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Summaries of Papers in this Issue

Determination of Fluorine in Silicates by Use of an Ion-selective Electrode Following Fusion with Lithium Metaborate

The determination of fluorine in silicate materials with a fluoride ion-selective electrode following fusion with lithium metaborate is described. The method is rapid, and the values obtained compare well with accepted values for standard rock and mineral samples. Samples containing up to 2.7% of fluorine have been analysed by this method.

Keywords: *Fluorine determination*; *silicates*; *fluoride ion-selective electrode*

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Analyst, 1977, **102**, 409–413.

Determination of Total Sulphur in Naphthas

The determination of total sulphur in naphthas and other volatile hydrocarbons is reported. The method developed involves the combustion of the sample in oxygen and conductimetric high-frequency micro-titration of the sulphate ions in the absorbing solution of hydrogen peroxide with standardised barium perchlorate solution. An aliquot is prepared by controlled evaporation to dryness (~0.5 ml) and then diluted with ethanol - water mixture (3 + 2). The method has been established for samples containing 0.4–50 p.p.m. *m/m* of sulphur. Results for precision determinations are given.

Keywords: *Total sulphur determination*; *naphtha*; *petrochemicals*; *combustion*; *high-frequency titration*

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Analyst, 1977, **102**, 414–423.

Enzymatic Determination of Maltose by Amperometric Measurement of the Rate of Oxygen Depletion

An enzymatic method for the rapid, direct kinetic measurement of maltose is described. Maltose is hydrolysed to glucose by α-glucosidase, and the glucose reacts with oxygen in the presence of glucose oxidase to form gluconic acid and hydrogen peroxide. The rate of oxygen depletion is measured with a Clark oxygen electrode. The glucose oxidase reagent contains sufficient amounts of α-glucosidase impurity for a maximum reaction rate to be obtained within 2 min after addition of maltose to the reagent. The reaction rate is obtained directly by recording the derivative of the change in amperometric current. Glucose already present in samples can be destroyed by incubation with purified glucose oxidase reagent for 10 min. Fructose, lactose and galactose do not interfere. Interference from starch (constant over a wide range of starch concentrations) can be readily corrected for by adding starch to standards, and sucrose interference can be minimised by adjustment of the pH. Excellent results were obtained for the recovery of maltose from pooled serum samples.

Keywords: *Maltose determination*; *enzymatic analysis*; *oxygen electrode*; *blood serum*

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Analyst, 1977, **102**, 424–428.

Determination of Barium in Calcium Carbonate Rocks by Carbon Furnace Atomic-emission Spectrometry

A simple and rapid method is described for the determination of barium at 0.5–30 $\mu g\ g^{-1}$ concentrations in calcium carbonate and limestone rocks. The sample is dissolved in nitric acid, caesium is added as an ionisation suppressor and the resulting solution is analysed by using carbon furnace atomic-emission spectrometry with automatic background correction using wavelength modulation. Spectral interference from CaOH band structure is low in the carbon furnace compared with flame atomisation and is removed by background correction. Other matrix interferences are negligible. The reproducibility is 5.9% at the 2.4 $\mu g\ g^{-1}$ level and the detection limit is 0.036 $\mu g\ g^{-1}$ of barium in rock samples.

Keywords: *Barium determination*; *calcium carbonate rocks*; *carbon furnace atomic-emission spectrometry*

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Analyst, 1977, **102**, 429–435.

Determination of Bismuth in Steels and Cast Irons by Atomic-absorption Spectrophotometry with an Induction Furnace: Direct Analysis of Solid Samples

A graphite induction furnace has been constructed within a Perkin-Elmer 300S atomic-absorption spectrophotometer for the determination of bismuth in 2–12-mg samples of steels and irons that are dropped into the furnace. Calibration graphs of peak absorbance *versus* mass of bismuth are best constructed by use of standard alloys of roughly similar composition to the samples being analysed but, in the absence of suitable standard alloys, semi-quantitative results can be obtained if calibration graphs are prepared using small volumes of a standard bismuth nitrate solution.

