indicated that the cadmium was distributed uniformly throughout the tomato soup, and was retained in the solution after digestion.

TABLE V

ADDITIONAL RESULTS FROM LABORATORY B

Sample	Lead found, p.p.m.	Mean, p.p.m.	Mean recovery, per cent.
Cheese savoury	0·06, 0·05	0·06	
+ 1 p.p.m. of lead	1·00, 1·03	1·02	
Recovery of added lead	0·94, 0·98	0·96	96·0
Cheese savoury with 0·5 p.p.m. of lead	0·48, 0·50	0·49	
+ 1 p.p.m. of lead	1·42, 1·43	1·43	
Recovery of added lead	0·94, 0·93	0·94	94·0

TABLE VI

DETERMINATION OF LEAD IN TOMATO SOUP

Laboratory	Lead found in soup, p.p.m.	Mean, p.p.m.	Lead found in soup + 0·5 p.p.m. of added lead, p.p.m.	Mean, p.p.m.	Recovery of added lead, p.p.m.	Mean, p.p.m.
A	0·16, 0·16	0·16	0·80, 0·76	0·78	0·64, 0·60	0·62
B	0·01, 0·08, 0·02	0·04	0·72, 0·59, 0·44	0·58	0·62, 0·51, 0·42	0·52
D	0·15, 0·10, 0·08, 0·08, 0·10, 0·10, 0·17, 0·17, 0·08, 0·06, 0·08, 0·08	0·10	0·62, 0·60, 0·58, 0·60, 0·58, 0·71 0·58, 0·83, 0·60, 0·60, 0·58, 0·64	0·63	—	0·53
F	0·09, 0·05, 0·05	0·06	0·61, 0·59, 0·57, 0·57, 0·57, 0·57	0·58	0·55, 0·53, 0·51, 0·51, 0·51, 0·51	0·52
G	0·08, 0·06, 0·05	0·06	0·44, 0·42, 0·43	0·43	0·38, 0·36, 0·37	0·37

APPENDIX

Method for the Determination of Lead

Principle

After destruction of organic matter the lead is complexed with ammonium pyrrolidine dithiocarbamate, the complex is extracted into 4-methylpentan-2-one and this solution is aspirated into the burner of an atomic-absorption spectrometer.

Reagents

4-Methylpentan-2-one. Low in lead grade.

Ammonium pyrrolidine dithiocarbamate (APDC) solution. Place approximately 1·5 g of APDC in a porosity 4 sintered-glass crucible, wash with 20 ml of acetone and suck dry by means of a water-pump. Weigh 1·0 g of the washed and dried APDC, dissolve it in 100 ml of water containing 0·1 g of mercaptoacetic acid and neutralise the solution to pH 6–7.

Standard lead solution, 10 $\mu g\ ml^{-1}$.

Apparatus

Use an atomic-absorption spectrometer with an air - acetylene burner and a suitable lamp.

Preparation of Sample

Destroy the organic matter in an appropriate amount of sample by wet oxidation by using one of the recommended methods.[1,2,6] If sulphuric acid and 50 per cent. hydrogen peroxide solution are used for the wet oxidation it is essential that the instructions given in the Sub-Committee's reports[1,2] are followed for the particular type of sample under test, otherwise low recoveries will be obtained. When oxidation is complete, dilute the cold solution with 10 ml of water, add 2 ml of 10 per cent. sodium sulphite solution, and boil until white fumes of sulphur trioxide appear.

Procedure

Dilute the digest from 25 g of sample (or other suitable mass, depending on the expected lead content) to 50 ml with water, cool and transfer the solution to a separating funnel. Add 2 ml of the APDC solution, shake and allow to stand for 5 min. Accurately add 10·0 ml of 4-methylpentan-2-one and shake the mixture vigorously for 1 min. Allow the layers to separate, discard all of the aqueous layer and filter the organic layer through a small dry Whatman No. 541 filter-paper into a suitable stoppered flask.

Calibration

Prepare standard solutions so as to cover the required range of lead contents by adding portions of the standard lead solution to 5 ml of sulphuric acid and diluting each standard to 50 ml with water. Add 2 ml of APDC solution and 10 ml of 4-methylpentan-2-one, and proceed as described for the sample solution.

