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Summaries of Papers in this Issue

Coulometric Microtitration of Arsenic(III) and Isoniazid Using a Vitreous Carbon Generating Electrode

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V. J. JENNINGS, A. DODSON and A. HARRISON
Department of Chemistry and Metallurgy, Lanchester Polytechnic, Priory Street, Coventry, CV1 5FB.

Analyst, 1974, **99**, 145–148.

Gel Supports for Electrophoresis with Pyridine - Acetic Acid Media

Agar gels that are suitable for use with an 80 per cent. solution of pyridine in water and cross-linked polystyrene gels in pyridine - acetic acid are effective support media for electrophoresis. They provide the means for widening the application of electrophoresis to materials themselves insoluble in aqueous media but soluble in pyridine - acetic acid.

Illustrative examples of the electrophoresis of methylene blue, rhodamine B, alizarin red, alizarin blue and eosin are given.

D. LEIGHTON, G. J. MOODY and J. D. R. THOMAS
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Analyst, 1974, **99**, 149–152.

Detection of *Curcuma zedoaria* and *Curcuma aromatica* in *Curcuma longa* (Turmeric) by Thin-layer Chromatography

It is difficult to determine the genuineness or otherwise of powdered turmeric (*Curcuma longa*) when it is admixed with *C. zedoaria* or *C. aromatica*.

A simple and rapid thin-layer chromatographic technique is described that involves a three-step colour sequence for the detection of camphor and camphene, the active principles of these adulterants, which are absent in turmeric.

A. R. SEN, P. SEN GUPTA and N. GHOSE DASTIDAR
Central Food Laboratory, 3 Kyd Street, Calcutta-16, India.

Analyst, 1974, **99**, 153–155.

A Rapid Method for the Semi-quantitative Determination of Volatile *N*-Nitrosamines in Alcoholic Beverages

A method is described for the rapid extraction of *N*-nitrosamines from strong alcoholic drinks with methylene chloride after saturating the water - ethanol phase with magnesium perchlorate. The nitrosamines are then determined by oxidation to nitramines and clean-up of the oxidation products by adsorption chromatography on a column containing two layers of different grades of alumina, followed by gas chromatography with an electron-capture detector. The method is equally applicable to beverages with low alcohol content such as beer.

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Unit of Environmental Carcinogens, International Agency for Research on Cancer, 150 Cours Albert Thomas, 69008 Lyon, France.

Analyst, 1974, **99**, 156–162.

A Combustion Method for the Determination of Total Sulphur in Limestones

A method is described for the determination of total sulphur in limestones. The sample is mixed with tungsten trioxide and quartz flour and placed in a boat of refractory material. The boat and contents are inserted into an aluminous porcelain combustion tube maintained at a temperature of about 1200 °C in a resistance-type tube furnace. The combustion tube carries a coil of copper gauze at the outlet end to reduce sulphur trioxide. Nitrogen is used as carrier gas and the sulphur dioxide evolved is absorbed in dilute hydrochloric acid containing potassium iodide and starch as indicator, titration with standard potassium iodate solution being carried out as the analysis proceeds.

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Analyst, 1974, **99**, 163–165.

A Comparison of Two Procedures for the Determination of Organobromine by the Schöniger Oxygen-flask Method

A statistical comparison of the argentimetric and mercurimetric methods of finish in the oxygen-flask determination of organobromine has been carried out. While no statistical difference was found to exist, the mercurimetric method is recommended for use in laboratories that lack the facilities required for the potentiometric determination.

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Analyst, 1974, **99**, 166–167.

Automated *in situ* Preparation of Azomethine H and the Subsequent Determination of Boron in Aqueous Solution

A procedure for the preparation of azomethine H and the use of the reagent in the same system for the determination of boron in solution is described.

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Department of Inorganic and Analytical Chemistry, University of Pretoria, Pretoria, Republic of South Africa.

Analyst, 1974, **99**, 168–170.

Determination of Polyoxyethylene *p*-t-Nonylphenyl Ethers in Pomades

A method is proposed for the determination of polyoxyethylene *p*-t-nonylphenyl ethers, $RO(CH_2CH_2O)_nH$ (R = *p*-t-nonylphenyl), in pomades. The method is based on the evaluation of the number average degree of polymerisation of the polydispersity ($\bar{n}$) by vapour pressure osmometry and by thin-layer chromatography, and on the spectrophotometric determination of the apparent mean molar absorption coefficient ($\bar{\varepsilon}$) at 620 nm of the bisammonium tetrathioisocyanatocobaltate(II) complexes extracted into 1,2-dichloroethane.

The method has been tested for values of $\bar{n}$ in the range 6·5 to 21·5 and for Weibull distributed polydispersions. Significant systematic errors arise only in mixtures of polydispersions with very different values of $\bar{n}$. The accuracy and intralaboratory reproducibility have been evaluated by analysing samples of a pomade of known composition.

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Analyst, 1974, **99**, 171–177.

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Coulometric Microtitration of Arsenic(III) and Isoniazid Using a Vitreous Carbon Generating Electrode

BY V. J. JENNINGS, A. DODSON AND A. HARRISON

(Department of Chemistry and Metallurgy, Lanchester Polytechnic, Priory Street, Coventry, CV1 5FB)

A small-scale coulometric titration cell and apparatus is described in which a vitreous carbon rod anode is used to generate bromine. The amount of the latter required to titrate from 3 to 15 μg of arsenic(III) or isoniazid with a precision of 2 per cent. is found by using an integrating digital milliammeter. The titration end-points were obtained by use of a form of differential electrolytic potentiometry.

PREVIOUS work has shown that bromine can be generated at a vitreous (or glassy) carbon electrode with a current efficiency of at least 99·9 per cent.[1] In the present work results obtained by using a vitreous carbon electrode to generate bromine in the coulometric microtitration of arsenic(III) and isoniazid (isonicotinylhydrazide) are reported.

There is general interest in analytical methods for determining microgram amounts of both substances, arsenic because of its toxicity and isoniazid because of its use as a chemotherapeutic agent for the treatment of tuberculosis.[2]

Conventional oxidimetric titration methods have been reported for the determination of arsenic(III)[3] and isoniazid[4] in which the oxidising agent is potassium bromate, occasionally in the presence of added bromide ions. For the latter condition the chemical reactions are—

$$As^{3+} + Br_2 = As^{5+} + 2Br^- \quad .. \quad .. \quad .. \quad (1)$$

for arsenic(III) and

$$C_5H_4N.CONHNH_2 + 2Br_2 + H_2O = C_5H_4N.COOH + 4Br^- + 4H^+ + N_2 \quad (2)$$

for isoniazid. Coulometric titrations with electrolytically generated bromine at a platinum anode have been reported for determining arsenic(III)[5,6] and isoniazid.[7] A major difficulty in micro-titrations in which the initial concentration of the titrand is approximately 10^{-5} M is in the location of the end-point, as the detection system must be sensitive to concentration levels of about 10^{-7} M in the vicinity of the equivalence point if an end-point precision of 1 per cent. is to be achieved. In the present work, a form of differential electrolytic potentiometry has been used to locate the end-point because it is known to provide an end-point detection method of high sensitivity in, for example, the coulometric determination of hydrazine with bromate.[8]

In differential electrolytic potentiometry, the potential difference between two platinum electrodes that have been polarised by the passage of microampere currents is measured. For arsenic(III) and isoniazid titrations it is expected that the complete titration graph of potential *versus* amount of bromine generated would show a relatively high potential plateau region and a sharp fall in this potential at the end-point. However, in order to decrease the time required to complete a titration in the present work, it was decided that instead of attempting to plot a complete graph, the amount of titrant required to produce an arbitrarily chosen decrease in potential of 100 mV from the initial start potential would be taken as the end-point value.

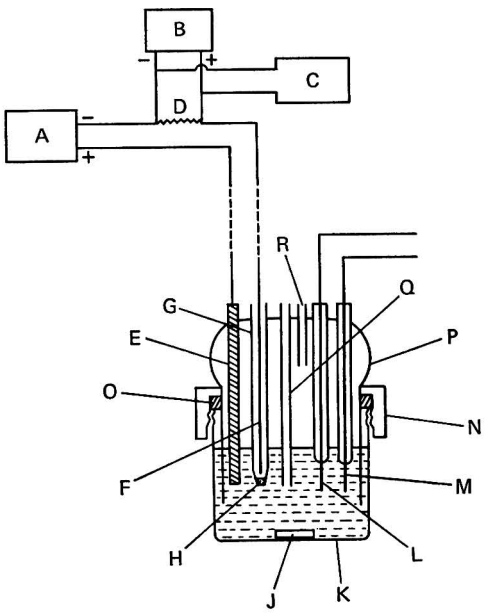
In a conventional coulometric titration, the current is maintained at a constant value and the elapsed time from the start to the end-point is used to determine (from Faraday's laws of electrolysis) the amount of titrant generated. After using some commercially available coulometric titration apparatus, we have found that it is not always a simple matter to maintain a constant current and there are difficulties in synchronising the current flow with the

timing system. There have been attempts to use a low-inertia integrating meter as a coulometer in series with the titration cell[9] in order to measure the charge directly. Integrating electronic digital milliammeters have recently become commercially available and such an instrument was used successfully in this work.

Experimental

Reagents—

Reagents of analytical-reagent grade quality were used when possible. The isoniazid (isonicotinylhydrazide pure, Koch-Light Laboratories Ltd.) was recrystallised from solution in ethanol and dried at 110 °C for 2 hours. Its melting-point after recrystallisation was 170 to 171 °C.



A	Griffin constant-current, 1- to 10-mA supply unit (S576–130)
B	Time Electronics Digital Integrator Type TS100A (100-mV range)
C	Beckman Research pH meter (mV mode) (also used to measure the potential across L and M)
D	ALMA Type SIT 50Ω ± 0·05 per cent. resistor
E	Le Carbone (Great Britain) Ltd. 3-mm diameter vitreous carbon rod anode (V25)
F	Johnson Matthey Ltd. thermopure platinum wire cathode, 0·5-mm diameter
G	Glass cathode compartment
H	Porous plug (coil of Whatman No. 541 filter-paper)
J	Magnetic stirrer bar
K	Bottom half of glass cell, capacity 3 ml
L & M	D.E.P. 0·5-mm diameter platinum wire electrodes
N	Sovirel bored cap for sliding joint, size 22 mm
O	PTFE wrapped silicone rubber sealing ring (22 × 16)
P	Top half of glass cell
Q	White-spot nitrogen inlet
R	Nitrogen outlet (also used for adding sample with hypodermic syringe)

Fig. 1. The cell and coulometer circuit

Isoniazid solution, 0·01 M—This was prepared by dissolving 0·2 g of the solid in distilled water and making the volume up to 500 ml in a Grade A calibrated flask.

Arsenic(III) oxide solution, 0·01 M—This was prepared in a similar manner to that previously described.[1]

APPARATUS—

The cell and coulometer circuit are shown in Fig. 1. The integrator display, which was a six-digit totalising magnetic counter with manual zero re-set, was calibrated by passing a known (2 mA) constant current for a given length of time (measured on a stopwatch to $\pm 0{\cdot}1$ s). The mean value of 1 integrator unit was equivalent to a charge of $6{\cdot}652 \times 10^{-4}$ C (theoretical value, $6{\cdot}666 \times 10^{-4}$ C) and the relative standard deviation from the mean value was 0·1 per cent. for timed periods of 100 s. The surface area of the vitreous carbon rod exposed to the electrolyte solution was 0·16 cm^2 with a current density of 12 mA cm^{-2}.

The polarising current of about 10^{-6} A for the differential electrolytic potentiometric platinum electrodes was supplied by a 90-V Exide Dymax dry battery (DM 256) in series with a Welwyn Electric Ltd. 100 MΩ (± 5 per cent.) carbon film resistor. The current density at the differential electrolytic potentiometric electrodes was about 12×10^{-6} A cm^{-2}. The potential across the electrodes was measured on a Beckman Research pH meter used in its millivolt measuring mode.

PROCEDURE—

For carrying out titrations the method of successive aliquot additions, as described previously,[10] was used. To the cell was added 2 ml of electrolyte solution that was 0·2 M in potassium bromide and 1·0 M in sulphuric acid. White-spot nitrogen was bubbled through the cell for 5 minutes so as to remove any titratable volatile impurity, then an aliquot of titrand was added by use of a 10-μl precision syringe (S.G.E. Pty. Ltd.). The potential across the two differential electrolytic potentiometric indicator electrodes was measured and the amount of charge, in integration units, required to decrease this potential by 100 mV was found. A further aliquot of titrand was then added and the above procedure repeated.

Naturally, the result for the first aliquot included any blank value, that is, the charge required to cause the 100-mV potential change in the absence of sample. This result was therefore high and was rejected. With the isoniazid titrations it was found that the result for the second aliquot could also be high so that this result was again rejected. It is believed that some pre-conditioning of the differential electrolytic potentiometric electrodes is necessary for isoniazid titration, as has been found by other workers in the coulometric titration of sulphur dioxide with bromine.[11]

The results are given in Tables I to IV. It was observed that there was a slow downward drift of the initial differential electrolytic potential after the addition of each aliquot.

TABLE I

COULOMETRIC TITRATIONS OF 10 μl OF 0·01 M ARSENIC(III) OXIDE SOLUTION
10 μl of solution ≡ 15·13 μg of arsenic(III)

Experiment series	A	B	C	D	E
Number of individual results in series	8	8	8	7	10
Mean amount of arsenic(III) found/μg	15·16	15·31	15·19	15·39	15·18
Relative standard deviation, per cent.	2·1	1·8	1·0	1·8	1·9
Error, per cent.	+0·1	+1·2	+0·4	+1·4	+0·4

DISCUSSION AND CONCLUSION

ARSENIC(III)—

The results in Tables I and II show that the accuracy and precision are of the order of 1 and 2 per cent., respectively, except for the smallest amount of sample (3 μg). At that level there is an accumulation of random errors, for example, the fact that there is an experimental limit to the minimum incremental amount of bromine that can be generated. A general limit on the precision level is imposed by the use of a microsyringe, with which the volume delivered is subject to a probable random error of 1 per cent.

TABLE II

COULOMETRIC TITRATIONS OF 2 TO 8 μl OF A 0·01 M ARSENIC(III) OXIDE SOLUTION

Volume of As_2O_3 solution taken/μl	2	4	6	8
Number of individual results in series	8	8	8	8
Amount of arsenic(III) present/μg	3·03	6·05	9·08	12·11
Mean amount of arsenic(III) found/μg	3·15	6·14	9·04	12·22
Relative standard deviation, per cent.	2·5	1·5	0·8	1·9
Error, per cent.	+4·2	+1·4	−0·4	+1·0

A least-squares fit of the results given in Tables I and II shows that if x μg of arsenic(III) are taken, then the y μg of arsenic(III) found is given by the equation—

$$y = 1{\cdot}002x + 0{\cdot}07$$

ISONIAZID—

The results in Tables III and IV are similar to those for arsenic(III) although the precision is lower and there is an appreciable increase in the error. A least-squares fit of the results given in Tables III and IV shows that if x μg of isoniazid are taken, then the y μg of isoniazid found is given by the equation

$$y = 0{\cdot}981x + 0{\cdot}390$$

The error may be partly due to the possibility that the solid, although recrystallised, was not absolutely pure. However, it is also likely that at these concentration levels the rate of reaction of isoniazid with bromine and the instrumental response of the end-point detection system limit the precision and accuracy that can be attained.

TABLE III

COULOMETRIC TITRATIONS OF 5 μl OF 0·01 M ISONIAZID SOLUTION
5 μl of solution ≡ 6·88 μg of isoniazid

Experiment series	A	B	C	D	E
Number of individual results in series	8	8	8	9	8
Mean amount of isoniazid found/μg	6·97	7·11	7·35	7·24	7·23
Relative standard deviation, per cent.	2·0	1·6	4·0	3·1	2·2
Error, per cent.	+1·4	+3·5	+7·3	+5·6	+5·5

It is concluded that the apparatus and technique described in this paper are suitable for the coulometric titration of down to 3-μg amounts of arsenic(III) and isoniazid and that the method could be applied to the determination of large numbers of samples as a result of the speed with which these titrations can be carried out.

TABLE IV

COULOMETRIC TITRATIONS OF 2 TO 10 μl OF 0·01 M ISONIAZID SOLUTION

Volume of isoniazid solution taken/μl	10	8	6	4	2
Number of results in series	8	7	8	8	8
Amount of isoniazid taken/μg	13·75	11·00	8·25	5·50	2·75
Mean amount of isoniazid found/μg	13·85	11·22	8·58	5·56	3·16
Relative standard deviation, per cent.	1·6	1·3	1·9	2·8	2·7
Error, per cent.	+0·7	+2·0	+4·1	+1·1	+14·8

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Gel Supports for Electrophoresis with Pyridine - Acetic Acid Media

By D. LEIGHTON, G. J. MOODY and J. D. R. THOMAS

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Agar gels that are suitable for use with an 80 per cent. solution of pyridine in water and cross-linked polystyrene gels in pyridine - acetic acid are effective support media for electrophoresis. They provide the means for widening the application of electrophoresis to materials themselves insoluble in aqueous media but soluble in pyridine - acetic acid.

Illustrative examples of the electrophoresis of methylene blue, rhodamine B, alizarin red, alizarin blue and eosin are given.

Zone electrophoresis, although usually performed in aqueous buffer - electrolyte systems, has also been effected in non-aqueous systems, and the work recorded to date indicates a wider perspective for electrophoresis. For example, Paul and Durrum[1] have separated oil-soluble dyes by electrophoresis in absolute ethanol, nitromethane - glacial acetic acid and pyridine - glacial acetic acid systems on filter-paper supports. Also, the electrophoretic behaviour of arsenazo III and its analogues on filter-paper in glacial acetic acid and mixed formic acid - chloroacetic acid media has been reported,[2] and several inorganic ions that were difficult to separate in aqueous solutions were readily resolved in a methanol - acetone - hydrochloric acid medium.[3]

Apart from filter-paper, thin-layer supports (as prepared for chromatography) are suitable for electrophoresis in non-aqueous media (Leighton, D., Moody, G. J., and Thomas, J. D. R., unpublished work), but the enormous capacity for resolution achieved with the various gel supports in aqueous systems[4] cannot always be extended to non-aqueous media because of gel solubility and stability problems, especially with polyacrylamide. The characteristics of electrophoretic systems based on agar gels for aqueous pyridine media, and cross-linked polystyrene gels for pyridine - acetic acid buffer electrolytes, are described in this paper.

Experimental

Preparation of agar gels—

The appropriate amount of special Noble Agar (Difco Laboratories, Detroit), but ideally 2·25 g, was dissolved in 30 cm^3 of water by heating the mixture on a boiling water bath and 120 cm^3 of pyridine were added. After cooling it on ice until it became viscous, the mixture was poured into a 0·6-cm deep horizontal trough formed by an aluminium frame sealed to a 20 × 16-cm glass plate with high-vacuum silicone rubber grease. The agar surface was levelled flush with the top of the aluminium frame with a glass rod and the whole kept on ice until the gel was firm. The frame was removed and slits were cut midway along the gel layer for sample application.

Preparation of cross-linked polystyrene gels—

A mixture of styrene and divinylbenzene (50 to 60 per cent. *m/m* in ethylvinylbenzene) monomers in appropriate proportions, and containing 0·60 g of benzoyl peroxide initiator, was added to 120 cm^3 of a pyridine - acetic acid - perchloric acid mixture (Table I) and the whole poured into a trough similar to that described above for agar gels, except that the top side of the aluminium frame was now covered with a glass plate. In order to facilitate filling of the trough, one 16-cm edge of the frame was cut away. For gelling, the assembly, which was held together by strong rubber bands, was placed in a boiling water bath with the open end just above the water level. Between 1 and 3 hours were usually required for gelling, depending on the concentration of the divinylbenzene.