Samples of alloys can be added to the furnace at 2–5-min intervals. Information is presented on the calibration graphs and on the accuracy, precision and limits of detection of the method for 40 high-purity irons and steels and for nine cast irons. With steels containing more than 0.04 $\mu g\ g^{-1}$ of bismuth, relative standard deviations of 3–10% are usually achieved. The limit of detection for bismuth in steels is 0.004 $\mu g\ g^{-1}$ when using this method.

Keywords: *Bismuth determination*; *steel and cast iron analysis*; *atomic-absorption spectrophotometry*; *induction furnace*; *direct analysis of solids*

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The Analyst

Determination of Fluorine in Silicates by Use of an Ion-selective Electrode Following Fusion with Lithium Metaborate

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The determination of fluorine in silicate materials with a fluoride ion-selective electrode following fusion with lithium metaborate is described. The method is rapid, and the values obtained compare well with accepted values for standard rock and mineral samples. Samples containing up to 2.7% of fluorine have been analysed by this method.

Keywords: *Fluorine determination*; *silicates*; *fluoride ion-selective electrode*

The determination of fluorine in silicate rocks has been one of the most difficult and time-consuming procedures in the analysis of rocks and minerals. The introduction of the steam distillation method of Willard and Winter[1] revolutionised the separation and determination of fluorine. As use of the method was expanded, many modifications were proposed, such as those by Ingamells.[2] Several colorimetric methods have been introduced[3–6] that eliminated the tedious thorium nitrate titration used in the steam distillation method. Pyrohydrolysis separation methods have been introduced that incorporate the use of either colorimetric reagents[7] or a fluoride ion-selective electrode[8] in the determination step.

The development of the fluoride ion-selective electrode by Frant and Ross[9] resulted in many new procedures for the determination of fluorine in rocks and minerals.[10–17] Proposed methods combine fusion of the samples with various fluxes and solution in various acids, followed by the determination of fluoride with an ion-selective electrode. These methods offer significant improvements over the older steam distillation methods that employ titration or colorimetry, but some separation, filtration or leaching steps are required, which restrict the handling of large numbers of samples that are often encountered in geological investigations.

The need for a rapid, reliable fluorine determination led to the development of a procedure that combines the versatile lithium metaborate - nitric acid dissolution technique[18–21] with the fluoride ion-selective electrode, and provides a direct, rapid and accurate method for the determination of fluorine in a wide range of silicate samples. No separation, leaching or filtration steps are required.

Experimental

Reagents

Lithium metaborate. Anhydrous $LiBO_2$.

Nitric acid, 4% *V*/*V*.

Standard fluoride solution. Heat reagent-grade sodium fluoride in a platinum crucible at a low red heat (640 °C) for 1–2 h. Cool, weigh exactly 0.2210 g, dissolve it in water and dilute to 1 l. Transfer into a polyethylene bottle for storage.

1 ml ≡ 100 μg of fluoride.

Dilute standard fluoride solution. Dilute 100.0 ml of the standard fluoride solution to 1 l and transfer into a polyethylene bottle for storage.

1 ml ≡ 10 μg of fluoride.

Complexing buffer solution. Add 18.2 g of DCTA (1,2-diaminocyclohexane-*NNN'N'*-tetraacetic acid) to 1.5 l of water and then 40% *m*/*V* sodium hydroxide solution dropwise

until the DCTA dissolves. Add 300 g of sodium citrate dihydrate and 60 g of sodium chloride, adjust the pH to 6.85 with hydrochloric acid and dilute to 2 l with water. This solution is approximately 0.5 M in citrate, 0.5 M in sodium chloride and 0.03 M in DCTA. The addition of 50 ml of this buffer solution to the sample aliquot produces a final pH of approximately 5.5.

Instrumentation

An Orion 801–A, line-operated specific ion meter, with an Orion 94–09 fluoride ion activity electrode and an Orion 90–02 double junction reference electrode were used for all determinations. Orion 90–00–02 inner filling solution and Orion 90–00–03 (10% potassium nitrate) outer filling solution were used in the reference electrode. All measurements were made on solutions stirred with polyethylene-coated magnetic stirring bars.