Measurement

Set up the instrument with a lead lamp and the monochromator adjusted to either 217·0 nm or 283·3 nm, whichever gives the greater signal to noise ratio (see Note 1). Prepare a calibration graph (see Note 2) by aspirating the standard solutions and plotting the mean signal response against lead content. Aspirate the solution obtained from the sample, record the mean signal response and read off the lead content from the calibration graph. If a large number of samples is being examined one or more standard solutions must be re-aspirated at intervals during the course of the analysis.

NOTES—

1. Aspirate the ketone when setting the zero of the instrument and between all readings. Care must be taken with some burner assemblies, *e.g.*, the 3-slot Boling, when changing solutions as the removal of the ketone renders the flame weak, with a tendency to flash back.
2. The calibration graph will be linear over the range 0–30 μg of lead; above 30 μg the graph will exhibit a slight curvature.

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Book Reviews

CHEMICAL PHASE ANALYSIS. By ROLAND S. YOUNG. Pp. viii + 138. London and High Wycombe: Charles Griffin & Co. Ltd. 1974. Price £4·50.

By simply scanning this title the reader is unlikely to obtain an immediate indication of the book's coverage, though it is not easy to suggest anything better or more succinct.

In industry and commerce, the analyst is often required to report on the extent to which specified valency states of a particular element are present in a sample. A typical everyday example involves the determination of ferrous, ferric, and metallic iron in certain commercially produced iron oxide powders. This typifies the analytical problems associated with the thirty-four elements, ranging from aluminium to zirconium, that are dealt with in Chemical Phase Analysis.

Some of the determinations are disposed of in under a single page, and many with less convincing details than those given for the more frequently encountered and better understood example just quoted. Most of the procedures referred to (not necessarily tailor-made for immediate application) have already been published (at least in principle) elsewhere, but the book appears to be unique in that it collates the information for the first time, albeit very briefly on occasions, under a single cover.

Recognising the place of more sophisticated (instrumental) approaches to these problems, there are many occasions on which it is expedient and much cheaper to use relatively inexpensive, simple chemical procedures for this work, and therein lies the potential value of this publication.

It is doubtful, however, whether the author has sufficiently emphasised the empirical nature, or made adequate critical appraisals, of many of the methods referred to. In this connection, indications of precision and comparisons between values for the same constituent, established by at least two procedures (when additional methods exist), would have been useful.

The author rightly and fairly indicates that the publication provides "valuable information," and that "for process control, an approximate result obtained quickly, can be very useful"; comments that could be appropriately embodied in a brief summary of the book's uses and limitations.

W. T. ELWELL

ATOMIC ABSORPTION SPECTROMETRY. Edited by MAURICE PINTA. Translated by K. M. GREENLAND and F. LAWSON. Pp. xxii + 418. London: Adam Hilger Ltd. 1975. Price £35.

This book is the English edition of the French original, which was published in 1971. The main objective of the authors of the French edition was a new presentation of the subject that would differ as widely as possible from related works in the English language. The result was a synthesis of the individual expertise of a number of practising specialists, each of whom contributed sections to the volume. Although 4 years have elapsed since the publication of the original French edition, and much progress has been made in the technique and practice of atomic-absorption spectrometry during that time, the text is most useful in its English edition. This results partly from the revision of the material in several chapters, but principally from the fact that the coverage of the various fields of application of the technique was comprehensive in the earlier edition. The most important techniques and procedures in these areas have not changed appreciably.

The volume contains 16 chapters. The first five chapters, which constitute approximately one-third of the book, are concerned with basic concepts, instrumentation, radiation sources and atomisers, interferences and the determination of optimal analytical conditions. The principal part of the text, in 11 chapters, encompasses the most important applications of atomic-absorption spectrometry and its use in the analysis of rocks and soils, ores, metals and alloys, nuclear fuels and shield materials, water and vegetable matter; the applications of the technique in biochemistry, toxicology and civil engineering are also discussed. Two concluding chapters are devoted to indirect methods of analysis, isotopic analysis and sundry applications, and industrial products, wines, glass and ceramics, coal and coal ash and air, atmosphere and gas analysis.

This is a very fine book, well produced and containing a wealth of detail concerning practical procedures of analysis by atomic-absorption spectrometry. The only disappointing feature is the absence of much information on the techniques of electrothermal atomisation, which are at present assuming major importance in the practice of atomic-absorption spectrometry. Nevertheless, this volume should be assured of a wide readership by practising analytical chemists.