After gelling, the top glass plate and the aluminium frame were removed, thus leaving the gel resting on the other plate. Samples for electrophoresis were placed into slits cut midway along the gel layer.

ELECTROPHORESIS—

Electrophoresis was carried out in the same way as for aqueous systems by using a Shandon power pack (Catalogue No. SAE 2525) and a Baird and Tatlock horizontal electrophoresis tank (Catalogue No. C40/4000) constructed of silica. Contact between the gel and buffer - electrolyte was effected with wicks made of filter-paper. The test solutions used were 1 per cent. solutions of the dye in the appropriate buffer.

TABLE I

PARAMETERS OF POLYSTYRENE GEL FORMATION

	Gel constituents						
Gel No.	Styrene monomer/ cm³	Divinyl-benzene monomer/ cm³	Benzoyl peroxide/ g	Buffer composition: pyridine to acetic acid ratio, *V*/*V* (120 cm³ used in each instance)	Buffer pH	Gelling time/ hours	Gel character
1		19·2				1·0	Cloudy and rubbery
2		14·4				1·25	
3		12·0				1·5	Yellow and rather brittle
4	24	9·6				1·5-2·0	
5		7·2				2·0(*a*)*	Clear yellow
6		6·0		100 : 1·0 + 0·5		2·0(*a*)*	
7		4·8		part of 71 to 73 per cent.	5·45	3·0(*b*)*	
8		3·6		perchloric acid		6·0(*c*)*	Polymer formed but no gel
9	36					1·5	
10	48					1·0	
11	60	7·2	0·6			1·0	
12	72					0·75	
13	84					0·75	Clear yellow
14		4·8		100:0	9·2	1·0	
15		7·2				2·0	
16				100:1·0	8·45	1·0	
17				100:7·16	6·7	1·0	
18	24			100:19·2	5·98	1·5	
19		4·8		100:33·8	5·45	1·5	
20				100:40·6	5·33	1·5	Translucent
21				100:48·7	5·09	1·0	White opaque precipitate
22				100:66·7	4·73	0·5	

* Following 4-t-butylcatechol inhibitor extraction (by shaking with four times the monomer volume o 0·05 M sodium hydroxide solution) from styrene and divinylbenzene monomers, the gelling times were 1·25 (*a*) 2 (*b*) and 6 (*c*) hours.

RESULTS AND DISCUSSION

AGAR GELS—

The development of agar gels was directed towards a purely non-aqueous pyridine system but the agar would not dissolve in water containing more than 80 per cent. of pyridine; the study of agar gel parameters was therefore based on varying the amount of agar in a mixture of 30 cm³ of water and 120 cm³ of pyridine. The qualities of gels obtained after 1 hour, with the various amounts of agar used parenthesised, were as follows: no gel formed even after 8 hours (0·9 g), very soft gel (1·2 g), firm gel (1·5, 2·25, 3·0 and 3·75 g), very firm gel (4·5 g). Firm and very firm gels were suitable for testing by electrophoresis and typical results for dye systems are summarised in Table II.

Migration of methylene blue and rhodamine B to the cathode, and of alizarin blue and eosin to the anode, is as expected from the cationic and anionic residues of these respective pairs of dyes. Although the zero migration of alizarin red in the buffers with high pyridine contents is rather surprising, agar gels based on aqueous pyridine mixed solvent media clearly provide suitable supports for zone electrophoresis in buffer media containing very substantial amounts of pyridine, especially for the gel based on 2·25 g of agar in 30 cm³ of water *plus* 120 cm³ of pyridine (Table II).

TABLE II

ELECTROPHORETIC MOBILITIES OF DYES ON GEL SUPPORTS IN PYRIDINE - ACETIC ACID MEDIA

Gel constituents	Buffer	Buffer pH	Constant voltage/ V	Starting current/ mA	Time/ minutes	Migration distances/cm: Cathode: Methylene blue	Cathode: Rhodamine B	Origin: Alizarin red	Anode: Alizarin blue	Anode: Eosin
Agar gels—										
Water (30 cm³) + agar (1·5 g) + pyridine (120 cm³)	Water - pyridine (1 + 4 *V*/*V*)	8·8	1000	35	40	0·5	0·3	0	0·3	0·5
Water (30 cm³) + agar (2·25 g) + pyridine (120 cm³)			1000	35	45	5·5	1·5	0	1·6	3·0
Water (30 cm³) + agar (4·5 g) + pyridine (120 cm³)			1000	35	45	0·5	0·3	0	0·3	0·5
Water (120 cm³) + agar (2·25 g) + pyridine (30 cm³)	Water - pyridine (4 + 1 *V*/*V*)	8·5	300	5	180	2·0	1·2	0·8 (to anode)	0·8	1·2
Cross-linked polystyrene gels—										
Pyridine (120 cm³) + acetic acid (1·2 cm³) + 71–73 per cent. perchloric acid (0·6 cm³) + styrene (24 cm³) + divinylbenzene (6·0 cm³) + benzoyl peroxide (0·6 g) (gel No. 6)	Pyridine - acetic acid - perchloric acid (71–73 per cent.) (100 + 1·0 + 0·5)	5·45	1000	8	180	3·5	2·0	0	0·3	0·4
Pyridine (120 cm³) + acetic acid (2·25 g) + 71–73 per cent. perchloric acid (0·6 cm³) + styrene (24 cm³) + divinylbenzene (4·8 cm³) + benzoyl peroxide (0·6 g) (gel No. 7)			600	7	180	3·5	2·0	0	1·0	0·8
Pyridine (120 cm³) + styrene (24 cm³) + divinylbenzene (4·8 cm³) + benzoyl peroxide (0·6 g) (gel. No. 14)	Pyridine	9·2	600	4	45	9·0	2·2	0	5·7*	4·5*
Pyridine (120 cm³) + styrene (24 cm³) + divinylbenzene (7·2 cm³) + benzoyl peroxide (0·6 g) (gel No. 15)			600	3	45	1·8	0·4	0	0·7	0·9
Pyridine (90 cm³) + acetic acid (30 cm³) + styrene (24 cm³) + divinylbenzene (4·8 cm³) + benzoyl peroxide (0·6 g) (gel No. 19)	Pyridine - acetic acid (3 + 1 *V*/*V*)	5·45	600	5	90	6·5	1·6	0	2·0	1·5
		5·45	600	5	240	9·5†	4·8	0	6·5	4·5
Silica gel—										
Silica gel G (Merck)	Pyridine - acetic acid (3 + 1 *V*/*V*)	5·45	1000	1	240	0·1	0	0	0·6	0·8
	Pyridine - water (1 + 4 *V*/*V*)	8·5	300	4	180	0·4	0·8	0	0·3	1·9

*Tailing.
† Moved to end of gel (9·5 cm) in 165 minutes.

CROSS-LINKED POLYSTYRENE GELS—

The several parameters examined for polystyrene gel formation are summarised in Table I. An alternative photopolymerisation process that involved gelling under an ultraviolet fluorescent lamp gave gels of similar quality to those given by the thermal method described above but the required gelling times of at least 3 days are rather too long for convenience.

The general features for successful gel formation are bound by the requirements that the ratio of divinylbenzene to polystyrene should not be less than 1:5 (gel No. 7) or as high as 2:5 (gel No. 3), although the former stipulation can be relaxed when the amount of polystyrene monomer is increased (gels Nos. 9 to 13). With respect to pH (a relative, empirical quantity only for the mixed solvent media used in this study), stable gels were obtained for the pH range 9·2 to 5·45 (gels Nos. 14 to 19), but under more acidic conditions a precipitate formed during the thermal treatment stage (gels Nos. 21 and 22).

Despite the presence of 4-t-butylcatechol inhibitor in the styrene and divinylbenzene monomer constituents, gelling times were of the order of 1 to 3 hours, but removal of the inhibitor reduced the times by about one third [see (*a*) and (*b*) in Table I for gels Nos. 5 to 7]. Such a reduction was considered to be an insufficient advantage for the removal of the inhibitor to be introduced into the procedure for the preparation of standard gels.

A further variant assessed with the view of simplifying gel preparation was the preforming of cross-linked polystyrene slabs followed by soaking in buffer electrolyte. Even though translucent slabs were obtained, these slabs, when swelled by floating on the buffer electrolyte, became brittle and snapped readily and were therefore unsuitable as supports for electrophoresis.

Gels Nos. 4 to 7 and 9 to 19 (Table I) were of suitable quality for electrophoresis and the most economical in terms of monomer materials are gels Nos. 6 and 7, and 14 to 19. Representative samples of these gels were tested for the electrophoresis of dyes and typical results are summarised in Table II. The migration behaviour is the same as for agar gels, with methylene blue and rhodamine B migrating to the cathode, alizarin blue and eosin migrating to the anode and alizarin red remaining immobile. Migration distances are rather less for the more cross-linked gel No. 6 than for gel No. 7 and also less for gel No. 15 than for gel No. 14. The conditions corresponding to gel No. 14 provide for the efficient separation of all five dyes. Reducing the pH from 9·2 (gel No. 14) to 5·45 (gel No. 19) lowers the migration rates, especially for the anionic dyes, but the separation is still effective. Migration rates were even less for a buffer of similar acidity on a silica gel G support. This feature and the reduced diffusion for the polystyrene gel confirm the suitability of the polymer gel as a support medium.

CONCLUSION

Although not as convenient or as elegant to use as purely aqueous systems, agar gels make suitable stable supports for electrophoresis in media of high pyridine content. Similarly, cross-linked polystyrene gels that are stable in pyridine - acetic acid provide another electrophoresis gel support medium for the separation of materials that are difficultly soluble in water and for which pyridine and acetic acid can emphasise the differences in the polar character of such materials.

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Detection of *Curcuma zedoaria* and *Curcuma aromatica* in *Curcuma longa* (Turmeric) by Thin-layer Chromatography

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It is difficult to determine the genuineness or otherwise of powdered turmeric (*Curcuma longa*) when it is admixed with *C. zedoaria* or *C. aromatica*. A simple and rapid thin-layer chromatographic technique is described that involves a three-step colour sequence for the detection of camphor and camphene, the active principles of these adulterants, which are absent in turmeric.

TURMERIC is the dried rhizome or bulbous root of *Curcuma longa* Linn., a perennial herb of the ginger family (*Zingiberaceae*), which is extensively cultivated in India, as well as in China and the East Indies. It is not a true spice, but rather a condiment that is used to a very large extent in the preparation of curries, pickles and many spicy Indian foods. It is also one of the chief ingredients of curry powder.

The adulteration of ordinary spices and condiments is exceedingly prevalent in India and probably the most subject to admixture is turmeric. The fact that this condiment is frequently offered for sale in a ground condition furnishes an opportunity for the incorporation of various cheaper vegetable substances. The use of a microscope is important for the detection of these additions, but when the adulterants belong to the same genus (*Curcuma*), even experts in microscopy find it difficult to decide whether or not a sample of turmeric is genuine.

In general, turmeric can readily be identified by the brilliant greenish yellow colour of the "paste balls," oleoresin cells that contain the valuable yellow colouring principle, curcumin. Curcumin is, however, also found in several other species of *Curcuma*,[1] *e.g.*, *C. aromatica* Salisb. and *C. domestica* Valeton. "The Wealth of India"[2] and the "Glossary of Indian Medicinal Plants",[3] however, appear to be confused and class *C. domestica* with *C. longa*, while Watt[4] and Bailey[5] did not mention it. Guenther[6] categorically differentiated *C. domestica* as a different species. It may be mentioned here that similar confusion had also arisen when Guibourt[7] classed *C. caesia* Roxb. as *C. longa*. Watt[4] suspected that so-called forms of *C. longa* may prove to be the tubers of different species. "The Wealth of India"[8] has mentioned its substitution with *C. aromatica*, as well as its close resemblance to *C. zedoaria* Roscoe, and from long experience we have found that both of these species, particularly the latter (local name Kachura, Soti), are being used extensively as adulterants. Although the latter species do not contain curcumin, this fact is of little significance when they are mixed with *C. longa* in the powdered form.

Parry[9] suggested that because the starch grains are usually more or less gelatinised as a result of the method of preparation, the presence of some well defined starch grains may indicate the presence of an adulterant. However, although most of the starch is swollen, grains may occasionally be found that still exhibit the characteristic scitaminaceous shape; moreover, while scalding is a very commonly used process, it is not obligatory and hence the presence of long, lens-shaped unaltered starch grains cannot be taken as indicative of adulteration.

The statutory chemical standards prescribed[10] are of minor importance, as all of these species belong to the same genus. Consequently, the detection of adulteration of turmeric will remain very difficult until their characteristic differences are established.

The flavouring substances present in the essential oils of *C. aromatica* and *C. zedoaria* contain sufficient amounts of camphor and camphene (both of which are absent in *C. longa*) to permit their identification, thus providing a useful indication of the presence of *C. aromatica*

or *C. zedoaria*. The relatively simple and rapid thin-layer chromatographic technique described below is suitable for differentiating these compounds and is of value for quality control purposes. A number of solvent systems, as well as different spraying reagents, have been tried. Although the solvent with which optimum separation was achieved is not new,[11] the detection by means of a three-step colour sequence has not previously been attempted.

Experimental and Results

Solvent system—

Ethyl acetate - n-hexane (3 + 17).

Chromogenic reagents—

(1) *Concentrated sulphuric acid* (50 *ml*) - *nitric acid* (*as oxidising agent*) (0·5 *ml*).

(2) *Anisaldehyde - sulphuric acid reagent*—A freshly prepared mixture containing 0·5 ml of anisaldehyde, 9 ml of ethanol (95 per cent.), 0·5 ml of concentrated sulphuric acid and 0·1 ml of glacial acetic acid.

Thin-layer chromatography—

Any convenient type of applicator can be used. Clean 10 × 20-cm thin-layer chromatographic plates with detergent and rinse them thoroughly. Coat them to a thickness of 500 μm with a 1 + 2 slurry of silica gel G in water. (An adsorbent layer of this thickness results in higher sensitivity and less background, and does not tend to peel when sprayed.) Air-dry the coated plates in a vertical position for half an hour and then dry them for 1 hour at 110 °C so as to activate the layers. Cool, wipe the backs and edges of the plates free from excess of silica gel G and store them in a desiccating storage cabinet until needed. The adsorbent layer must be uniform from plate to plate in order to obtain reproducible results.

Steam distillation (as recommended by the British Pharmacopoeia[12]) for 4 hours is the most convenient method of separating the essential oils from other plant constituents. The separated oils (from 20 to 25 g of sample) are mixed with a volatile solvent (benzene) so as to make an approximately 1 per cent. solution and a 10-μl spot is applied to the plate 2 cm from the bottom edge. Different samples can be spotted 2 cm apart. Spots are identified by comparison with volatile oils from authentic materials run alongside.

The developing tank should be prepared at least 3 hours before use, so as to allow the tank to become saturated and to shorten the developing time. The solvent level should not be higher than the spotting line, a depth of 1 cm being generally sufficient. As the solvent travels rapidly (10 to 12 minutes), the plate must be removed from the tank as soon as the solvent front reaches the previously marked line, 10 cm from the origin.

Identification—

The identification of the substances was achieved by means of a three-step sequence: colour produced with sulphuric acid - nitric acid (chromogenic reagent No. 1), fluorescence and the subsequent colour produced with anisaldehyde - sulphuric acid (chromogenic reagent No. 2), as described below.

After allowing the solvent to evaporate, the chromatogram is sprayed with chromogenic reagent No. 1. The entire chromatographed portion must be uniformly sprayed in order to achieve optimum results (10 ml of reagent are sufficient). The plate is allowed to stand for 1 minute, so as to enable the layer to become saturated with the reagent. Although the patterns obtained are somewhat similar near the origin, the spots obtained with *C. longa* with R_F values of 0·55 and above are different from those obtained with the other two varieties (see Table I).

Inspection under ultraviolet light (365 nm) showed that with *C. zedoaria* and *C. aromatica* the spots fluoresce at R_F 0·55 while with *C. longa* there is no such fluorescence in that zone, hence both species can be clearly differentiated from turmeric in this way.

If the chromatogram is then further sprayed with chromogenic reagent No. 2, a contrasting pattern of colour differentiation is revealed. Orange (R_F 0·72) and deep pink (R_F 0·55) spots of camphene and camphor are characteristic of *C. zedoaria* and *C. aromatica*. Neither of these spots is given by oils of *C. longa*, which show only one bluish violet spot (R_F 0·75) and another dirty brown spot (R_F 0·60) closer to the region of the camphene and camphor spots (see Table I).

The spots can also be made visible by spraying the plate with chromogenic reagent No. 2 alone, a similar pattern being observed after heating the plate for 2 minutes at 110 °C.

The colour reactions described generally depend on the substance, amount of reagent used, temperature and duration of heating. The R_F values obtained should not be regarded as absolute as they may be influenced by the presence of other extractives from the sample. For this reason, the identity of an isolate should be confirmed by re-chromatographing the suspected material against known standards. For identification purposes, a 5 per cent. addition of *C. zedoaria* or *C. aromatica* to *C. longa* can be clearly distinguished from the *C. longa* by the R_F values, the colour of the spots and their fluorescence.

TABLE I

COLOUR REACTIONS IN THE DIFFERENT DETECTION METHODS
Figures in parentheses are R_F values

Chromogenic reagent No. 1				Chromogenic reagents No. 1 and No. 2 (daylight)	
Daylight		Ultraviolet			
C. zedoaria and *C. aromatica*	*C. longa*	*C. zedoaria* and *C. aromatica*	*C. longa*	*C. zedoaria* and *C. aromatica*	*C. longa*
Violet (0·77)	Bluish violet (0·75)			Violet (0·77)	Bluish violet (0·75)
Pink (0·72)				Orange (0·72)	
Light pink (0·64)				Light Pink (0·64)	
Orange (0·60)	Brown (0·60)			Blue (0·60)	Dirty brown (0·60)
Dirty green (0·55)		Bluish fluorescence (0·55)		Deep pink (0·55)	
Greenish violet (0·46)	Light blue (0·46)			Violet (0·46)	Greenish violet (0·46)
Violettish pink (0·40)	Violet (0·40)			Light pink (0·40)	Pinkish brown (0·40)
Bluish violet (0·33)	Bluish green (0·33)			Bluish violet (0·33)	Bluish green (0·33)
Pink (0·23)	Light pink (0·23)			Violet (0·23)	Brown (0·23)

It may be further added that the above test can be applied to check for the presence of *C. caesia* and *C. domestica*,[6] as both of these species contain camphor, and the former also contains camphene.

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A Rapid Method for the Semi-quantitative Determination of Volatile *N*-Nitrosamines in Alcoholic Beverages

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A method is described for the rapid extraction of *N*-nitrosamines from strong alcoholic drinks with methylene chloride after saturating the water - ethanol phase with magnesium perchlorate. The nitrosamines are then determined by oxidation to nitramines and clean-up of the oxidation products by adsorption chromatography on a column containing two layers of different grades of alumina, followed by gas chromatography with an electron-capture detector. The method is equally applicable to beverages with low alcohol content such as beer.

THE *N*-nitrosamines have been widely investigated in studies on carcinogenesis, but while certain of them are carcinogenic to all animal species investigated, there is still no definite evidence of their carcinogenicity in man.