Sample Dissolution

Grind the sample to pass a 200-mesh sieve (ASTM), and mix 200 mg of the powder with 800 mg of anhydrous lithium metaborate. Transfer the mixture to a pre-ignited graphite fusion crucible and place it in a muffle furnace at 1050 °C for 10 min. Pour the molten material into a polypropylene beaker containing 80.0 ml of 4% *V*/*V* nitric acid. Cover the beaker and place it on a magnetic stirrer until the sample is completely dissolved (usually 5–10 min). Transfer the solution quantitatively into a 100-ml calibrated flask and dilute to volume to obtain a solution containing 2 mg of sample per millilitre.

Transfer a 40.0-ml aliquot (0.080 g of sample) into a 150-ml beaker containing a magnetic stirring bar and add 10.0 ml of water and 50.0 ml of the complexing buffer solution.

Fluoride Measurement

The concentration of fluoride in the sample solution is determined with the fluoride ion-selective electrode by the method of standard addition in which the change of potential resulting from the addition of a known volume of standard fluoride solution to the sample solution is used to determine the fluoride concentration in the sample solution.

The sample solution is stirred for 5 min to reach a steady potential reading before the addition of 10.0 ml of the 10 μg ml^{-1} standard fluoride solution. The solution is again stirred for 5 min after the addition of the standard fluoride solution.

The procedure can accommodate fluoride concentrations up to 0.4%. Higher concentrations are determined by taking smaller sample aliquots or increasing the concentration of the standard fluoride solution in the standard addition step while lower concentrations are determined by decreasing the concentration of the added standard fluoride solution.

Results and Discussion

Initially in this study, fusions were performed in covered platinum crucibles over gas burners and low results were consistently obtained on standard rock samples. As the interference of aluminium in the determination of fluoride is well known, several complexing and buffering solutions were investigated but no significant improvement in the results was obtained.

The loss of fluorine by volatilisation during the fusion was considered as a possible ex-

TABLE I

COMPARISON OF RECOVERIES OF FLUORINE FROM GRANODIORITE GSP-1 WITH LITHIUM METABORATE FUSIONS CARRIED OUT WITH GAS BURNER AND ELECTRIC MUFFLE FURNACE

Length of fusion period/min	Fluorine content, %	
	Gas burner	Electric muffle furnace
5	0.348	0.348
10	0.310	0.356
15	0.308	0.350
20	0.286	0.352
25	0.252	0.350

planation for the low recoveries. Fusions were then made in both graphite and platinum crucibles in an electric muffle furnace by the recommended procedure and the length of fusion time varied from 5 to 25 min. Identical amounts of fluorine were found over the different periods of fusion time and the recoveries were in good agreement with accepted values, suggesting that the material of the crucible was not a factor in the loss of fluorine.

TABLE II

COMPARISON OF RESULTS OBTAINED FOR FLUORINE BY THE PROPOSED METHOD AND BY THE DISTILLATION - TITRATION METHOD

Samples obtained from S. S. Goldich, Geology Department, Northern Illinois University, DeKalb, Ill., USA.

	Fluorine content, %	
Sample	Proposed method	Titrimetric method*
R-765	0.348, 0.348	0.35
R-852	0.144, 0.151	0.146
R-896	0.234, 0.227	0.241, 0.237
R-908	0.170, 0.172	0.179
R-2547	0.288, 0.292	0.291

* These results were obtained in the former Rock Analysis Laboratory, University of Minnesota, Minneapolis, Minn., USA.

A parallel set of samples were then fused in covered platinum crucibles over a gas burner and the fusion time was varied from 5 to 25 min. Results obtained for fluorine from a set of fusions of the sample Granodiorite GSP-1 are compared in Table I. It is evident that in the fusion with lithium metaborate over a gas burner, fluorine is lost. No explanation is offered for this loss.