G. F. KIRKBRIGHT

TECHNIQUE OF ELECTROORGANIC SYNTHESIS. *Techniques of Chemistry. Volume V, Parts I and II.* Edited by NORMAN L. WEINBERG. New York, London, Sydney and Toronto: John Wiley and Sons. Part I. 1974. Pp. x + 197. Price £20. Part II. 1975. Pp. xiv + 1070. Price £22.

Electroorganic synthesis has for many years been considered a black art, largely ignored both by organic chemists and electrochemists. In recent years, however, there have been considerable developments in electroanalytical techniques such as polarography and cyclic voltammetry, so that an extensive bibliography of organic electrode reactions is now available. Thus the organic chemist now has access to a uniquely important synthetic tool, which has the important feature that electrode-specific synthesis can be carried out, leading in many instances to products that are not readily attainable by other methods. Part I consists of six chapters, covering the main areas of theory and experimental technique. The chapter by Goodridge and King on experimental methods and equipment outlines the principles of electrode and cell design, measuring equipment and the development and scale-up of industrial cells. Techniques for the study of organic electrode reactions are reviewed by Conway and Rudd. This chapter surveys the role of polarography, cyclic voltammetry and coupled electrochemical - spectroscopic techniques in the study of the mechanism of electrode reactions. An extremely comprehensive and readable chapter on the electrooxidation of organic compounds is presented by the Editor of the volume (300 pages), while the final two chapters deal with oxidation of aliphatic and aromatic nitrogen functions (Nelson) and the anodic reactions of carboxylates (Utley).

The volume contains an excellent balance of theory and practice and provides a comprehensive background in electrochemical theory as well as the basic experimental facts on cells, instrumentation, electrodes, solvents and supporting electrolytes.

Part II consists primarily of a vast compilation of data on electrosynthetic reactions. The areas covered include electrochemical halogenations, electrochemical reduction of organic compounds, preparation of synthetic and naturally occurring *N*-heterocyclic compounds, organometallics and electrosynthesis of polymers.

Most of these chapters contain a large amount of information of value to electroanalytical chemists as well as those involved in the synthetic field. This is particularly so with the last section by Siegerman, which consists of a 400-page summary of oxidation and reduction potentials and electrolysis conditions of organic compounds.

As one might expect in such a collection of electrochemical data, one can find some minor errors of omission and of mechanistic interpretation, but this in no way detracts from the over-all value of the book.

Together, the two volumes comprise one of the key texts to this important and rapidly expanding field.

B. FLEET

Errata

SEPTEMBER (1975) ISSUE, p. 669, line 11: *for* "33 mg kg^{-1}" *read* "3 mg kg^{-1}."

OCTOBER (1975) ISSUE, p. 689: *The last two lines should read* "Small amounts of unused cyanogen bromide, used tissue wipes and glassware can be rendered harmless by treatment with sodium hypochlorite (10 per cent. available chlorine, diluted 2 or 3-fold) for 1–2 h before discharge, after dilution with a large volume of water."

NOVEMBER (1975) ISSUE, plate facing p. 812: *The caption should read* "Fig. 3. Photograph of flow cell."

PATENTS

BRITISH PATENT No. 1261948

Quinazoline Derivatives and Conversion of same to Benzodiazepine Derivatives. Owner desires commercial exploitation on reasonable terms by license or sale. Inquiries Fitzpatricks, 14-18 Cadogan Street, Glasgow G2 6QW and Warwick House, Warwick Court, London WC1R 5DJ.

APPOINTMENTS VACANT

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An Improved Field Test for Barbiturates and Hydantoins with a Modified Cobalt(II) Thiocyanate Reagent

The classical Dille - Koppanyi cobalt(II) acetate reagent has been rendered more sensitive and stable so as to provide a convenient single-fluid reagent for field testing for barbiturates. The test will also detect hydantoins and other hypnotics. Reagents based on cobalt(II) thiocyanate are essentially stable and are able to detect sub-microgram amounts of barbiturates in the keto form. The colour developed with barbiturates can be preserved indefinitely.

M. J. de FAUBERT MAUNDER

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

Analyst, 1975, **100**, 878–883.