In areas of Northern France, epidemiology studies are currently being carried out by us on the relationship between high alcohol consumption and cancer of the oesophagus.[1] In order to further these studies, an investigation of analytical methods for the determination of nitrosamines in home-distilled spirits is being undertaken, particular attention being paid to reduction in the time of analysis.

In a similar study of home-produced beers conducted in East Africa, Collis, Cook, Foreman and Palframan[2,3] found no evidence of the presence of nitrosamines in concentrations above 100 μg kg^{-1}, which was their minimum level of detection. However, such reliable evidence as is available on the presence of nitrosamines in foodstuffs usually indicates levels in the order of 1 to 10 μg kg^{-1}. It would seem reasonable to require a useful screening method for determining nitrosamines in spirits to be capable of detecting similar levels.

Isolation of nitrosamines from solutions containing appreciable amounts of ethanol presents problems in extraction and separation, as the lower homologues of the dialkylnitrosamines are freely soluble in water, ethanol and solvent phases. Distillation also presents problems, which arise from the formation of azeotropes. Crosby, Foreman, Palframan and Sawyer[4] attempted to concentrate the nitrosamines by using a spinning band column, but the concentration factor obtained was only modest and the technique is slow. An alternative method has been proposed by Sen and Dalpe[5] in which the alcohol is removed as an azeotrope with either benzene or toluene. The method, however, is somewhat lengthy for screening large numbers of samples.

The authors report a method of analysis for nitrosamines in alcoholic beverages, which involves the use of a simple liquid-liquid extraction of nitrosamines and is suitable for screening large number of samples. The final determination of the nitrosamines is made by gas chromatography of the nitramine derivatives according to the method of Sen,[6] after clean-up on a column of alumina.

EXPERIMENTAL

As methylene chloride has been found by most workers to be the most suitable solvent for the extraction of nitrosamines, attempts were first made to extract, with methylene chloride, a 1 + 1 water - ethanol mixture containing known amounts of seven different nitrosamines. After separating it, the solvent phase was dried over sodium sulphate and distilled in a Kuderna-Danish apparatus. The collected fractions of the distillate and the residue were then analysed for nitrosamines by gas chromatography. As expected, the co-extracted ethanol carried into the solvent phase an appreciable amount of water, with

the result that the nitrosamines passed into the distillate and were lost. The problem, therefore, was to prevent extraction of water and ethanol into the methylene chloride phase.

Calcium chloride is known to bond loosely with ethanol; therefore, extraction was attempted with simulated spirit (1 + 1 water - ethanol), after first saturating the solution with calcium chloride. However, on shaking the mixture in a separator, emulsions formed that were difficult to break down. As an alternative, magnesium perchlorate, which is soluble in ethanol and has a high affinity for water, was tried, with success. On separation, the volumes of the two layers were only slightly changed and the solvent could be completely distilled off without significant increase in the temperature of distillation. Ethanol was mainly retained in the water. A minor problem was encountered in differentiating the layers when the concentration of ethanol was varied about the ratio 1:1. As the difference in density between the perchlorate-saturated water - ethanol solution and methylene chloride is small, for relatively small variations in the percentage of ethanol, the methylene chloride may be found as either the upper or the lower layer. On addition of sufficient ethanol to bring its content to about 55 per cent., the two layers separate cleanly, with methylene chloride as the bottom layer. All subsequent extractions were made on solutions containing 55 per cent. of ethanol. Alternatively, if concentrations of ethanol are adjusted with water to be less than 45 per cent., the phases readily separate with methylene chloride as the upper layer. The method can thus be varied to suit the type of product to be analysed.

The partition between the two phases was checked on 10 p.p.m. solutions of nitrosamines in 1 + 1 water - ethanol and by measuring those in the methylene chloride extract in an ultraviolet spectrophotometer. Three extractions with methylene chloride were sufficient to remove all the nitrosamines from the perchlorate-saturated water - ethanol phase. This extraction technique was applied to solutions of ethanol in water containing 10 $\mu g\ kg^{-1}$ of each of seven nitrosamines and the methylene chloride extract analysed for the nitrosamines. The recoveries were generally found to be of a similar order to those found by Sen and Dalpe.[5]

The method was then applied to samples of nitrosamine-free calvados, both with and without addition of the nitrosamines. Accurate analysis was difficult owing to the presence of a large number of interfering peaks. Attempts to reduce their number by distillation of the calvados from alkaline and acidic solution were ineffective and, furthermore, led to lower recoveries, particularly of the higher-boiling nitrosamines. The problem was overcome by using an alumina column similar to that used by Telling[7] for the determination of nitrosamines in foodstuffs. The method required some modification as the neutral-grade alumina used by this author did not remove all the interfering peaks in the chromatograms originating from the calvados. Those peaks which remained were removed by using an alkaline grade, and an effective composite column of the two types of alumina was devised . Fig. 1 illustrates a chromatogram obtained from a calvados sample that contained 10 $\mu g\ kg^{-1}$ of each of seven nitrosamines before clean-up. Figs. 2 and 3 illustrate the chromatograms obtained from the same calvados sample with and without addition of the nitrosamines after clean-up on the alumina column.

Method of analysis for nitrosamines in calvados

Materials—

N-Nitroso compounds—Nitrosodimethylamine, nitrosodiethylamine, nitrosodipropylamine and nitrosodibutylamine were obtained from Eastman Kodak Ltd. and nitrosomethylpentylamine from Schuchardt, Munich, and all were distilled before use. Nitrosopyrrolidine was kindly supplied by Dr. Eisenbrand of the Krebsforschungszentrum, Heidelberg, and nitrosoethylmethylamine by Mr. J. F. Palframan of the Laboratory of the Government Chemist, London. All samples contained gas-chromatographic impurities in concentrations of less than 1 per cent.

Solvents—

Methylene chloride—Merck, analytical-reagent grade, was redistilled from sodium carbonate in 2-litre batches, the first 100 ml being rejected. All other solvents were of analytical-reagent grade.

Reagents—

Hydrogen peroxide solution, 50 per cent. m/V.

Trifluoroacetic acid—Merck, analytical-reagent grade, was redistilled before use.
Magnesium perchlorate—Prolabo, laboratory grade.
Sodium sulphate, anhydrous—Merck, analytical-reagent grade.

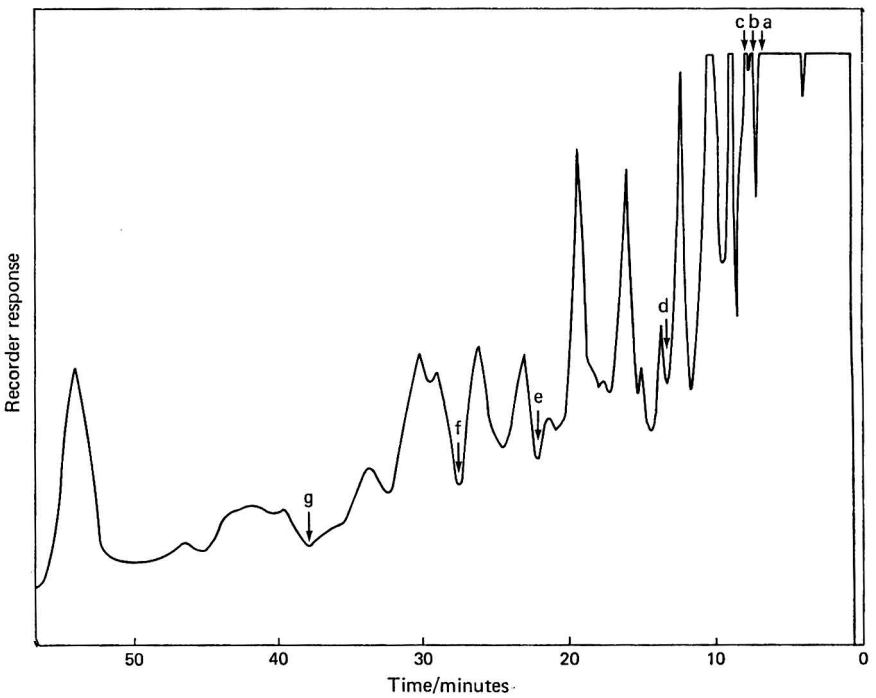

Fig. 1. Chromatogram illustrating the masking of nitramine peaks by other oxidation products: arrows indicate the position of the peaks for dimethylnitramine (a); ethylmethylnitramine (b); diethylnitramine (c); dipropylnitramine (d); methylpentylnitramine (e); dibutylnitramine (f); and pyrrolidylnitramine (g)

EXTRACTION—

The approximate percentage of ethanol in the calvados is first measured by means of a hydrometer. To a 50-ml aliquot of sample sufficient ethanol is added to bring its concentration to about 55 per cent. To this solution is then added sufficient anhydrous magnesium perchlorate to saturate the solution, the magnesium salt being added in successive amounts of about 10 g and the solution cooled under a tap after each addition (interaction between water and anhydrous magnesium perchlorate is exothermic). The saturated solution is then extracted with three successive 50-ml portions of methylene chloride, which are bulked and shaken in a second separator with 35 to 40 g of anhydrous sodium sulphate so as to remove any of the water - ethanol phase that may be carried through in suspension, and the methylene chloride layer is run off into a suitable apparatus for removal of the solvent. The sodium sulphate is finally washed with 10 ml of methylene chloride, which is added to the bulked extracts.

Two methods of evaporation have been carried out, (*a*) by using the Kuderna-Danish apparatus, and (*b*) by evaporation from a 250-ml round-bottomed flask immersed in a water-bath at 60 °C and flushed with a gentle current of nitrogen. In each instance the volume of solvent is reduced to between 0·5 and 1 ml. The results shown in Table I indicate that better recoveries for the more volatile nitrosamines are obtained by using the Kuderna-Danish apparatus. The second method, however, is much more rapid and shows little disadvantage for the higher nitrosamines.

PREPARATION OF NITRAMINES—

The nitrosamines in the residue are oxidised to nitramines with a mixture containing 5 ml of trifluoroacetic acid and 4 ml of 50 per cent. hydrogen peroxide solution.[6] After gently shaking it overnight at room temperature, the solution is cooled in an ice-bath, the pH adjusted to between 10 and 11 by dropwise addition of a 20 per cent. solution of potassium carbonate and then extracted with three successive 50-ml volumes of methylene chloride. The extracts are bulked in a separator and dried by shaking with 15 to 20 g of anhydrous sodium sulphate. The methylene chloride is then transferred into a flask as before. The sodium sulphate is washed with 20 ml of methylene chloride, which is added to the bulked extracts, and the volume of solvent reduced in the water-bath at 60 °C to slightly less than 10 ml. The concentrate is then transferred, with washing, into a graduated test-tube and its bulk is further reduced in a current of nitrogen to slightly more than 6 ml (it is important not to reduce the volume below this level as nitramines may be lost); 2 ml of hexane are then added and the bulk further reduced to between 0·7 and 0·5 ml (again, care must be taken not reduce the volume below 0·5 ml). The volume is finally adjusted to 5 ml with pentane and the solution is ready for the final clean-up by column chromatography.

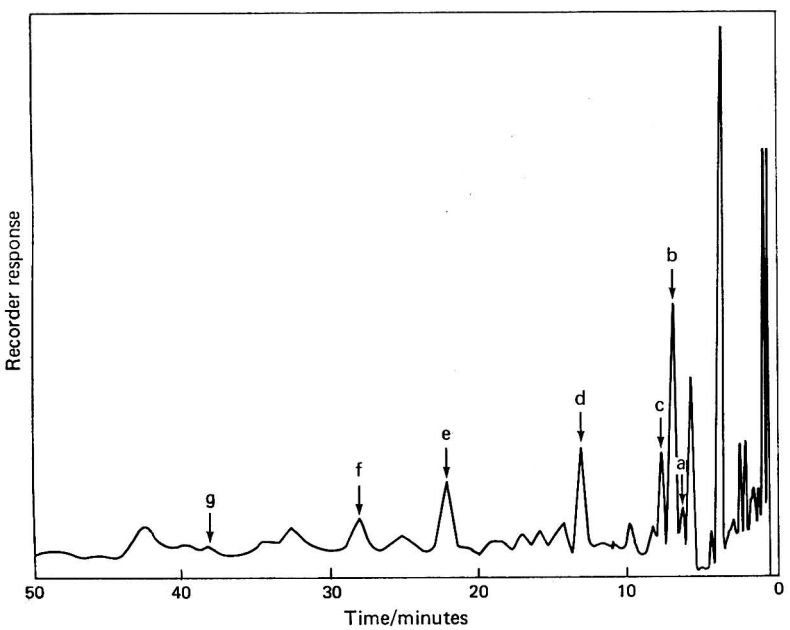

Fig. 2. Chromatogram illustrating the seven nitramines a to g (Fig. 1), gas chromatographed at 130 °C, as indicated by the arrows, after clean-up on the alumina column

COLUMN CHROMATOGRAPHY—

The column consists of three layers: a bottom layer of 3 g of basic aluminium oxide, activity II; a middle layer of 3 g of neutral aluminium oxide, activity III; and, at the top, a layer of 1 g of anhydrous sodium sulphate. A glass column, 1 cm in diameter and 25 cm in length, is used. Each type of oxide is prepared by first activating the appropriate Merck grade (*i.e.*, basic or neutral) of activity I by heating it in an oven for 3 hours at 240 °C. After cooling in a desiccator, 100 g of the activated material are shaken in a stoppered flask for 3 hours with the appropriate amount of water (3 g for basic aluminium oxide, activity II, and 6 g for neutral aluminium oxide, activity III). The stoppered flask is then left to stand overnight so as to enable the mixture to equilibrate.

Three grams of the basic aluminium oxide (activity III) are then agitated gently for half an hour with a sufficient volume of a 1 per cent. solution of diethyl ether in pentane

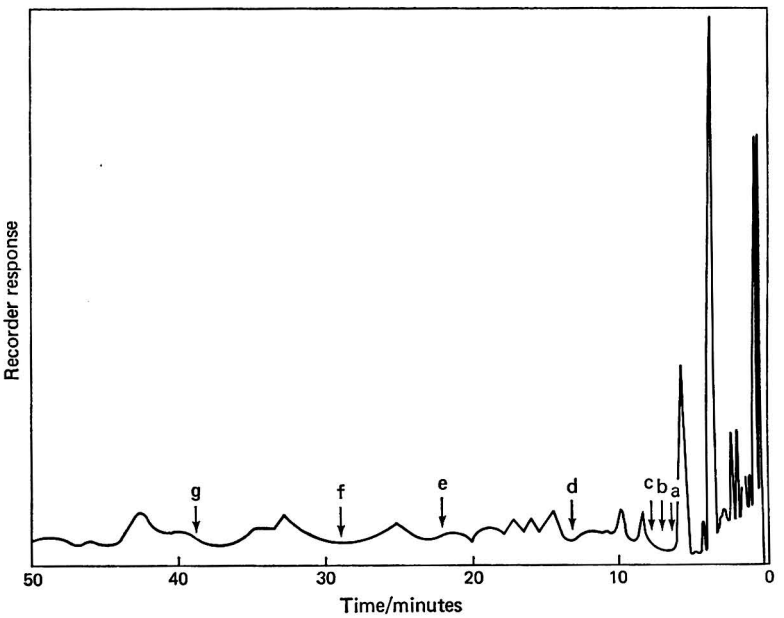

Fig. 3. Chromatogram obtained for a blank calvados after clean-up on the alumina column. Arrows indicate the positions at which the nitramines a to g (Fig. 1) would be found

to cover the solid and desorb gases from the alumina. The washed alumina is then added to the glass column and solvent run off from the bottom of the column until the top of the solvent layer is level with the upper surface of the alumina. This procedure is repeated with the neutral aluminium oxide (activity II) and then finally 1 g of anhydrous sodium sulphate is added to the top of the column. The pentane solution containing the nitramines is then added to the column and liquid run out until the upper level coincides with the upper surface of the column. The column is eluted successively, first with 25 ml of a 1 per cent. solution of diethyl ether in pentane, then with 40 ml of a 25 per cent. solution of diethyl ether in pentane and finally with diethyl ether alone. The first 25 ml of eluate are rejected, after which it is collected in 5-ml fractions. From each fraction a 5-μl portion is injected into the gas chromatograph. Finally, the solutions are bulked and the volume is reduced to 5 ml by evaporation on a water-bath. A suitable aliquot is then injected into the gas chromatograph. The minimum level of detection is about 1 $\mu g\ kg^{-1}$ of nitrosamine in the original sample. The volume of ether can be further reduced to 0·5 ml without loss of nitramine, giving a factor of 10 on the peak size. This step is preferable for accurate measurements of the concentrations below 10 $\mu g\ kg^{-1}$.

So far it has not been possible to adjust conditions so as to obtain complete separation of the individual nitramines on the alumina column. However, as the retention volume of each nitramine is reasonably reproducible, analysis of each fraction separately increases confidence in identification. The nitramines are eluted from the column in order of polarity, the least polar being eluted first, *i.e.*, in the order: dibutyl, dipropyl, diethyl, methylpentyl, ethylmethyl, dimethyl and, finally, the pyrrolidyl compound. The total volume of eluate is normally about 55 ml but varies slightly from batch to batch of the prepared alumina, and it is therefore necessary to run nitramine standards for each batch. The variation has been found not to be more than 5 ml for the nitramines that have the longest retention times when using freshly prepared alumina. Recovery of the nitramines after having passed through the column, the fractions having been bulked and the volume reduced, is 100 per cent.

GAS CHROMATOGRAPHY—

Pye 104 gas chromatographs fitted with nickel-63 electron-capture detectors were used with 6 foot × ¼ inch o.d. glass columns packed with 10 per cent. Carbowax 20M on DMCS-treated Chromosorb W. The columns were conditioned at 220 °C for 2 days before use. The determination of nitramines in the series dimethyl to the methylpentyl derivative was performed isothermally at 130 °C with a nitrogen flow of 50 ml min^{-1} through the column and a scavenge flow of 20 ml min^{-1} through the detector. The injection temperature was 170 °C and the chart speed 5 mm min^{-1}. The determination of the dibutyl- and pyrrolidyl-nitramines was performed on a second chromatograph at 160 °C. With a second instrument available this procedure was found to be more convenient than temperature programming.

RESULTS

The recoveries obtained in the replicate analyses of the aqueous ethanolic solution containing 10 $\mu g\ kg^{-1}$ each of seven nitrosamines are illustrated in Table I, where the results shown were obtained by using both methods for evaporation of the solvent. Table II shows the recoveries for the same concentration of nitrosamines in four different samples of calvados; in this instance only the rapid method of evaporation in the water-bath was used. Recoveries were good, with the exception of nitrosodimethylamine and nitrosopyrrolidine, losses of which, even when using a Kuderna-Danish apparatus, still tended to occur.

TABLE I

RECOVERY OF THE SEVEN NITROSAMINES FROM THE WATER - ETHANOL MIXTURE CONTAINING 10 $\mu g\ kg^{-1}$ OF EACH NITROSAMINE

	Recovery of each nitrosamine, per cent.							
	(*a*)				(*b*)			
Nitrosamine								
Nitrosodimethylamine	33	49	46	42	26	48	38	22
Nitrosoethylmethylamine	52	54	89	79	33	60	47	43
Nitrosodiethylamine	86	78	76	71	95	64	64	57
Nitrosodipropylamine	83	98	82	79	83	100	100	72
Nitrosomethylpentylamine	97	92	79	75	85	100	100	95
Nitrosodibutylamine	96	110	100	104	100	89	100	100
Nitrosopyrrolidine	70	70	43	40	30	50	70	50

(*a*) Methylene chloride removed by using a Kuderna-Danish apparatus.
(*b*) Methylene chloride removed by evaporation on a water-bath.

As acidic and alkaline distillation stages are frequently used in the clean-up of nitrosamines extracted from food, a number of analyses of both water - ethanol and calvados containing nitrosamines were carried out with the inclusion of a distillation stage. Distillation was carried out nearly to dryness and the distillate analysed by using the method described above. The results shown in Table III indicate considerable losses of higher nitrosamines, which, we feel, indicates the value of a liquid - liquid extraction technique in analysing alcoholic samples for nitrosamines.

TABLE II

RECOVERY OF SEVEN NITROSAMINES, ADDED AT THE 10 $\mu g\ kg^{-1}$ LEVEL, FROM FOUR DIFFERENT SAMPLES OF CALVADOS*

	Recovery of each nitrosamine, per cent.			
Nitrosamine				
Nitrosodimethylamine	48	82	25	25
Nitrosoethylmethylamine	75	83	75	73
Nitrosodiethylamine	45	71	44	49
Nitrosodipropylamine	88	99	93	83
Nitrosomethylpentylamine	99	102	80	98
Nitrosodibutylamine	102	103	63	58
Nitrosopyrrolidine	40	40	25	50

* Methylene chloride was removed by simple evaporation on a water-bath.

TABLE III

RECOVERY OF NITROSAMINES FROM THE DISTILLATE OF A WATER - ETHANOL MIXTURE AND FOUR DIFFERENT SAMPLES OF CALVADOS

Each sample was initially spiked with nitrosamines at the level of 10 $\mu g\ kg^{-1}$

Nitrosamine	Recovery of each nitrosamine, per cent.						
	(*a*)			(*b*)			
Nitrosodimethylamine	20	31	34	14	85	16	8
Nitrosoethylmethylamine	21	83	58	26	84	77	106
Nitrosodiethylamine	29	Trace	59	Trace	71	19	27
Nitrosodipropylamine	33	Trace	66	Trace	91	28	32
Nitrosomethylpentylamine	45	65	75	Trace	90	18	22
Nitrosodibutylamine	22	Trace	Trace	Trace	100	31	29
Nitrosopyrrolidine	Trace	Trace	Trace	Trace	Trace	25	37

(*a*) Water - ethanol mixture.
(*b*) Four different samples of calvados.

As an example of samples with low alcohol content, the liquid - liquid extraction technique was used for the analysis of a number of beers spiked with the seven nitrosamines as before. Recoveries again were in the same range as those for calvados. However, as interference from other oxidation products in the gas-chromatographic analysis was considerably less than for calvados, it was possible to eliminate the column clean-up procedure, thus considerably diminishing the time of analysis.

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A Combustion Method for the Determination of Total Sulphur in Limestones

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A method is described for the determination of total sulphur in limestones. The sample is mixed with tungsten trioxide and quartz flour and placed in a boat of refractory material. The boat and contents are inserted into an aluminous porcelain combustion tube maintained at a temperature of about 1200 °C in a resistance-type tube furnace. The combustion tube carries a coil of copper gauze at the outlet end to reduce sulphur trioxide. Nitrogen is used as carrier gas and the sulphur dioxide evolved is absorbed in dilute hydrochloric acid containing potassium iodide and starch as indicator, titration with standard potassium iodate solution being carried out as the analysis proceeds.

SINCE this Institute undertook a feasibility study for a survey of resources of limestones, the need for a rapid method for the determination of sulphur in such material became apparent. The classical method of precipitating sulphur as barium sulphate is time consuming and susceptible to errors. Many combustion methods[1–3] have been used, all of which involved somewhat different conditions. That proposed by Coller and Leininger[1] requires a high temperature and equipment that is not readily available in this laboratory. The method of Sen Gupta[2] in which vanadium pentoxide was used as a flux was found to give low recoveries when applied to the analysis of limestones, and tests made on the residues from the combustion boats in this method showed that some sulphur remained in them. Results improved when the heating time was increased from 30 to 50 minutes or longer, but in view of the number of samples to be analysed, this heating time was not practicable.

In an attempt to overcome these problems, a flux composed of tungsten trioxide mixed with quartz flour, and a furnace temperature of 1200 °C were used. Results were slightly improved but still tended to be low. Examination of the residue showed sulphur to be absent, so it was assumed that part of the sulphur had been lost as sulphur trioxide. Introduction of a piece of copper gauze, about 40 × 120 mm, in the form of a 40-mm long coil into the outlet end of the furnace tube where the temperature lay in the range 750 to 850 °C overcame the losses. Results obtained with the modified method compared favourably with certificate values and gravimetric determinations. Samples other than limestones were also analysed and the results indicated that the method could be used for a wide variety of geological materials regardless of the form in which sulphur was present. The presence of the copper gauze had no detrimental effect if sulphur were present as sulphide.

EXPERIMENTAL

APPARATUS—

The apparatus used is shown in Fig. 1 and consists of the following: a furnace capable of maintaining a temperature of 1200 °C; an aluminous porcelain combustion tube, 0·6 mm long and of 20 mm i.d.; combustion boats of high-alumina fireclay with over-all dimensions of length 72 mm, width 16 mm and depth 10 mm; oxygen-free (white-spot) nitrogen from a cylinder (further purification is unnecessary); a 100-ml tall-form measuring cylinder; and a multi-hole bubbler that reaches the bottom of the measuring cylinder.

REAGENTS—

Tungsten trioxide.
Dilute hydrochloric acid (1·5 + 98·5).
Potassium iodide solution, 3·0 per cent. m/V.
Quartz flour, 240 mesh B.S.S.
Starch solution, 2·0 per cent. m/V.

Standard potassium iodate solution, 0·006 25 N—Dissolve 0·223 g of potassium iodate (previously dried for 1 hour at 110 °C) in distilled water, dilute the solution to 1 litre in a calibrated flask and mix thoroughly.

1 ml of solution ≡ 0·10 mg of sulphur.

Copper gauze, 40 mesh.

PROCEDURE—

Mix, in a small bottle, 1 g of tungsten trioxide, 0·5 g of quartz flour and a known suitable amount of sample. To a measuring cylinder containing 80 ml of dilute hydrochloric acid (1·5 + 98·5) add 1 ml of potassium iodide solution and 1 ml of starch indicator. Place the measuring cylinder in position at the end of the combustion train. Pass nitrogen through the system at a steady rate (approximately 150 ml min^{-1}) and titrate the absorbing solution with the standard potassium iodate solution to a faint blue colour. (The intensity of the blue colour can be adjusted to suit individual needs.)

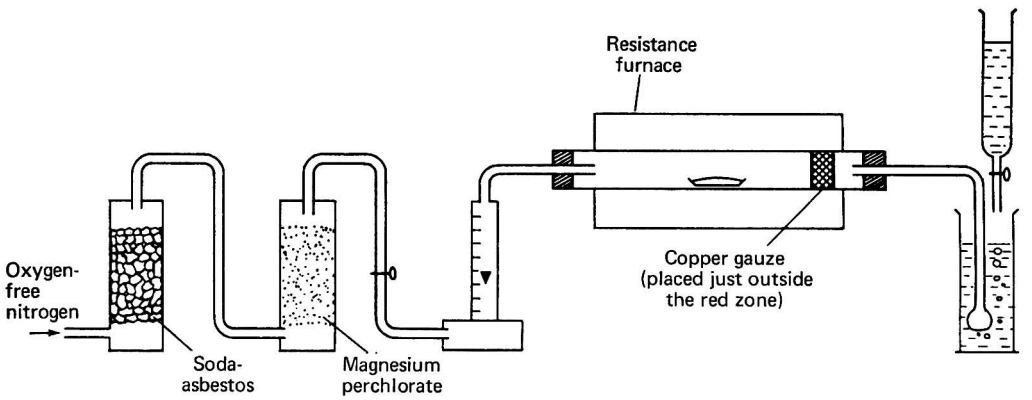

Fig. 1. Apparatus for the determination of total sulphur

Transfer the pre-mixed sample into a combustion boat (which has been ignited previously and then cooled) made of refractory material and insert the boat quickly into the hot zone of the furnace (1200 °C). Continue to pass nitrogen through the system and titrate the absorbing solution so as to maintain the blue colour. Continue passing the nitrogen for about 15 minutes or until the colour of the absorbent remains unchanged for 2 to 3 minutes. Wash the inside of the bubbler by closing the screw-tap and again opening it. Any condensation appearing on the glass tube should be vaporised either by increasing the nitrogen flow-rate at the end of the run or by heating the tube gently with a burner. If the colour of the absorbent fades, add another drop of titrant and ensure that the colour remains unchanged after a further 2 minutes. Note the volume of potassium iodate solution required. Carry out a blank test by using tungsten trioxide and quartz flour and make any necessary changes to the volume of titrant used.

$$\text{Sulphur, per cent.} = \frac{v}{100 \times w}$$

where v ml is the volume of potassium iodate solution used after correcting for the blank and w g the amount of sample taken.

RESULTS

Results obtained on standard materials by using the above method are shown in Table I.

Selected limestone samples covering the whole range of sulphur contents encountered in the feasibility study were analysed by both the combustion method and the barium sulphate gravimetric method, which was applied after decomposition of the sample by fusion with sodium carbonate. The results of these determinations are shown in Table II.

TABLE I

RESULTS OBTAINED ON STANDARD MATERIALS

Sample	Total sulphur, per cent. Certificate value	Value by combustion method
Argillaceous limestone N.B.S. No. 1a	0·27	0·28
Portland cement N.B.S. No. 177 ..	0·637	0·623
B.C.S. iron ore "A"	0·063	0·055
U.S. Geological Survey:		
Standard rock BCR-1	0·04	0·040
Standard rock GSP-1	0·03	0·033

The coefficients of variation obtained for the argillaceous limestone and Derbyshire limestones Nos. 4 and 11 were 6·07, 6·02 and 3·15 per cent., respectively, based on twelve determinations in each instance.

The procedure described is satisfactory for all types of samples tested, although the heating period can be varied slightly, depending upon the form in which the sulphur is present. For example, if the sulphur is present entirely as sulphide the heating period can be reduced to less than 15 minutes. The copper gauze turns black after three or four runs and should be replaced; it can, however, be re-used after the oxide layer has been removed. In order not to melt the copper, it is important to place the gauze just outside the red zone, otherwise its purpose is not served.

TABLE II

RESULTS OF ANALYSIS OF LIMESTONE SAMPLES

Sample	Total sulphur, per cent, by gravimetric method	combustion method
Derbyshire limestone No. 4 ..	0·05	0·04
No. 11 ..	0·50	0·45
No. 12 ..	1·95	1·92
Calcareous marl	0·22	0·22

CONCLUSIONS

A rapid combustion - titrimetric method has been developed for the determination of sulphur in limestones. By using a flux composed of a mixture of tungsten trioxide and quartz flour, good sulphur recoveries are obtained over a wide range of sample compositions. Inclusion of a coil of copper gauze in the cooler part of the furnace ensures that any sulphur trioxide evolved is reduced to dioxide. The method is comparable in accuracy with the barium sulphate gravimetric method but has the advantage of greater speed.

The author thanks Mr. P. J. Moore for helpful discussion with this paper, and the Director of the Institute of Geological Sciences for permission to publish this work.

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A Comparison of Two Procedures for the Determination of Organobromine by the Schöniger Oxygen-flask Method

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A statistical comparison of the argentimetric and mercurimetric methods of finish in the oxygen-flask determination of organobromine has been carried out. While no statistical difference was found to exist, the mercurimetric method is recommended for use in laboratories that lack the facilities required for the potentiometric determination.

The oxygen-flask method for the determination of organically bound bromine still follows the basic combustion method developed for micro-scale work by Schöniger.[1,2] In all analyses, assuming that total combustion takes place, accuracy is dependent upon conversion of the organobromine into a suitable form for determination by a well established method of finish.

Various methods of finish have been recommended for the determination of bromine in organic compounds by the oxygen-flask method.[3,4] Iodimetric, argentimetric and mercurimetric titrations have all been extensively used but controversy remains over their relative merits. There is also disagreement over the need to add hydrogen peroxide to the absorbing solution.[3,5] Childs, Meyers Cheng, Laframboise and Balodis,[6] for example, demonstrated that for bromine determinations absorption of combustion products is rapid if a solution of either hydrazinium sulphate or hydrogen peroxide in sodium hydroxide solution is used. We have, therefore, carried out a statistical comparison of the argentimetric and mercurimetric titrations, both with and without the use of hydrogen peroxide.

Experimental

Apparatus—

The oxygen-flask combustion unit manufactured by Thomas and Co., Philadelphia, U.S.A., was used because of the safety features it incorporates. We have found the method involving ignition with an infrared lamp by remote control to be of particular value in introducing inexperienced workers to oxygen-flask procedures.

Absorption solution—

For each determination in the statistical study, the absorption solution consisted of 10 ml of approximately 0·1 M sodium hydroxide solution containing 0·3 ml of 100-volume hydrogen peroxide. After ignition, the flask was left to cool for 10 minutes before being shaken in order to assist absorption of the combustion products in the absorption solution.

Methods of finish—

Argentimetric finish—Titration of the neutralised solution with 0·01 M silver nitrate solution was carried out potentiometrically by using essentially the procedure recommended for chlorine determinations by the Analytical Methods Committee.[7] For our determinations, the absorption solution was transferred from the combustion flask into a 100-ml beaker with 40 ml of distilled water. The pH of the solution was adjusted to between 6·0 and 6·2 measured with a glass - calomel pH electrode. The titration was carried out by using a glass - silver electrode system with the potential measured on a millivoltmeter.

Mercurimetric finish—The traditional method of titration of bromide ions with standard mercury(II) nitrate, using diphenylcarbazone with bromophenol blue as a screen, gives poor results in aqueous solution. The modified procedure developed by Cheng[8] and applied extensively by White,[9] which involves the use of an 80 per cent. ethanolic solution, was used for our determinations.

Following the combustion procedure the flask was washed with 10 ml of distilled water and acidified with 0·1 M nitric acid to the yellow colour of bromophenol blue; 100 ml of absolute ethanol and 0·5 ml of 0·1 M nitric acid were added and the pH was adjusted to 3·6. After addition of diphenylcarbazone solution (0·5 ml of a 0·1 per cent. ethanolic solution), the solutions were titrated with 0·01 M mercury(II) nitrate solution to the first appearance of a purple colour.

Results and discussion

All determinations were carried out on micro-analytical reagent grade 3-bromobenzoic acid (Br content 39·75 per cent.) with 10 to 20-mg samples. Corrections for blanks were applied to all results.

An initial set of results obtained in the absence of hydrogen peroxide in the absorption solution were discarded as they were found to be irregular and valueless from an analytical point of view. This finding was in sharp contrast with Steyermark's views,[5] but confirmed those expressed by Schöniger,[1,2] Macdonald[3] and Childs *et al.*[6]

The results obtained for bromine, per cent., were as follows:

Argentimetric finish 39·68, 39·35, 39·78, 39·85, 39·57, 39·57, 39·86, 39·36, 39·13, 39·56 (ten determinations)
Mean .. .. 39·57 Standard deviation .. .. 0·237

Mercurimetric finish 40·00, 39·71, 39·56, 39·84, 39·75, 39·51 (six determinations)
Mean .. .. 39·71 Standard deviation .. .. 0·202

Statistical comparison of results—

Student's *t*-test[10] was used and for the above calculated standard deviations *t* was found to be 1·21. As the value of *t* obtained lies between + 1·76 and −1·76, there is no statistically significant difference between the two means. At this level, $P = 0{\cdot}2$ to 0·3.

From a comparison of precision by using the *F* distribution with the above values, *F* is 1·38. At the 5 per cent. level, $F_{0{\cdot}05}$ is given as 4·77 and as the value found for *F* is less than 4·77, there is no significant difference in the precision of the means obtained by the two methods.

Conclusions

This investigation has shown that for argentimetric and mercurimetric finishes it is essential to include hydrogen peroxide in the absorbent solution. Our initial results were wholly unrelated to each other in the absence of hydrogen peroxide.

The statistical analysis of the results supports Steyermark's contention[5] that there is no significant difference between the results obtained by these two methods. It is, therefore, apparent that even a poorly equipped laboratory that lacks the facilities for potentiometric titrations can obtain reproducible results for the determination of bromine by using the less sophisticated mercurimetric finish. Of the two methods it has, in any event, the great advantage of being more rapidly carried out.

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Automated *in situ* Preparation of Azomethine H and the Subsequent Determination of Boron in Aqueous Solution

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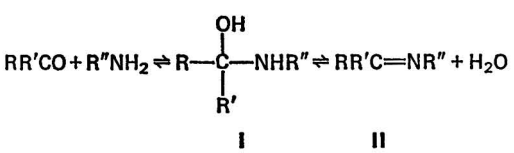
A procedure for the preparation of azomethine H and the use of the reagent in the same system for the determination of boron in solution is described.

A drawback to the automated procedure recently described for the automated determination of boron in plant tissue[1] is the time needed to prepare the required reagent, azomethine H, and the fact that, unless stored in a desiccator, it is unstable, resulting in changes in the calibration graphs. Moreover, aqueous solutions of the reagent tend to hydrolyse rapidly, unless they are refrigerated, and should not be stored for longer than 1 day because of their loss of sensitivity. In continuous-flow procedures, this tendency towards hydrolysis leads to considerable drift during the determination.

In this paper a procedure is described whereby the azomethine is prepared *in situ* in a continuous-flow system and is subsequently used for the determination of boron in aqueous solutions. This procedure results in a system in which the above-mentioned problems are overcome, thus increasing the effectiveness of the basic method.

The formation of Schiff's bases from a carbonyl compound and a primary amine is catalysed by the presence of an acid, a carbinolamine (**I**), being formed first, which rapidly eliminates water to give the Schiff base (**II**)—



I **II**

From studies with semicarbazide Hammett[2] concluded that the addition of a proton to the carbonyl group gives a carbonium ion, $RR'COH^+$ (R′ is hydrogen or an alkyl radical), which adds rapidly to the base. Deprotonation of this adduct is the rate-determining stage, leading to the formation of the carbinolamine (**I**). The condensation between benzaldehyde and aniline has been shown to be first order with respect to aldehyde, amine and acid catalyst.[3]

It appears, therefore, that the reaction between aldehydes and amines is rapid. The possibility of preparing azomethine H from salicylaldehyde and H-acid (8-amino-1-naphthol-3,6-disulphonic acid) in a continuous-flow procedure, and then utilising it to determine boron in the same system, is reported here.

Experimental

Apparatus—

Technicon AutoAnalyzer-II equipment.

Reagents—

All reagents are of analytical-reagent grade, unless otherwise specified.

Method and results—

In view of the work carried out by Deming and Morgan[4] on their optimisation, the reaction conditions for the determination of boron with azomethine H were studied on the basis of the

TABLE I

COMPARISON OF RESULTS FOR BORON CONTENT OBTAINED BY USE OF ORIGINAL AND MODIFIED PROCEDURES

Automated procedure,[1] p.p.m.	Suggested procedure, p.p.m.
38	36
9	10
84	81
24	26
33	33
6	5

suggested simplex model. Three variables, *viz.*, pH, concentration of H-acid and concentration of salicylaldehyde, have to be considered in order to optimise the reaction conditions for the preparation of the azomethine. By using the earlier flow system[1] the optimum conditions for the determination of boron were found to be a pH of 6·35 (previously established[1] as 5·1) and an azomethine H concentration of 9·5 g dm^{-3}. The following reactant conditions were finally selected.