Determination of Aluminium in Dental Enamel by the Carbon Cup Atomic-absorption Method

The possibility of using the carbon cup atomisation technique for the atomic-absorption determination of aluminium in dental enamel has been investigated. Various parameters that influence the sensitivity and accuracy of the measurements were studied by use of a standard electronic detection system (Varian AA-5; chart recorder f.s.d., 1 s) and oscilloscopic (Tektronix) recording of the absorption signal. The latter system enabled the atomic- and non-specific absorption signals to be studied as a function of the time of atomisation. Thus, increasing the rate of heating of the carbon cup in the atomisation step resulted in a substantial enhancement of the aluminium peak absorption signal. The presence of calcium in the solution (7·2 mg ml^{-1}, in the form of the phosphate) produced a two-fold increase in sensitivity for aluminium.

For routine determinations of aluminium in tooth material (*in vitro*) 10 mg of finely ground and homogenised sample are dissolved in 6 N nitric acid and the solution is diluted to 0·5 ml; 5-μl aliquots are sampled for a single determination. A single calibration graph obtained by the standard addition method is adequate for one set of samples. No correction due to non-specific absorption is necessary, but the blank value (nitric acid) should be checked from time to time.

The absolute sensitivity of the method ($3{\cdot}5 \times 10^{-11}$ g of aluminium) permits the determination of aluminium in tooth material both *in vitro* and *in vivo* at microgram per gram concentration levels with a general precision of 8 per cent.

In vivo analysis can be carried out on 300–600 μg of sample obtained by a chemical biopsy method. The main problem encountered in *in vivo* analysis lies in obtaining an aluminium-free support material for etching purposes. In addition, "clean room" facilities are recommended for improving the accuracy.

F. DOLINŠEK, J. ŠTUPAR and M. ŠPENKO

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Analyst, 1975, **100**, 884–890.

Effects Due to the Type of Glass Used in the Discharge Tubes on the Calibration of Emission Spectrometers for the Measurement of Nitrogen-15

The influence of the type of glass used in the discharge tubes on the calibration of a spectrometer that is used for the measurement of nitrogen-15 by emission spectrometry has been investigated. Discrepancies in the values obtained for the same experimental samples measured with a Statron, Model NOI-5, emission spectrometer and with a Model NOI-4 emission spectrometer were traced to the use, with the latter instrument, of sealed glass calibration standards supplied by the manufacturer and prepared with glass different from that supplied for the preparation of experimental samples.

Three consignments each of Pyrex and Rasotherm glass were used to prepare discharge tubes containing nitrogen gas at six different levels of known nitrogen-15 abundance. Both the type and the consignment of glass had a considerable effect on the values obtained for apparent nitrogen-15 abundance. By applying a simple calibration expression appropriate to each batch of glass, corrected values in good agreement were obtained.

Results for experimental samples measured with the NOI-5 and NOI-4 emission spectrometers were in good agreement when each instrument was calibrated with standards prepared from the same consignment of glass as was used for the experimental samples.

C. P. LLOYD-JONES, JILL ADAM

University of Bristol, Department of Agriculture and Horticulture, Research Station, Long Ashton, Bristol, BS18 9AF.

and D. N. SALTER

National Institute for Research in Dairying, Shinfield, Reading, RG2 9AT.

Analyst, 1975, **100**, 891–895.

PTFE Apparatus for Vapour-phase Decomposition of High-purity Materials

The determination of ultra-trace impurities in high-purity inorganic materials is often limited by the "blank" value of the solvent acids used for the decomposition of the samples. A device is described with which the problem is overcome by means of decomposition of the material in the vapour phase of acid mixtures produced in a sealed vessel heated to temperatures of up to 250 °C. It is shown that no contamination of the sample occurs when using relatively impure acid mixtures.

J. F. WOOLLEY

Standard Telecommunication Laboratories Limited, London Road, Harlow, Essex, CM17 9NA.

Analyst, 1975, **100**, 896–898.

Determination of Small Amounts of Lead in Organic Matter by Atomic-absorption Spectrometry

Report prepared by the Metallic Impurities in Organic Matter Sub-Committee.

ANALYTICAL METHODS COMMITTEE

The Chemical Society, Burlington House, London, W1V 0BN.

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