H-acid—A 2·5-g amount of H-acid was dissolved in 250 cm^3 of distilled water and the pH was adjusted to 2·25.

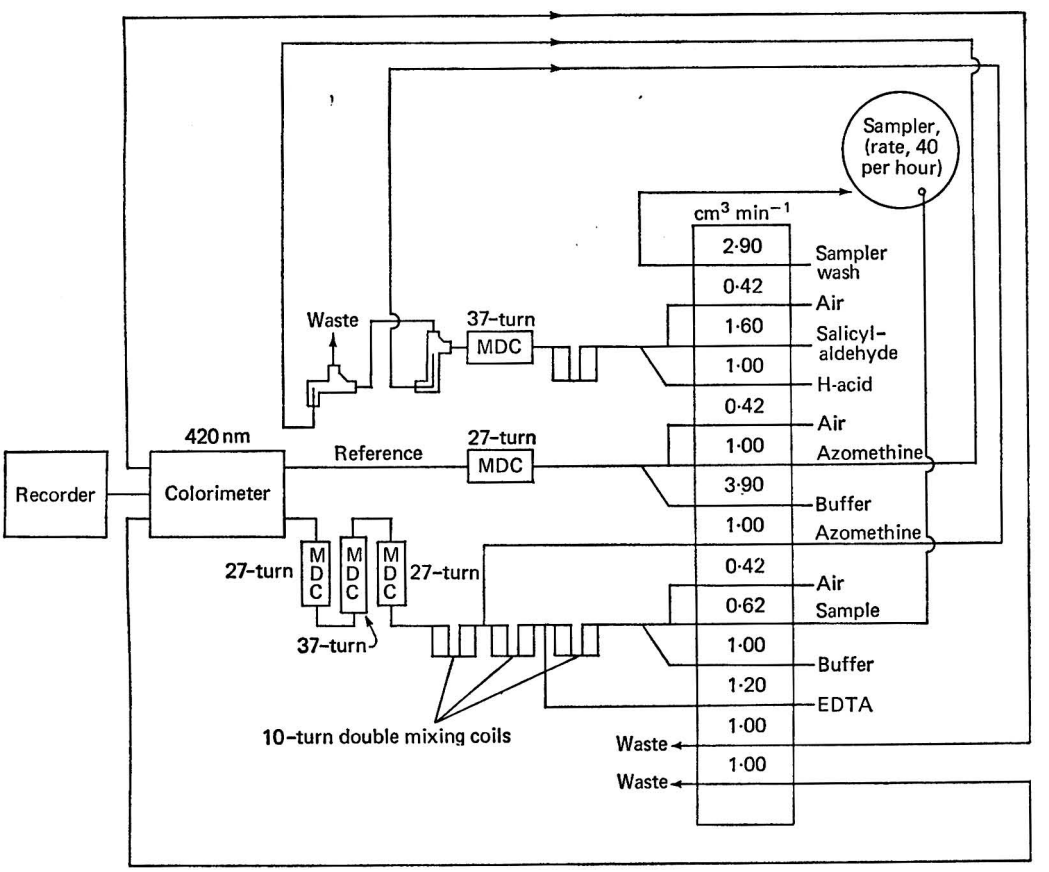


Fig. 1. Flow diagram for the preparation of azomethine H and the determination of boron in aqueous solution. MDC = mixing delay coil

Salicylaldehyde solution—A 1·0-cm³ volume of salicylaldehyde was dissolved in 250 cm³ of 80 per cent. ethanol.

Samples of plant material were analysed by the procedure previously reported[1] and the results compared (Table I) with those obtained by the system described in the present paper and illustrated diagrammatically in Fig. 1.

Conclusion

A more effective automated azomethine procedure for the determination of boron in a variety of materials is described in which the preparation of the azomethine is incorporated into the flow system.

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Determination of Polyoxyethylene *p*-t-Nonylphenyl Ethers in Pomades

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A method is proposed for the determination of polyoxyethylene *p*-t-nonylphenyl ethers, $RO(CH_2CH_2O)_nH$ (R = *p*-t-nonylphenyl), in pomades. The method is based on the evaluation of the number average degree of polymerisation of the polydispersity ($\bar{n}$) by vapour pressure osmometry and by thin-layer chromatography, and on the spectrophotometric determination of the apparent mean molar absorption coefficient ($\bar{\epsilon}$) at 620 nm of the bisammonium tetrathioisocyanatocobaltate(II) complexes extracted into 1,2-dichloroethane.

The method has been tested for values of $\bar{n}$ in the range 6·5 to 21·5 and for Weibull distributed polydispersions. Significant systematic errors arise only in mixtures of polydispersions with very different values of $\bar{n}$. The accuracy and intralaboratory reproducibility have been evaluated by analysing samples of a pomade of known composition.

POLYOXYETHYLENE oligomers derived from alkylphenols, $RO(CH_2CH_2O)_nH$ (where R is an alkylphenyl group and n is the degree of polymerisation), are often determined in products containing them by the method of Brown and Hayes,[1] which is based on the extraction of the complexes, formed by the polyoxyethylene chain with bisammonium tetrathioisocyanatocobaltate(II) $[(NH_4)_2Co(NCS)_4]$, into an organic phase, followed by spectrophotometric determination in the visible or ultraviolet region. This method gives relative concentration values with respect to a polyoxyethylene compound taken as a standard, as, under similar conditions, the apparent specific absorbance of the complexes in the organic phase varies with the degree of polymerisation of the oligomers and with the nature of the hydrophobic group (R) and that of the extracting solvent.

If, for homogeneous oligomers with a definite R group, a relationship can be found between n and the apparent molar absorption coefficient (ϵ), within a certain range of n values, this relationship can, in principle, be made use of for the determination of the oligomers in this range, provided that n can be determined with the aid of other systems. The commercial polydisperse products are characterised by the number average degree of polymerisation ($\bar{n}$). Their determination involves many accurate operations because of the presence of variously reacting oligomers. For example, with polyoxyethylene *p*-t-nonylphenyl ethers (PENPEs) (R = *p*-t-nonylphenyl), the curve $\bar{\epsilon}$ (apparent mean molar absorption coefficient) as a function of $\bar{n}$ shows an increasing trend for the value of $\bar{n}$ to be greater than 5 to 6, with a maximum at about 20 to 25, which is controlled by the solubility of the complexes in the organic phase.[2] Observations made on homogeneous compounds ($0 \leqslant n \leqslant 9$) substantially confirmed this trend.[3] Within a limited range of $\bar{n}$ values ($\bar{n}$ always higher than 6) a linear relationship may fit the results, provided the sample is homogeneous or characterised by a narrow distribution of relative molecular masses, as occurs in the Weibull distributed oligomers.[4] Suitable choice of solvent and of experimental conditions are important factors for extending the practical applicability of this empirical relationship.

Within the above limitations, a method for the determination of PENPE with $6{\cdot}5 \leqslant \bar{n} \leqslant 21{\cdot}5$ in a typical pomade is suggested and discussed. The method is based on the extraction and determination by absorption spectrophotometry of the complexes formed with bisammonium tetrathioisocyanatocobaltate(II), in conjunction with the evaluation of $\bar{n}$ for the polyoxyethylene compound by vapour pressure osmometry and by thin-layer chromatography, coupled with direct photometry of the layer.[5–7] The matrix generally contains hydrophobic substances such as stearic acid, which are first eliminated so as to avoid interferences in the vapour pressure osmometry and in the thin-layer chromatography.

METHOD

APPARATUS—

A Perkin-Elmer 402 ultraviolet - visible recording spectrophotometer was used with 1-cm glass cells for measurement of the absorbance of solutions in the visible range.

REAGENTS—

Ethanol, 95 per cent.—RP, Erba.

Bisammonium tetrathioisocyanatocobaltate(II)—This reagent was prepared according to the method of Greff, Setzkorn and Leslie[2] by dissolving 620 g of ammonium thiocyanate (Fisher, Certified) and 280 g of cobalt(II) nitrate hexahydrate (Fisher, Certified) in water and making the volume up to 1 litre.

Standard aqueous solutions of polyoxyethylene p-*t-nonylphenyl ethers, 250 mg* l^{-1}—These solutions were prepared by using compounds with $\bar{n}$ values of 6·5, 7·5, 8·6, 9·7, 10·8, 12·9, 15·0 and 21·5 (Chemische Werke Hüls), which had previously been dried at 50 °C and at a pressure of 0·5 mm of mercury for 2 hours. The solutions should be freshly prepared. The value of $\bar{n}$ was controlled by vapour pressure osmometry, by using a Hewlett-Packard Mechrolab vapour pressure osmometer.

1,2-*Dichloroethane*—RP, Erba, freshly distilled.

Sodium chloride—AnalaR grade.

Sodium hydroxide solution, 2 M—This solution was freshly prepared from Merck pro analysi reagent.

Diethyl ether—Merck pro analysi reagent.

Chloroform—Merck pro analysi reagent.

PROCEDURES—

Preparation of the sample and extraction of the complexes—Weigh exactly 0·500 g of pomade into a 25-ml beaker, add 4·0 ml of ethanol and heat the mixture for a few minutes at 40 °C in order to dissolve the pomade almost completely. Transfer the solution into a 500-ml calibrated flask, wash the residue in the beaker with 1·0 ml of ethanol and then with 10 to 20 ml of water (40 to 50 °C), adding the wash liquids to the contents of the flask. Finally, wash the beaker with 20 to 25-ml portions of cold water, and add these to the liquid in the flask until a total volume of at least 300 ml is obtained. Mix the contents of the flask for a few seconds and make the volume up to the mark, thus obtaining a relatively stable, opalescent aqueous suspension.

With a calibrated pipette, transfer 30·0 ml of suspension into a 50-ml calibrated flask, together with 10·0 ml of bisammonium tetrathioisocyanatocobaltate(II) reagent. Mix the solution and allow it to stand for at least 1 hour. Add 10·0 ml of 1,2-dichloroethane (accurately measured with a calibrated pipette), and extract by shaking the flask vigorously for 3 minutes. Transfer the contents into a 100-ml separating funnel the tap of which is lubricated with water, and, after separation of the phases, transfer the organic layer dropwise into a 15-ml centrifuge tube fitted with a polyethylene tap and centrifuge it for 5 minutes at 3000 r.p.m.

Determination of PENPE by absorption spectrophotometry—Measure the absorbance of the clear organic extract at 620 nm in 1-cm cells against a blank consisting of the pure solvent. Taking the dilution into account, the percentage (*m*/*m*) of PENPE in the pomade is:

$$\text{PENPE, per cent. } m/m = 133{\cdot}3\,\frac{A}{4\bar{a}p} \quad (\text{for } 6{\cdot}5 \leqslant \bar{n} \leqslant 21{\cdot}5)$$

where A is the absorbance, p g is the amount of sample in 1000 ml of solution, $\bar{a}$ l g^{-1} cm^{-1} is the apparent mean specific absorption coefficient, which is calculated for a given value of $\bar{n}$ by means of the expression $\bar{a} = \bar{\epsilon}/\bar{M} = (\beta\bar{n} + \alpha)/(220 + 44{\cdot}05\bar{n})$, where $\beta = 130{\cdot}0$ l mol^{-1} cm^{-1} per oxyethylene group, and $\alpha = -335{\cdot}8$ l mol^{-1} cm^{-1} at 20 °C. $\bar{M}$ is calculated from the number average degree of polymerisation found by vapour pressure osmometry and by thin-layer chromatography.

Determination of $\bar{n}$ *by vapour pressure osmometry and by thin-layer chromatography*—Extract the PENPE from 250 ml of sample solution by introducing 40 g of powdered sodium chloride and 20 ml of 2 M sodium hydroxide solution, followed by extraction twice with 50 ml of

diethyl ether that has been saturated with water. Combine the extracts, evaporate the solution in a small conical centrifuge tube and take up the residue with 5 ml of anhydrous diethyl ether. Centrifuge the solution for 5 minutes at 3000 r.p.m., transfer the clear liquid to another tube, evaporate to dryness at 60 °C and a pressure of 0·1 mm of mercury, and determine the $\bar{M}$ value of the residue in 1,2-dichloroethane by vapour pressure osmometry in a concentration range of 2 to 8 $\times$ 10^{-3} M.

Take up the residue of another sample with 2·00 ml of chloroform and use this solution (approximately 1 to 2 per cent. *m/m* of PENPE) for thin-layer chromatography.

Discussion of the method

Preparation of the sample—

The experiments were carried out with a pomade that had the following composition: stearic acid 17·5, propane-1,2-diol 5·0, benzoic acid 0·10, PENPE ($\bar{n}$ = 9·7) 5·25, gum tragacanth 1·2 and water 70·75 per cent. *m/m*. The preliminary dissolution of stearic acid with ethanol and its precipitation by addition of an excess of water allows the preparation of a dispersion, which is stabilised by the surface-active agent, from which it is possible to take a representative aliquot.

Extraction of the complexes and preparation of calibration graphs—

Compared with other organic phases previously used, 1,2-dichloroethane has the following general advantages: it has lower volatility (with good reproducibility of results), a density greater than that of the aqueous phase, resulting in easy separation by centrifugation, and is a more suitable solvent for insoluble complexes.[8] The last feature is illustrated by Fig. 1, in which absorbances obtained with various organic phases (other conditions being identical) are compared. With 1,2-dichloroethane absorbances are enhanced and limiting factors due to the solubility are avoided.

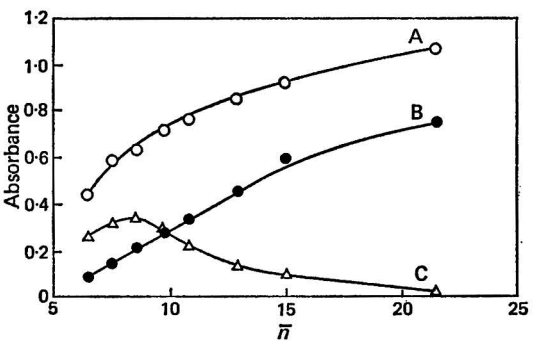

Fig. 1. Absorbances (× 5) at 620 nm and at 20 °C (b = 1 cm) of extracts with various solvents (A, 1,2-dichloroethane; B, chloroform; and C, benzene) as a function of the number average degree of polymerisation ($\bar{n}$) of PENPEs. Ratio of organic to aqueous phase 1:4 *V/V*. Concentration of PENPE in aqueous phase 25 mg l^{-1}

Blank tests show that 1,2-dichloroethane reacts slowly with the thiocyanate ions to give ethylene thiocyanate, $NCSCH_2CH_2SCN$ (see Note), which is extracted into the organic solvent. As this compound absorbs at 240 nm, with a broad band, in 1,2-dichloroethane, its presence makes the measurement at the maximum for the complexes in the ultraviolet region (321 nm) less reproducible. Therefore the maximum at 620 nm was preferred, although its sensitivity is five times lower than at 321 nm. At 620 nm, interferences caused by ultraviolet chromophores are also avoided.

Note—

This substance was crystallised from organic extracts and identified by its melting-point (90 °C, in agreement with that given in the literature[9]), by elemental analysis, and by infrared and nuclear magnetic resonance spectroscopy.

TABLE I

POLYNOMIAL REGRESSION ANALYSIS OF $\bar{\epsilon}$ *versus* $\bar{n}$ RELATIONSHIPS

Coefficients of the polynomial $\bar{\epsilon} = \alpha + \beta\bar{n} + \gamma\bar{n}^2$

	1-degree polynomial		2-degree polynomial		
	α	β	α	β	γ
1,2-Dichloroethane	−335·85	130·01	−266·18	118·20	0·4325
Chloroform	−683·16	110·79	−650·62	105·53	0·1832

Analysis of the variance

Number of observations	Source of variation	Degrees of freedom	Sum of squares	*F*
1,2-Dichloroethane—				
1-degree polynomial—				
35	Due to regression	1	9 762 610	12 830
	Deviation about regression	33	25 110	
	Total	34	9 787 720	
2-degree polynomial—				
35	Due to regression	2	9 765 263	6958
	Deviation about regression	32	22 457	
	Total	34	9 787 720	
Chloroform—				
1-degree polynomial—				
10	Due to regression	1	3 191 217	1299
	Deviation about regression	8	19 647	
	Total	9	3 210 864	
2-degree polynomial—				
10	Due to regression	2	3 191 387	573
	Deviation about regression	7	19 477	
	Total	9	3 210 864	

For every PENPE considered, Beer's law at 620 nm holds at least in the concentration range 5 to 25 mg l^{-1} in the aqueous phase, with an intralaboratory reproducibility of 2 to 3 per cent., expressed as the coefficient of variation of the results from the interpolating line.

In order to define analytically the empirical relationship $\bar{\epsilon}$ *versus* $\bar{n}$, polynomial regression analysis has been applied to the results. $\bar{\epsilon}$ has been calculated as follows: $\bar{\epsilon} = (A/4c)/(220 + 44{\cdot}05\bar{n})$, taking into account the ratio (V/V) of the organic to the aqueous phase; c g l^{-1} is the concentration of PENPE in the aqueous phase. Table I shows the statistical

TABLE II

ACCURACY AND PRECISION IN THE DETERMINATION OF THE NUMBER AVERAGE DEGREE OF POLYMERISATION ($\bar{n}$) BY THIN-LAYER CHROMATOGRAPHY (3 OPERATORS)

Sample	$\bar{n}$ = 6·5 (by vapour pressure osmometry)	$\bar{n}$ = 7·5 (by vapour pressure osmometry)		$\bar{n}$ = 9·7 (by vapour pressure osmometry)	
	Standard	Standard	Extracted	Standard	Extracted
1	6·28	8·04	7·59	10·50	9·53
2	6·85	7·55	8·04	9·77	9·79
3	6·98	7·81	7·91	9·63	9·64
4	6·99	7·72	—	10·27	9·56
5	—	—	—	10·09	—
$\bar{x}$	6·77	7·78	7·85	9·96	9·63
s	±0·34	±0·20	±0·23	±0·26	±0·14
100 ($s/\bar{x}$)	±5·0	±2·6	±2·9	±2·6	±1·5
f	3	3	2	4	3
t-value observed	1·59	2·30	2·64	2·24	1·00
t-value theoretical (P = 0·99)	5·84	5·84	9·93	4·60	5·84

$\bar{x}$, average of N determinations; s, root mean square deviation; 100 ($s/\bar{x}$), coefficient of variation, per cent.; $f = N - 1$ degrees of freedom.

results calculated by using an IBM 7044 computer. Comparison of F values shows that a straight line fits the results better than a parabolic curve. The equation $\bar{\epsilon} = -335{\cdot}8 + 130{\cdot}0\bar{n}$ is the empirical relationship that is valid for Weibull distributed PENPEs in the range of $\bar{n}$ values from 6·5 to 21·5. Fig. 2 shows the regression lines observed in 1,2-dichloroethane and in chloroform.

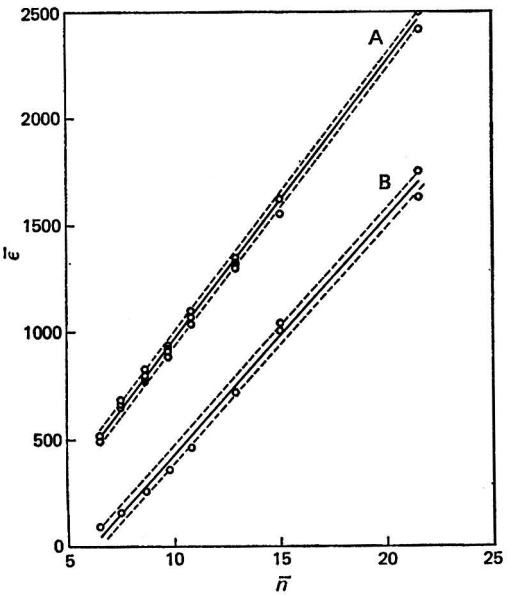

Fig. 2. Linear regressions of the apparent mean molar absorption coefficient ($\bar{\epsilon}$) as a function of number average degree of polymerisation ($\bar{n}$) at 20 °C, in two-phase extraction of PENPEs with 1,2-dichloroethane (line A) and chloroform (line B). Regression equations: A, $\bar{\epsilon} = -335{\cdot}8 + 130{\cdot}0\ \bar{n}$ ($s = \pm 28$ and $N = 35$); and B, $\bar{\epsilon} = -683{\cdot}2 + 110{\cdot}8\ \bar{n}$ ($s = \pm 49$ and $N = 10$). s is the root mean square deviation of the estimate and N is the number of results (in the diagram many of the results are superimposed)

EXTRACTION OF PENPE AND DETERMINATION OF $\bar{n}$—

Diethyl ether was used for the extraction of the PENPE from the pomade, in combination with salting-out. In order to minimise the extraction of interfering organic acids, the aqueous phase was made strongly alkaline. The results obtained by thin-layer chromatography and vapour pressure osmometry in the determination of $\bar{n}$ for the standard PENPEs are shown in Table II. The significance of the difference between the value for $\bar{n}$ found by vapour pressure osmometry, and taken as a true value, and the average value found by thin-layer chromatography was evaluated by the Student t-test.[10] In every instance, the observed t-values were smaller than the critical values at the 99 per cent. probability level, so that it is justifiable to assume that there is no significant difference.

PRECISION AND ACCURACY OF THE METHOD—

Table III shows the results of the analysis of two samples taken from the pomade that contained 5·25 per cent. (m/m) of PENPE ($\bar{n} = 9{\cdot}7$). The accuracy of the method is acceptable, as shown by the t-test applied to the comparison of the true value and the average value found. In both samples, the t-value, calculated from N observations, is smaller than the theoretical critical value tabulated at 99 per cent. probability and for $f = N - 1$ degrees of freedom.

TABLE III

EVALUATION OF THE PRECISION AND ACCURACY OF THE RESULTS OBTAINED FROM THE ANALYSIS OF TWO SAMPLES (A AND B) OF A STANDARD POMADE CONTAINING 5·25 PER CENT. *m/m* OF PENPE ($\bar{n} = 9{\cdot}7$)

	PENPE, per cent.	
Analysis No.	A	B
1	5·34	5·36
2	5·39	5·25
3	5·27	5·22
4	5·25	5·29
$\bar{x}$	5·31	5·28
s	±0·064	±0·058
$100\ (s/\bar{x})$	1·2	1·1
t-value observed	1·88	1·04
t-value theoretical ($P = 0{\cdot}99$)	5·84	5·84
f	3	3

$\bar{x}$, average; s, root mean square deviation; $100\ (s/\bar{x})$, coefficient of variation, per cent.

In Table IV some examples of the systematic errors that arise with a PENPE sample from a mechanical mixture of two polydispersions that had different $\bar{n}$ values are reported. In the range 6·5 to 12·9 the polydispersity can be controlled by thin-layer chromatography. Fig. 3 illustrates some distributions obtained by this method, which are plotted on Weibull probability logarithmic paper.* Weibull distributed samples appear as nearly straight lines. Unfortunately, thin-layer chromatography is not very efficient in resolving broad poly-

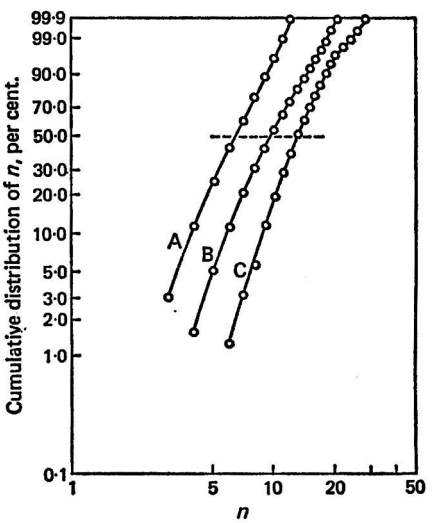

Fig. 3. Cumulative distributions of the degree of polymerisation (n) observed in Weibull distributed PENPE samples. Polydispersions are characterised by their number average degree of polymerisation ($\bar{n}$), which is immediately read out on the abscissa at the 50 per cent. value. Analyses performed by thin-layer chromatography: A, 6·5; B, 9·7; and C, 12·9

* Available from W. Heffer & Sons, Cambridge.

dispersities owing to the limited peak capacity of this technique. Further studies are in progress on the determination of $\bar{n}$ in truncated distribution by thin-layer chromatography.

TABLE IV

ANALYSIS OF MIXTURES AT A CONCENTRATON OF 25 mg l^{-1} OF PENPE IN THE AQUEOUS PHASE

$\bar{n}$ for the oligomers in the mixture ($1 + 1$ *m*/*m*)	$\bar{n}$ (by vapour pressure osmometry)	*A*		Difference (per cent.) $[100(X - \bar{x})/X]$	*t*-value	
		Observed ($\bar{x} \pm s$)	Calculated (X)		Observed	Theoretical ($P = 0.99$)
8·6 + 10·8	9·7	0·1443 ± 0·0019	0·1429	−1·0	1·5	5·84
7·5 + 12·9	10·2	0·1463 ± 0·0017	0·1479	+1·1	1·9	5·84
8·6 + 21·5	14·2	0·1695 ± 0·0013	0·1785	+5·0	13·8	5·84

$\bar{x}$, average; s, root mean square deviation calculated from $N = 4$ analyses; A, absorbance at 620 nm ($b = 1$ cm).

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Determination of iota-Carrageenan with 2-Thiobarbituric Acid

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A colorimetric method for the determination of iota-carrageenan is described, based on the measurement of the yellow colour developed by the reaction of the 3,6-anhydro-D-galactose residue with 2-thiobarbituric acid and hydrochloric acid. The method has a sensitivity equivalent to 0·01 mg ml^{-1} of carrageenan and can also be used to determine carrageenan in blood and urine in concentrations of 0·02 and 0·01 mg ml^{-1}, respectively. The method has advantages of sensitivity, relative specificity, a small sample-volume requirement and convenience over previously published methods for the determination of sulphated polysaccharides, thus providing a means of extending quantitative biological investigations of this group of substances.

The determination of sulphated polysaccharides still presents difficulty and is normally subject to appreciable error, especially when their determination in biological material is required. Several methods have been suggested for the determination of sulphated polysaccharides; the toluidine blue[1] and azure A[2] methods can be used, in which the excess of cationic dye, after completion of a metachromatic reaction between the dye and the macro-anionic sulphated polysaccharide, is determined. These methods lack specificity and require carefully standardised conditions, particularly of salt concentration, rendering them unsuitable for routine, accurate determinations in complex media in which the salt concentration varies, when mixtures of macro-anions occur and when dialysis is not always possible.

The determination of sulphated polysaccharides from the amount of the sulphate ester or their identification by infrared spectroscopy in media other than simple solutions requires prior removal of the sulphated polysaccharide by precipitation with a quaternary ammonium compound from the mixture,[3] followed by isolation and characterisation of the sulphated polysaccharide prepared from the macro-anion - quaternary ammonium complex and thereafter determination with one of the methods referred to.[1,2] Such methods have obvious disadvantages for the routine determination of sulphated polysaccharides in boilogical media in which they are likely to occur in small concentrations.

Some of the difficulties can be resolved for certain sulphated polysaccharides by adapting the resorcinol method,[4,5] in which 5-hydroxymethyl-2-furaldehyde, produced from fructose or 3,6-anhydro-D-galactose that has been liberated from the polysaccharide, reacts with resorcinol in the presence of 1,1-diethoxyethane[6,7] to form a coloured reaction product, which can then be determined. However, we have found that this method presents certain difficulties when applied to the determination of sulphated polysaccharides containing 3,6-anhydro-D-galactose, not only in biological fluids but even in aqueous solution.

The continuing need for a rapid, convenient method for the determination of sulphated polysaccharides containing 3,6-anhydro-D-galactose has been intensified by a recent report on the intestinal toxicity of sulphated polysaccharides after oral administration to experimental animals[8] and the demonstration that gastrointestinal absorption of certain members of this group of substances with low relative molecular mass can occur,[9,10] together with their traditional and apparently continuing use in foodstuffs, beverages and medicines. In order to provide a more accurate and sensitive method for the determination of sulphated polysaccharides containing 3,6-anhydro-D-galactose, the use of 2-thiobarbituric acid, previously suggested as a reagent for the quantitative determination of fructosides,[11] has been investigated, and is now reported.

Reagents and materials—

Sulphated polysaccharide containing 3,6-anhydro-D-galactose—Both native and degraded iota-carrageenans were used. The native carrageenan was obtained by extraction with hot

water from the red seaweed *Eucheuma spinosum*; degraded carrageenan was prepared from native carrageenan by treatment with 0·001 N hydrochloric acid followed by neutralisation with 0·001 N sodium hydroxide solution (after a predetermined degree of depolymerisation, monitored viscosimetrically, had occurred), precipitation with ethanol, dialysis and freeze-drying. Results for these carrageenans are summarised in Table I. Lambda-carrageenans were obtained by extraction from *Chondrus crispus* and *Gigartina pistillata* in the usual manner.

For purposes of spectrophotometric calibration crystalline methyl-3,6-anhydro-D-galactopyranoside was used.

TABLE I

PROPERTIES OF SULPHATED POLYSACCHARIDES CONTAINING 3,6-ANHYDRO-D-GALACTOSE

	Sulphate ester content ($-OSO_3Na$)*, per cent.	3,6-Anhydro-D-galactose content†, per cent.	Mass average relative molecular mass‡
Native ι-carrageenan	37·7	18·70 ± 0·21	800 000
Degraded ι-carrageenan	36·1	21·01 ± 0·21	25 000

* Determined by complete acid hydrolysis with 10 N hydrochloric acid followed by addition of barium chloride and a gravimetric finish.

† Determined by 2-thiobarbituric acid method described herein.

‡ Determined by light scattering.

2-Thiobarbituric acid, 1,1-diethoxyethane and resorcinol—Laboratory-reagent grade.

Charcoal—This was Norit A (Sigma), washed with 10 per cent. acetic acid followed by distilled water and heated to redness in air for 15 minutes.

Sugars—The sugars used were D-glucose (AnalaR), L(+)-rhamnose, D-xylose, D-arabinose, L-fucose, D-galactose, D-mannose, D-fructose (glucose-free) and D-ribose.

Heparin.

Chondroitin sulphate.

Blood—The blood was obtained by exsanguination from the ox, rat and guinea-pig; 4 parts of fresh blood were collected into 1 part of 3·8 per cent. *m/V* trisodium citrate solution. The plasma was used after centrifugation and removal of particulate sediment.

Urine—Freshly voided normal human urine was used.

EXPERIMENTAL

COLOUR DEVELOPMENT WITH 2-THIOBARBITURIC ACID—

(*a*) Determinations of monosaccharides and polysaccharides in a series of 2-ml aqueous solutions (the concentrations of which are noted below) were carried out by adding 2 ml of 2×10^{-2} M thiobarbituric acid solution and 2 ml of 10 N hydrochloric acid to each, heating them for 6 minutes at 100 °C, followed by cooling for 2 minutes in an iced water bath and measuring the absorbance at 432 nm within 30 minutes. Concentrations of the test substances used were: carrageenans, 1 to 40 mg per 100 ml; heparin and chondroitin sulphate, 10 to 2000 mg per 100 ml; methyl-3,6-anhydro-D-galactose, 0·025 to $0·25 \times 10^{-3}$ M; fructose, 0·1 to $0·25 \times 10^{-3}$ M; and other monosaccharides, 10^{-3} to 5×10^{-3} M.

(*b*) The determination of degraded carrageenan in blood and urine was carried out as follows.

Blood—A 1-ml volume of plasma was shaken for 60 minutes at 37 °C with 8 ml of saturated potassium chloride solution so as to dissociate the carrageenan - protein complexes. Protein was precipitated by adding 0·3 ml of 30 per cent. trichloroacetic acid and removed by centrifugation. The carrageenan in 2 ml of the supernatant liquid was determined by adding 1 ml of 3×10^{-2} M thiobarbituric acid followed by 1·5 ml of 10 N hydrochloric acid and the method conducted as described in (*a*) above. The final concentrations of the reagents in the reaction mixture were the same as in the procedure described in (*a*).

Urine—A 1-g amount of charcoal was added to 10 ml of fresh urine and shaken for 30 minutes. After filtration, 0·5 ml of 4 N sodium hydroxide solution was added to 2 ml of the filtrate and the mixture was then placed in a bath at 100 °C for 10 minutes, cooled in an iced water bath, 0·2 ml of 10 N hydrochloric acid added and a 2-ml aliquot treated as in (*a*) above.

MODIFIED RESORCINOL METHOD—

This method, intended for the determination of fructose and 3,6-anhydro-D-galactose, was carried out as described,[6,7] except that the 1,1-diethoxyethane concentration was varied.

RESULTS

2-THIOBARBITURIC ACID METHOD—

Colour development—The spectra obtained with the products of the interaction of 2-thiobarbituric acid with iota-carrageenan, methyl-3,6-anhydro-D-galactose and fructose are shown in Fig. 1, which shows a single peak at 432 nm in each instance. Beer's law is obeyed over the ranges of direct application and graphs of the absorbance at 432 nm *versus* the concentration of the substance in aqueous solution yield the equations shown in Table II. As would be expected, the lambda-carrageenans from *Chondrus crispus* and *Gigartina pistillata* contained less 3,6-anhydro-D-galactose than iota-carrageenan, which was verified by infrared studies.

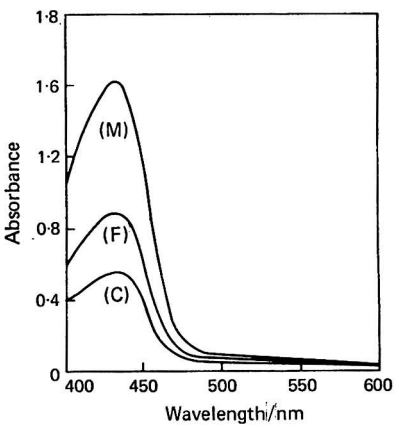

Fig. 1. Spectra for reaction product of $0{\cdot}2 \times 10^{-3}$ M methyl-3,6-anhydro-D-galactose (M), $0{\cdot}2 \times 10^{-3}$ M fructose (F) and 8 mg per 100 ml degraded ι-carrageenan (C) with 2-thiobarbituric acid

Colour stability—The first-order rate constants for loss of absorbance at 432 nm for thiobarbituric acid and degraded carrageenan (taken as being representative) in dark and light conditions were $12{\cdot}4 \times 10^{-4}$ and $29{\cdot}8 \times 10^{-4}$ min^{-1}, respectively, at 36·5 °C and $0{\cdot}5 \times 10^{-4}$ and $1{\cdot}1 \times 10^{-4}$ min^{-1}, respectively, at 20 °C.

TABLE II

EQUATIONS FOR COLOUR DEVELOPMENT BETWEEN 2-THIOBARBITURIC ACID AND CARRAGEENANS, METHYL-3,6-ANHYDRO-D-GALACTOSE AND FRUCTOSE

Degraded ι-carrageenan	$y = 0{\cdot}0715x$
Native ι-carrageenan	$y = 0{\cdot}0633x$
Carrageenan from *C. crispus*	$y = 0{\cdot}0463x$
λ-Carrageenan from *G. pistillata*	$y = 0{\cdot}0185x$
Degraded λ-carrageenan from *G. pistillata*	$y = 0{\cdot}0132x$
λ-Carrageenan from *C. crispus*	$y = 0{\cdot}0121x$
Methyl-3,6-anhydro-D-galactose	$y = 0{\cdot}3390x$
Fructose	$y = 0{\cdot}3110x$

y = absorbance at 432nm; and x = concentration/mg per 100 ml.

Specificity—The ratios of the colour intensity developed at 432 nm as a result of the reaction between 2-thiobarbituric acid and various sugars relative to that developed by equimolar amounts of methyl-3,6-anhydro-D-galactose and carrageenan are shown in Table III.

TABLE III

SPECIFICITY OF 2-THIOBARBITURIC ACID ASSAY FOR CARRAGEENAN CONTAINING 3,6-ANHYDRO-D-GALACTOSE

Sugar	Ratio A*	Ratio B†
Fructose	80·0	197·0
Ribose	2·0	5·0
Fucose	2·0	5·0
Mannose	1·8	4·0
Rhamnose	1·8	4·0
Xylose	1·2	2·5
Arabinose	0·6	1·4
Galactose	0·6	1·4
Glucose	0·6	1·4

* Ratio A = $100 \times \frac{\text{absorbance (432nm) using 1}\mu\text{mol of sugar}}{\text{absorbance (432nm) using 1}\mu\text{mol of methyl-3,6-anhydro-D-galactose}}$

† Ratio B = $100 \times \frac{\text{absorbance (432nm) using 1}\mu\text{mol of sugar}}{\text{absorbance (432nm) using 1}\mu\text{mol of carrageenan (repeating unit)}}$

The minimum theoretical relative molecular mass of the repeating carrabiose unit in carrageenan when sodium is the micro-cation is 405 (calculated on the structures shown by Rees[12]) and this value was used in calculating the equivalent molar concentrations. Chondroitin sulphate and heparin at a concentration of 200 mg per 100 ml showed absorbances at 432 nm of 0·055 and 0·017, respectively.

Determination of degraded carrageenan in blood and urine—Pre-treatments of blood and urine, as described in (*b*) above, required to reduce interference in the assay did not completely remove interfering substances; blank samples containing no carrageenan showed absorbance values of 0·11 to 0·17 (urine) and 0·05 to 0·08 (ox and guinea-pig blood). However, after accounting for blank values, assays of carrageenan in blood (2 to 100 mg per 100 ml) and urine (1 to 40 mg per 100 ml) yielded graphs that indicated conformity to Beer's law over the ranges of direct application and the equations in Table IV were derived from the average values obtained.

TABLE IV

EQUATIONS FOR COLOUR DEVELOPMENT BETWEEN 2-THIOBARBITURIC ACID AND DEGRADED ι-CARRAGEENAN IN URINE AND BLOOD

Degraded i-carrageenan in—	
Urine	$y = 0{\cdot}0403x$
Control	$y = 0{\cdot}0473x$
Ox blood	$y = 0{\cdot}0110x + 0{\cdot}045$
Control	$y = 0{\cdot}0108x$

y = absorbance at 432 nm (corrected for blank values in blood and urine); and x = concentration of degraded ι-carrageenan/mg per 100 ml. Blood and urine controls were aqueous solutions of degraded ι-carrageenan, treated in a similar manner to plasma and charcoal-treated urine test samples.

Reproducibility—In aqueous solution, the coefficient of variation of the absorbance at 432 nm (for a fifteen-sample series) was not more than 6·6 per cent. at degraded carrageenan concentrations of 1 to 2 mg per 100 ml, and not more than 5·5 per cent. at higher concentrations. In blood, the coefficient of variation of the absorbance at 432 nm was not more than 6 per cent. at a degraded carrageenan concentration of 20 mg per 100 ml of blood (five-sample series). The reproducibility of the results for urine was less than that in aqueous solution or in blood because of the difficulty of removing all interfering substances from the samples. Coefficients of variation at degraded carrageenan concentrations in the range from 3 to 45 mg per 100 ml of urine were 5 to 11 per cent. In a six-sample series containing eight to twelve different concentrations of degraded carrageenan, the correlation coefficients between the absorbance at 432 nm and concentration were not less than 0·994.

RESORCINOL METHOD—

While the results of Yaphe and Arsenault[7] were, in general, confirmed for fructose and for 3,6-anhydro-D-galactose, it was found that the development of the measured absorbance peak at 555 to 558 nm was time dependent and that it occurred after formation of another peak, at 515 to 520 nm, which decreased as the size of the measured peak increased. Further, significant deviations from Beer's law at 555 to 558 nm occurred at the upper concentration ranges for degraded iota-carrageenan, 16 to 96 mg per 100 ml, although these deviations could be decreased by adjusting the heating time for colour development from 10 to 15 minutes and increasing the 1,1-diethoxyethane concentration from 2·56 to 12·9 μmol per 100 ml of reagent.

Further modifications to the 1,1-diethoxyethane concentration and heating time were required for carrageenan in urine and unacceptably high blank values could not be overcome when determining carrageenan in blood. Also, for fructose, 3,6-anhydro-D-galactose and carrageenan, a single concentration of 1,1-diethoxyethane did not yield maximum colour development at 555 to 558 nm over the entire range of concentrations investigated. Excess or insufficient 1,1-diethoxyethane caused distortion of the spectra and lowering of the absorbance peak.

DISCUSSION

The use of 2-thiobarbituric acid provides the basis for a method for the determination of 3,6-anhydro-D-galactose and polysaccharides that contain this sugar. Such determinations have previously involved the use of resorcinol reagents, which are believed to form coloured condensation products with 5-hydroxymethyl-2-furaldehyde or its derivative, laevulinic acid, resulting from hydrolysis of the polysaccharide and ring rearrangement of the liberated 3,6-anhydro-D-galactose. We have found, however, that the resorcinol method is unsuitable for routine direct application in the determination of carrageenan containing 3,6-anhydro-D-galactose both in aqueous solution and in biological fluids because of spectral distortion effects that result from the varying 1,1-diethoxyethane requirements for optimal colour development of a variety of test substance concentrations and also because of high blank values. Recoveries of carrageenan from urine were varied (140 per cent. at 10 mg per 100 ml to 70 per cent. at 120 mg per 100 ml) and the method was found to be virtually inapplicable to assays in blood samples.

The proposed method, involving the use of 2-thiobarbituric acid, avoids the difficulties associated with the resorcinol method because measurements are made with reference to a single-peak spectrum and Beer's law is obeyed over the entire ranges of direct application for both 3,6-anhydro-D-galactose and sulphated polysaccharides containing this sugar, in aqueous solution, blood and urine.

The method shows high specificity for 3,6-anhydro-D-galactose, for carrageenan that contains this sugar and for fructose (Table III). The low absorbances yielded by a number of monosaccharides are comparable with those found for resorcinol,[7] indicating a corresponding lack of interference in the 2-thiobarbituric acid method. In calculating the relative molecular mass of the carrabiose unit used for comparison of these sugars, the minimum theoretical value was used and thus the results given in Table III show the maximum interference in the assay that could be caused by these sugars. In addition, colour development in the 2-thiobarbituric acid method by the sulphated polysaccharides heparin and chondroitin sulphate is less than 0·5 per cent. of that developed by corresponding amounts of carrageenan containing 3,6-anhydro-D-galactose. Neither of these polysaccharides contains 3,6-anhydro-D-galactose but both might easily cause confusion in the determination of any one sulphated polysaccharide in biological fluids when other methods of assay are used.

Fructose develops a strong colour with thiobarbituric acid and might be expected to cause interference, especially in blood and urine. However, when using the pre-treatments described, the interference due to normal levels of all substances is reduced to a value that is widely accepted as being unavoidable in a colorimetric assay of biological materials. In any experiment in which fructose may occur together with carrageenan, treatment of the sample by heating it with 4 N sodium hydroxide solution prior to assay with 2-thiobarbituric acid will destroy any fructose present without affecting the assay.

Chloral hydrate has recently been reported[13] as developing a colour when treated with thiobarbituric acid under mild, non-acidic conditions (pH 9·5). We have found, however,

that this substance does not interfere in the assay of carrageenan with thiobarbituric acid.

Methods that have been used for the determination of carrageenan, which involve the use of cationic dyes, for example toluidine blue, are less suited to the determination of small amounts as toluidine blue is a general reagent for macro-anions in acidic solution, which leads to low specificity and susceptibility to interference from other macro-anions, when such methods are applied to complex materials. Methods that involve the precipitation and isolation of the sulphated polysaccharide prior to infrared examination and determination with toluidine blue,[14] apart from incorporating a positive identification step rendered necessary by the non-specificity of the test, are tedious, tend to be subject to substantial quantitative error and require relatively large sample volumes (20 ml of blood; 10 ml of urine) that are impractical when the experiment calls for small laboratory animals, which may be required to survive the experiment.

The need for a rapid, reliable method for the determination of carrageenan containing 3,6-anhydro-D-galactose in the biological fluids of large numbers of small animals has recently intensified, not only as a result of the unknown metabolism of carrageenans and recognition of their toxicity, but also because they find continued use in biological investigations[9,10] and in foodstuffs. The method described, involving the use of 2-thiobarbituric acid, is suitable for such investigations, which have hitherto been hindered by the lack of a suitable assay technique.

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Determination of Trace Amounts of Lead in Steel and Cast Iron by Atomic-absorption Spectrometry with the Use of Carbon Furnace Atomisation

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A method is described for the determination of 1 to 150 p.p.m. of lead in steel and cast iron involving the use of atomic-absorption spectrometry and carbon furnace atomisation. Samples are dissolved in nitric or perchloric acid and analysed directly without pre-concentration.

The determination of levels of lead greater than 0·01 per cent. in steels can be performed satisfactorily by atomic-absorption spectrometry using flame atomisation.[1,2] However, at lower levels the method lacks sensitivity and a Study Group set up by the Chemical Analysis Committee of BISRA preferred a method based on solvent extraction and spectrophotometric determination with dithizone, and this method is now adopted as the British Standard Method (BS 1121).[3] Various atomic-absorption procedures have been developed for the determination of low levels of lead in steel and these procedures have been reviewed by Scholes[2] and by Hofton and Hubbard.[4] In all instances, complex solvent extraction procedures have been found necessary and the time of analysis can be very long. The presence of small amounts of lead can cause hot rupture during the rolling and forging of stainless steels and can cause the formation of a graphite structure known as Widmanstatten graphite, which may lead to catastrophic failure of cast irons. There is therefore a need for a rapid method for the determination of lead in steels and cast irons in the range 0·0001 to 0·01 per cent. Recently, colleagues at the University of Strathclyde[5] have developed a rapid method for determination of lead at these levels based on anodic stripping voltammetry. The great sensitivity of this technique makes possible the direct analysis of solutions of steels with interference only from high concentrations of copper and molybdenum.

Owing to the small sample size required, carbon furnace and carbon filament atomisation provide a considerable improvement in absolute sensitivity over flame atomisation for all elements.[6] However, Fernandez and Manning[7] also demonstrated an improvement in the limit of detection in terms of concentration by a factor of 50 for the determination of lead by carbon furnace atomisation compared with flame atomisation. It seemed possible, therefore, that this technique might also offer a rapid method for the determination of lead in steel and cast iron, provided that the problems of the matrix could be overcome. Interferences have been reported[7,8] in the determination of lead by this technique but it has been found[8] that these are serious only when the solutions are prepared in chloride media. Provided that the lead solutions are prepared in oxy-anion media, *e.g.*, nitric acid, there is no interference from large amounts of iron and other metals.[8] By using these conditions, we have developed a simple and rapid method for the determination of small amounts of lead in steel and cast iron.

Experimental

Reagents—

Reagents of the highest available purity were used throughout.

Stock lead solution (100 *p.p.m. of lead*)—Dissolve 0·16 g of analytical-reagent grade lead nitrate in water, transfer the solution to a 1-litre calibrated flask and dilute to the mark with water and sufficient AnalaR nitric acid to make the final solution 10^{-2} M in nitric acid.

Iron solution (10 000 *p.p.m.*) *for interference studies*—Dissolve 0·5 g of BCS 149/3 iron in 10 ml of 40 per cent. nitric acid, transfer the solution into a 50-ml calibrated flask and dilute to the mark with water.

APPARATUS—

The instrument used for all measurements was a Perkin-Elmer 306 atomic-absorption spectrometer equipped with an HGA-70 heated graphite atomiser and a deuterium arc background corrector and coupled to an Electronik 19 strip-chart recorder. A Perkin-Elmer Intensitron hollow-cathode lamp was used as the source.

The design and operation of the HGA-70 has been described in detail elsewhere.[7,9] Samples are atomised in a graphite tube, 5·3 cm long and 1 cm in diameter, under an argon atmosphere. Samples were transferred to the centre of the tube by means of 50 or 20-μl Eppendorf micropipettes. The HGA-70 has variable time and temperature selectors for sequentially drying, charring and atomising the samples and, once set, this sequence of operations proceeds automatically. In a typical lead determination, the sample or standard is introduced into the centre of the tube and is then dried at 100 °C. Samples can then be charred at an intermediate temperature of 490 °C and are finally atomised at 2200 °C. Only the atomic-absorption signal obtained during the atomisation period is recorded, as shown in Fig. 1. Both the time of drying and the volume of sample introduced into the tube are varied according to the sample being analysed.

PROCEDURE A: MILD STEELS AND CAST IRONS IN THE RANGE 0·0001 TO 0·0010 PER CENT. OF LEAD—

(*i*) *Preparation of calibration solutions*—Dilute 10 ml of stock lead solution (100 p.p.m.) to 1 litre with water. This solution should be freshly prepared every day. Transfer 0, 1·0, 2·0, 3·0, 4·0 and 5·0 ml of this solution into 100-ml PTFE beakers each containing 0·5 g of BCS 149/3 iron and add 10 ml of 40 per cent. nitric acid. When the iron has dissolved, transfer the solutions into 50-ml calibrated flasks and dilute each solution to the mark with water. The solutions contain the equivalent of 0, 0·0002, 0·0004, 0·0006, 0·0008 and 0·0010 per cent. of lead in steel when 0·5 g of steel sample is used to prepare 50 ml of solution.

(*ii*) *Preparation of sample solutions*—Weigh 0·5 g of sample into a 100-ml PTFE beaker and dissolve it in 10 ml of 40 per cent. nitric acid. Transfer the solution to a 50-ml calibrated flask and dilute to the mark with water.

For the analysis of solutions, follow procedure E, below.

PROCEDURE B: MILD STEELS AND CAST IRONS IN THE RANGE 0·0010 TO 0·010 PER CENT. OF LEAD—

(*i*) *Preparation of calibration solutions*—Dilute 10 ml of stock lead solution (100 p.p.m.) to 100 ml with water. Prepare this solution freshly every day. Transfer 0, 1·0, 2·0, 3·0, 4·0 and 5·0 ml of this solution into 100-ml PTFE beakers, each of which contains 0·5 g of BCS 149/3 iron, and add 10 ml of 40 per cent. nitric acid. When the iron has dissolved, transfer the solutions into 50-ml calibrated flasks and dilute to the mark with water. These solutions contain the equivalent of 0, 0·0020, 0·0040, 0·0060, 0·0080 and 0·0100 per cent. of lead in steel when 0·5 g of steel sample is used to prepare 50 ml of solution.

(*ii*) *Preparation of sample solutions*—Proceed as in procedure A (*ii*) above. For the analysis of solutions, follow procedure E, below.

PROCEDURE C: MILD STEELS IN THE RANGE 0·0100 TO 0·0150 PER CENT. OF LEAD—

(*i*) *Preparation of calibration solutions*—Transfer by microburette 0, 0·50, 0·60, 0·70 and 0·80 ml of the stock lead solution (100 p.p.m.) into 100-ml PTFE beakers, each of which contains 0·5 g of BCS 149/3 iron, and add 10 ml of 40 per cent. nitric acid. When the iron has dissolved, transfer the solutions into 50-ml calibrated flasks and dilute to the mark with water. These solutions, when diluted five-fold, contain the equivalent of 0, 0·0100, 0·0120, 0·0140, 0·0160 per cent. of lead in steel when 0·5 g of sample is used to prepare 250 ml of solution.

(*ii*) *Preparation of sample solutions*—Proceed as in procedure A (*ii*) above. Before injection of the sample, dilute each solution five-fold.

For the analysis of solutions, follow procedure E, below.

PROCEDURE D: STAINLESS STEELS IN THE RANGE 0·0010 TO 0·0100 PER CENT. OF LEAD—

(*i*) *Preparation of calibration solutions*—Dilute 10 ml of the stock lead solution (100

p.p.m.) to 100 ml with water. This solution should be freshly prepared each day. Transfer 0, 1·0, 2·0, 3·0, 4·0 and 5·0 ml of this solution into 100-ml PTFE beakers, each of which contains 0·5 g of BCS 149/3 iron, and add 10 ml of 60 per cent. *m/m* perchloric acid. When the iron has dissolved, transfer the solutions into 50-ml calibrated flasks and dilute to the mark with water. These solutions contain the equivalent of 0, 0·0020, 0·0040, 0·0060, 0·0080 and 0·0100 per cent. of lead when 0·5 g of steel sample is used to prepare 50 ml of solution.

(*ii*) *Preparation of sample solutions*—Weigh 0·5 g of sample into a 100-ml PTFE beaker and dissolve it in 10 ml of 60 per cent. *m/m* perchloric acid. Transfer the solution into a 50-ml calibrated flask and dilute to the mark with water.

For the analysis of solutions, follow procedure E, below.

PROCEDURE E: OPERATION OF THE INSTRUMENT—

The instrument is operated under the following conditions:

	Procedure		
	A	B, C	D
Wavelength/nm	283·3	283·3	283·3
Lamp current/mA	8	8	8
Spectral band width/nm	0·7	0·7	0·7
Drying temperature/°C	100	100	100
Drying time/s	40	30	40
Charring temperature/°C	—	—	490
Charring time/s	—	—	30
Atomisation temperature/°C	2200	2200	2200
Atomisation voltage/V	8	8	8
Atomisation time/s	10	10	10
Volume of sample solution/μl	50	20	50
Scale expansion	×3	×1	×3
Argon flow-rate/l min^{-1} (at 40 p.s.i.)	1·5	1·5	1·5

Sequentially inject samples and standards into the graphite tube and record the atomic-absorption signal during the atomisation step. Interpolate sample concentrations from a calibration graph obtained from the standards.

RESULTS AND DISCUSSION

INTERFERENCES IN THE DETERMINATION OF LEAD—

Studies[7,8] on interferences in the determination of lead by atomic-absorption spectrometry using carbon furnace atomisation have indicated that elements such as sodium, iron, calcium and aluminium depress the lead signal when solutions are prepared in chloride media. No interference was found, however, when solutions were prepared in chloride-free media by using nitrate salts and nitric acid. This procedure evidently prevents the volatilisation of lead as a molecular chloride. Use of an oxy-anion medium leads to the formation of the relatively involatile oxides and recent evidence[10] suggests that these oxides are efficiently reduced by the carbon from the graphite tube, liberating metal atoms directly in the gaseous state.

In the determination of lead in steel at levels down to 0·0001 per cent., a ratio of 10^6 would exist between iron and lead concentrations. No interference from 10 000 p.p.m. of iron was found on the signal of 0·01 p.p.m. of lead in nitrate media under the conditions described in procedure E. It would therefore be possible to analyse solutions of cast iron and steel by direct comparison with standard solutions that contain only lead in the appropriate solvent. However, a smoke signal is given by the iron matrix after the atomic-absorption signal of lead, and although the effect of this signal is removed by the background corrector, it was decided, as a precautionary measure, to add the appropriate concentration of pure iron (BCS 149/3) to the calibration solutions to match that in the samples.

DETERMINATION OF LEAD IN STEELS AND CAST IRONS—

The above procedures were applied to the determination of lead in a range of standard steels and cast irons. Undissolved silica and carbon were allowed to settle to the bottom

of the calibrated flask before withdrawing the appropriate aliquot for analysis, but the solution could be filtered if required. In our view, analysis at the levels described should be made as simple as possible so as to avoid possible sources of contamination. The results are shown in Table I. Results obtained by anodic stripping voltammetry[5] are also given for comparison.

TABLE I

DETERMINATION OF LEAD IN STEELS AND CAST IRONS

	Lead, per cent.		
Sample	Certificate value*	Results by procedure indicated	Anodic stripping[5]
D1 (cast iron)	0·00012	A: 0·00013, 0·00010 0·00014, 0·00015 0·00015	—
D2 (cast iron)	0·0028	B: 0·0031, 0·0029 0·0027, 0·0028 0·0029	0·0022
D5 (cast iron)	0·0004	A: 0·00035, 0·00037 0·00037, 0·00036 0·00036, 0·00033	0·0004
D6 (cast iron)	0·0022	B: 0·0024, 0·0025 0·0024, 0·0023 0·0024	0·0024
D7 (cast iron)	0·0038	B: 0·0039, 0·0041 0·0042, 0·0041 0·0040	0·0038
D8 (cast iron)	0·0018	B: 0·0018, 0·0020 0·0018, 0·0019 0·0017	0·0017
D9 (cast iron)	0·0070	B: 0·0070, 0·0077 0·0069, 0·0078 0·0075	0·0066 to 0·008
BCS 330 (mild steel) ..	0·003	B: 0·0025, 0·0026 0·0028, 0·0026 0·0028, 0·0026	0·0022
BCS 326 (mild steel) ..	0·014	C: 0·015, 0·014 0·014, 0·015 0·014, 0·015	0·013
BCS 328 (mild steel) ..	0·015	C: 0·014, 0·015 0·016, 0·015 0·015, 0·015	0·015
BCS 334 (stainless steel) ..	0·0011	D: 0·0010, 0·0010 0·0009, 0·0009 0·0010	0·0010
BCS 335 (stainless steel) ..	0·0015	D: 0·0015, 0·0014 0·0013, 0·0013 0·0013	0·0013

* The cast iron samples were provided by the British Cast Iron Research Association, Blantyre, Nr. Glasgow and the certificate values for these samples are the analytical results supplied by them which were obtained by a spectrophotometric method. We are grateful to Mr. J. Sneddon for providing these samples and results.

The results obtained by atomic-absorption spectrometry are averages from three measurements on each solution. Typical results for sample BCS 326 and a 0·00014 per cent. lead standard are shown in Fig. 1. The results suggest that the method would be satisfactory for the determination of lead in steel and cast iron. A larger range of results was obtained for sample D9 and a similar range was obtained by anodic stripping voltammetry, probably indicating that this sample is inhomogeneous. Ten samples can be analysed in approximately 1 hour, most of this time being taken up in dissolution of the sample; a single sample can be analysed in about 15 minutes.

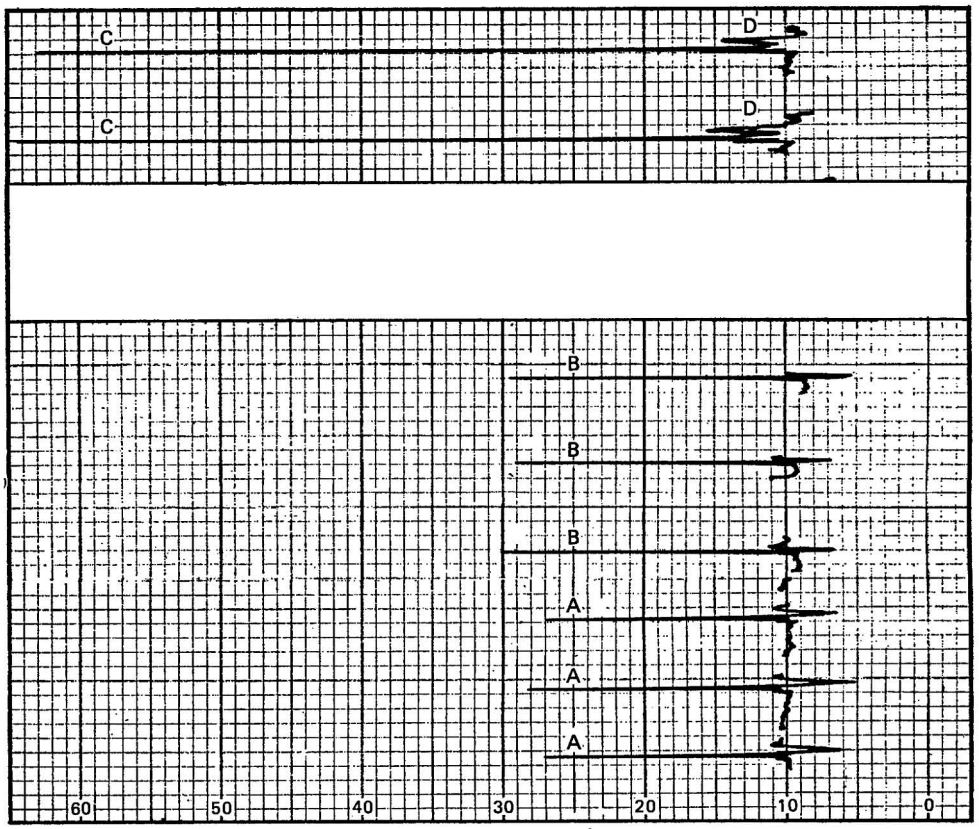

Fig. 1. Atomic-absorption signals for solutions prepared as in procedure A for (A) a standard solution containing the equivalent of 0·0001 per cent. of lead, *i.e.* 0·01 p.p.m. of lead, in 10 000 p.p.m. of iron (signal includes a small lead blank from BCS 149/3); (B) sample D1 (both A and B at × 3 scale expansion); and (C) a standard solution containing 0·01 p.p.m. of lead and 10 000 p.p.m. of iron at × 10 scale expansion showing a small residual smoke smoke signal at D

The reproducibility of the method was tested in two ways (Table II). The reproducibility of the instrument was tested by carrying out ten readings on the same sample solution. The reproducibility of the method as a whole was then tested at two concentration levels, by carrying out ten complete analyses of two standard steel samples. The results in Table II indicate that the main contribution to the standard deviation is from the instrument reproducibility, the relative standard deviation of sample D1 being greater than that of sample BCS 330 as a × 3 scale expansion was used for D1 and no scale expansion for BCS 330. At the concentration levels being determined, the precision is considered to be adequate. There appear to be small differences between the results on samples BCS 330 and D1 in Tables I and II. However, calculations involving all of the results for these samples only increase the relative standard deviations to 4·2 per cent. for BCS 330 and 13·6 per cent. for D1. The detection limit (2σ) and sensitivity (1 per cent. absorption) for the lowest concentration range, procedure A, were found to be 0·000 04 and 0·000 008 per cent. of lead, respectively. At this lowest concentration range, a blank was detected due to lead in the pure iron added to the standard solutions, and this blank was subtracted from the readings on the standard solutions.

As mentioned above, if samples are atomised after only a drying step, smoke is given out during atomisation but only after the lead signal has been obtained. Without the background corrector, a large background absorption signal is obtained from the smoke but

use of the background corrector effectively suppresses this signal. The small residual background signal can be seen at ×10 scale expansion in Fig. 1. Investigations designed to reduce the smoke signal by varying the charring temperature and time resulted only in loss of lead during the charring step at temperatures that were still ineffective in removing the smoke. For determinations on solutions in nitric acid, a charring step was therefore omitted. With perchloric acid, which was necessary for dissolving stainless steels, an explosive evolution of smoke occurred during atomisation if no charring step was included. A charring temperature of 490 °C effectively removed most of the smoke from these solutions without a significant loss of lead.

TABLE II

REPRODUCIBILITY TESTS IN THE DETERMINATION OF LEAD

	Instrument reproducibility, per cent. of lead	Reproducibility of separate determinations, per cent. of lead	
	BCS 330	BCS 330	D1
	0·0027	0·0025	0·00014
	0·0028	0·0026	0·00014
	0·0028	0·0024	0·00019
	0·0027	0·0025	0·00014
	0·0027	0·0026	0·00012
	0·0026	0·0026	0·00014
	0·0027	0·0025	0·00015
	0·0027	0·0026	0·00016
	0·0026	0·0025	0·00013
	0·0026	0·0024	0·00014
Mean	0·0027	0·0025	0·00015
Certificate value	0·003	0·003	0·00012
Standard deviation	0·000075	0·000082	0·00002
Relative standard deviation, per cent.	2·8	3·3	13·3
95 per cent. confidence limits, per cent. of lead	—	±0·00019	±0·000045

To our knowledge, no previous publications have described the application of carbon furnace atomisation to the determination of trace elements in iron and steel. The determination of chromium in steel by use of a tantalum filament has been reported,[11] but only at levels of chromium above 0·3 per cent. In the carbon furnace, lead appears to be released readily from the iron matrix and it would seem possible that other volatile elements could be determined by this technique. With less volatile elements, background correction would be essential and the release of the element from the iron matrix might be more difficult.

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Book Reviews

VOCABULAIRE DES AGENTS DE SURFACE, TERMS ET DÉFINITIONS (VOKABULARIUM VON TENSIDEN BEGRIFFE UND DEFINITIONEN; VOCABULARY OF SURFACE ACTIVE AGENTS, TERMS AND DEFINITIONS). (Trilingual.) 2nd Edition. Pp. xiv + 135. Paris: Comité International des Dérivés Tensio-Actifs (C.I.D.) Commission de Terminologie. 1972. Price 20F.

The second edition of this work by the Terminology Commission of the C.I.D. is welcome, but further effort must still be devoted to this field. Readers will find this work very helpful; the indexing is good for the three different languages used in the definitions. One is left, however, with the feeling that a small percentage of the definitions have received insufficient thought, are too theoretical to be helpful to the practising analyst and one or two can be misleading to those not conversant with the detergent field. For example, soap would be better defined as from a mixture of fatty acids containing 8 to 24 carbon atoms; loss of surface-active characteristics is only one requirement for a biodegradable surface-active agent; biodegradability is also referred only to loss of surface activity; procedures used to measure degree of biodegradability may assess components that have lost their surface activity and different procedures give different degrees of biodegradation; saponification products from neutral fat can also include fatty alcohols and sterols.

The text could also benefit by stating how chelating and sequestering agents differ from one another.

G. F. LONGMAN

ATLAS OF THERMOANALYTICAL CURVES. Volume 2. (TG-, DTG-, DTA-CURVES MEASURED SIMULTANEOUSLY.) Edited by G. LIPTAY. Pp. 161 (loose-leaf). London, New York and Rheine: Heyden & Son Ltd. 1973. Price £11·50; $31·65; DM94.50.

This Atlas is the latest in a useful series. The layout is slightly different from that of the first volume in that small samples heated at a slow rate were printed in the first volume on transparent sheets which could be superimposed on results for larger samples heated quickly which were printed in black on white paper. In the present volume, both plots are printed on the same sheet of white paper. To make the best use of the Atlas, it is best to read the Preface which is included in Volume 1 but omitted from Volume 2.

The results quoted without critical comment but with precision serve to illustrate the advantages and disadvantages of the combination of techniques employed. Thus to quote a few examples, the data for barium oxalate hemihydrate do not indicate that, as formed from the interaction of the soluble barium salt and oxalic acid, the precipitate may be found to have the formula $BaC_2O_4.H_2C_2O_4.2H_2O$ and not $BaC_2O_4.\frac{1}{2}H_2O$ as used in the study quoted in the volume. Furthermore, the Atlas fails to indicate the way in which the atmosphere plays a decisive part in the oxalate decompositions while other materials are relatively unaffected by the surrounding atmosphere. This is recognised in some of the coals quoted, which are also reported heat-treated in nitrogen as well as static air. All of the results are clearly presented and the notes are easy to understand. The comments made by the reviewer are only meant to indicate to possible users of the Atlas recognised limitations in the techniques and in no way to detract from the advantage offered by these two volumes in providing typical TG, DTG and DTA curves for some 125 different materials.

It should also be noted that the list of references provided is not a complete list and, indeed, this point is made in the preface to Volume 1. As an indication of possible material for further volumes, it would seem feasible to include repeats of some of the materials already quoted using atmospheres other than static air and crucibles other than platinum.

D. DOLLIMORE

THE DETERMINATION OF SULPHUR-CONTAINING GROUPS. Volume 1. THE ANALYSIS OF SULPHOXIDES, SULPHONYL HALIDES, THIOCYANATES, ISOTHIOCYANATES AND ISOCYANATES. By M. R. F. ASHWORTH. *An International Series of Monographs, No.* 2. Pp. viii + 149. London and New York: Academic Press. 1972. Price £3·20.

In this short, yet surprisingly comprehensive monograph, a chapter is dedicated to each of the sulphur groups named in the title. Curiously, isocyanates are included, although they contain no sulphur. Each chapter has a similar format, beginning with a brief description of the occurrence

and use of compounds containing the sulphur group in question, and followed by sections dealing with chemical and physical methods of determination (and, frequently, of detection) of the group. Much of the information concerning the analytical methods is presented in tabular form, and usually in chronological order, which frees the text from a great deal of unnecessary detail while retaining comprehensive coverage.

The chemical methods include descriptions of the various decomposition methods, and gravimetric, titrimetric, spectrophotometric and polarographic finishes. A number of the methods are specific for the particular functional group, but many are simply variations of general methods of sulphur determination. Usually only brief outlines of the various methods are given, but occasionally complete procedures are included. The physical methods are concerned mainly with nuclear magnetic resonance, ultraviolet and infrared spectroscopy and the various chromatographic techniques, and are generally dealt with in less detail than the chemical methods.

The book is well produced, appears to be free from typographical errors, and contains an extensive author index as well as the subject index. It should be an essential reference text for all organic analysts.

A. TOWNSHEND

MASSENSPEKTRENSAMMLUNG VON LÖSUNGSMITTELN, VERUNREINIGUNGEN, SÄULENBELEGMATERIALIEN UND EINFACHEN ALIPHATISCHEN VERBINDUNGEN. By MARGOT SPITELLER and G. SPITELLER. Pp. xiv + 243. Vienna and New York: Springer-Verlag. 1973. Price DM58; $21.50.

This is a useful compilation of the mass spectra of common solvents, gas - liquid chromatographic packings and simple compounds. It is clearly designed to aid those searching for spurious or inexplicable peaks in the display from a combined gas - liquid chromatograph - mass spectrometer system. The information is tabulated under a variety of headings so that the whole makes a very useful laboratory manual. A brief discussion of the fragmentation patterns of the spectra introduces the compilation.

D. BETTERIDGE

DYNAMIC MASS SPECTROMETRY. Volume 3. Edited by D. PRICE. Pp. viii + 340. London, New York and Rheine: Heyden & Son Ltd. 1972. Price £8·75; $21.50; DM72.

In the second volume of this series, the Editor stated that "dynamic mass spectrometry is intended to promote the growth of this technique by providing a medium for the publication of its current developments and applications," and Volume 2 did indeed try to fulfil this aim. Volume 3 is rather a mixture. It is devoted to time-of-flight mass spectrometry, but it contains two excellent long review articles on the applications of inhomogeneous oscillatory electric fields in ion physics and on the application of dynamic mass spectrometers to problems in gas analysis. Both review articles are supported by extensive bibliographies containing nearly 500 references. Much of the remainder of the book is devoted to the proceedings of a European Symposium on Time-of-Flight Mass Spectrometry held 2 years ago, and is of primary interest to present and prospective users of time-of-flight mass spectrometers. It is hard to resist the conclusion that this series is in danger of falling between two stools. If its aim is to cover systematically the development of dynamic mass spectrometry, one would think that a deliberate and larger selection of review articles (and some research papers) would be appropriate. To mix these with unselected contributions to a symposium produces a whole which is neither comprehensive nor sufficiently typical.

It is to be hoped that future volumes will include more research papers on the applications of dynamic mass spectrometers in the life sciences and space research as well as the latest developments in related topics such as chemical ionisation, gas chromatography, data handling, surface studies and other *selected* topics.

J. M. B. BAKKER

ORGANIC REAGENTS IN METAL ANALYSIS. By K. BURGER. *International Series of Monographs in Analytical Chemistry, Volume* 54. Pp. 268. Oxford, New York, Toronto, Sydney and Braunschweig: Pergamon Press. 1973. Price £5·80.

The main emphasis of the book is on the determination of trace amounts of metals (within the broadest interpretation of the word metals) in, *e.g.*, high-purity materials, although the information, as presented, should enable the analyst to provide his own "tailor-made" procedures. It may be reasoned that much of the information is already freely available elsewhere, but the initial approach on this occasion is somewhat unusual in that the book deals, in detail and expertly, with the complex chemical reactions involved, from a co-ordination chemistry viewpoint (Chapter 1).

In Chapter 2, established analytical procedures are reviewed in the light of the author's personal experience. The two main headings in this chapter are "The Methods of Application of Selective Organic Reagents," and "Some Important Organic Metal Reagents," the latter including phenanthroline and its related compounds, oximes, flavones, dithiocarbamates and arsonic acids.

Chapter 3 has about the same number of pages as each of the other two chapters, and includes Summarising Tables (*e.g.*, an alphabetical list of organic reagents with supporting details, and a similar list in which the metals appear first), References (496), Author Index and Subject Index.

Dr. Burger is Professor of Inorganic and Analytical Chemistry at the L. Eövös University in Budapest, and this book is an English translation of his 1969 Hungarian publication, supplemented by several recent references and published procedures. While the potential value of this monograph should not be underrated, current trends in, for example, instrumental methods for determining trace amounts of metals may mean that authoritative publications such as this are likely to have the greatest appeal to research workers in the more conventional fields of analysis.

W. T. ELWELL

PARAMAGNETIC LANTHANIDE SHIFT REAGENTS IN NMR SPECTROSCOPY: PRINCIPLES, METHODOLOGY AND APPLICATIONS. By J. REUBEN. *Progress in Nuclear Magnetic Resonance Spectroscopy, Volume 9, Part 1.* Pp. viii + 70. Oxford, New York, Toronto, Sydney and Braunschweig: Pergamon Press. 1973. Price £2.

Nuclear magnetic resonance and its many abilities are by now well known. Most failures to obtain the expected information arise when chemical shifts are so small that lines overlap and the spin - spin coupling pattern has complex second-order features. This has resulted in the continual progress to higher frequencies with instruments such as the expensive 220-MHz spectrometers with superconducting magnets. In 1969, C. C. Hinkley realised that the addition of paramagnetic rare earth complexes (often called shift reagents) to a solution could usually increase chemical shifts, and the idea has proved so simple and practical that over 200 papers on organic applications appeared in the next 3 years. A card-index file on these has been essentially incorporated into the main section of the book. Since the style is that of the less happy features of annual reports and the interest of individual papers relates to specific organic compounds rather than to the technique, the outcome is decidedly stodgy.

A typical reagent is $Eu(fod)_3$, where (fod) stands for the anion of the enol form of 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctane-4,6-dione. The mode of action appears to be to form complexes with the molecules of interest and the size and magnitude of the shift depend on the stability of the complex and the dominance of contact or pseudo-contact features. The stability question is covered in this text, but the rather difficult contact and pseudo-contact features, each of which can lead to shifts of either sign, are quoted rather than discussed. This is a pity as already, apparently, wrong deductions have been made by those who overlook the $(3 \cos^2 \theta - 1)$ angular term in the pseudo-contact shift or make other assumptions such as a ligand-independent g-factor anisotropy. Some mention of distance determination is made, but its reliability is not discussed. Also, it seems, the enhanced chemical shifts may make difficult quantitative analyses by nuclear magnetic resonance rather easier.

To summarise, this book is valuable as being the chief review to date in English, but for anyone who hoped for a critical review and a clear explanation of the more theoretical features of lanthanide shift reagents, it is something of a disappointment.

D. H. WHIFFEN

APPOINTMENTS VACANT

Determination of iota-Carrageenan with 2-Thiobarbituric acid

A colorimetric method for the determination of iota-carrageenan is described, based on the measurement of the yellow colour developed by the reaction of the 3,6-anhydro-D-galactose residue with 2-thiobarbituric acid and hydrochloric acid. The method has a sensitivity equivalent to 0·01 mg ml^{-1} of carrageenan and can also be used to determine carrageenan in blood and urine in concentrations of 0·02 and 0·01 mg ml^{-1}, respectively. The method has advantages of sensitivity, relative specificity, a small sample-volume requirement and convenience over previously published methods for the determination of sulphated polysaccharides, thus providing a means of extending quantitative biological investigations of this group of substances.

W. ANDERSON and W. BOWTLE

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Analyst, 1974, **99**, 178–183.

Determination of Trace Amounts of Lead in Steel and Cast Iron by Atomic-absorption Spectrometry with the Use of Carbon Furnace Atomisation

A method is described for the determination of 1 to 150 p.p.m. of lead in steel and cast iron involving the use of atomic-absorption spectrometry and carbon furnace atomisation. Samples are dissolved in nitric or perchloric acid and analysed directly without pre-concentration.

F. SHAW and J. M. OTTAWAY

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Analyst, 1974, **99**, 184–189.

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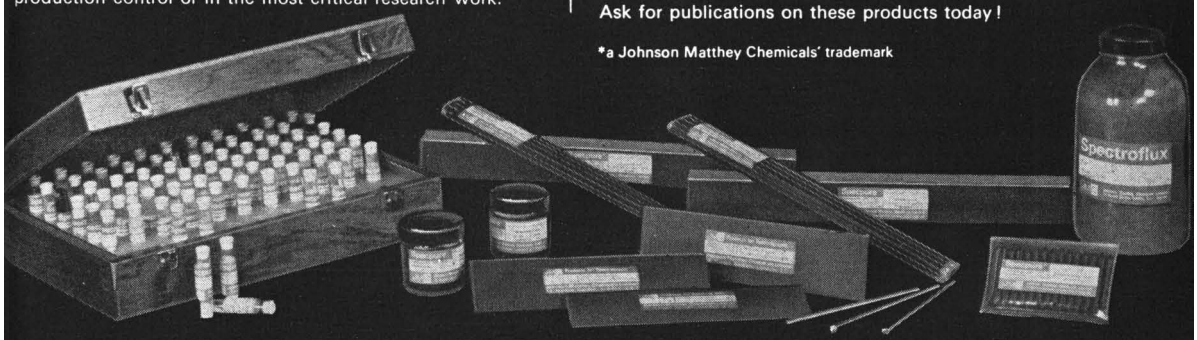

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