The results obtained for fluorine on a series of rock samples previously analysed by the Willard - Winter distillation - thorium nitrate titration method at the former Rock Analysis

TABLE III

COMPARISON OF RESULTS OBTAINED FOR FLUORINE IN VARIOUS STANDARD ROCKS BY PROPOSED METHOD WITH ACCEPTED VALUES

			Fluorine content		
Source	Material	Designation	Proposed method, p.p.m.	Accepted values, p.p.m.	Reference
USGS*	Granite	G-1	690	690	22
	Diabase	W-1	240	250	22
	Peridotite	PCC-1	20	15	23
	Dunite	DTS-1	20	15	23
	Andesite	AGV-1	430	435	23
	Basalt	BCR-1	510	470	23
	Granite	G-2	1310	1290	23
	Granodiorite	GSP-1	3600	3200	23
			Proposed method, %	Accepted values, %	
CRPG†	Granite	GH	0.321	0.30	24
	Granite	GA	0.054	0.05	24
	Granite	GR	0.099	0.10	24
	Basalt	BR	0.109	0.10	24
	Biotite	Mica-Fe	1.58	1.56	24
	Phlogopite	Mica-Mg	2.72	2.70	24

* United States Geological Survey, Reston, Va., USA.
† Centre de Recherches Petrographiques, Nancy, France.

TABLE IV

COMPARISON OF RESULTS OBTAINED FOR FLUORINE BY THE PROPOSED METHOD AND OTHER METHODS

		Fluorine content, %				
Sample	Description	Proposed method	Method 1*	Method 2†	Method 3‡	Accepted values§
1E/S1	Quartz monzonite	0.133 0.133	0.129	0.129	0.137	0.12
2F/MC17	Migmatite	0.099	0.109	0.100	0.093	0.09
3E/P1	Tourmaline granite	0.240 0.240 0.239	0.249 0.251 0.256	0.245	0.250	0.24
4S/T1	Tonalite	0.075 0.078	0.077 0.079 0.076	0.062	0.072	0.07
5G/G1	Nepheline syenite	0.056	0.062	0.053	0.048	0.05
6I/C2	Hornfels	0.069	0.064	0.063	0.059	0.06
7S/A1	Eucrite	0.004 0.003 0.004	0.000 0.002	0.002	0.005	0.01
8E/M1	Diabase	0.055	0.053 0.057	0.054	0.056	0.05
9E/H1	Marine shale	0.080	0.089 0.086 0.091	0.081	0.085	0.07
10I/C1	Semi-pelitic schist	0.084	0.096	0.075	0.078	0.08

* Method 1. Modified Willard - Winter distillation with thorium nitrate titration.
† Method 2. Modified Willard - Winter distillation with fluoride ion-selective electrode potentiometric measurement.
‡ Method 3. Leaching method with colorimetric determination with xylenol orange.
§ Accepted values given by Ingamells, Table I.[2]

Laboratory, University of Minnesota, are compared with those obtained by the proposed method in Table II. The results obtained for fluorine in some standard rock samples are compared with the "accepted" values in Table III. In addition, samples (as described in Table II, reference 2) were independently analysed by different analysts, each using a

TABLE V

VALUES OBTAINED BY THE PROPOSED METHOD FOR FLUORINE CONTENT OF NEW STANDARD SAMPLES

Source	Material	Designation	Fluoride content by proposed method, %	Reference
CRPG	Diorite	DR-N	0.066	24
	Serpentine	UB-N	0.009	24
	Disthene	DT-N	0.005	24
	Bauxite	BX-N	0.088	24
	Granite	GS-N	0.092	25
	Feldspar	FK-N	0.004	25
CCRM*	Gabbro	MRG-1	0.028	26
	Syenite	SY-2	0.53	26
	Syenite	SY-3	0.71	26
NIM†	Granite	NIM-G	0.423	27
	Dunite	NIM-D	0.015	27
	Norite	NIM-N	0.014	27
	Pyroxenite	NIM-P	0.014	27
	Lujavrite	NIM-L	0.430	27
	Syenite	NIM-S	0.017	27

* Canadian Certified Reference Materials Project, Energy, Mines and Resources Branch, Ottawa, Ontario, Canada.
† National Institute for Metallurgy, Aukland Park, South Africa.

different technique, and also by the proposed ion-selective electrode procedure. The results are presented in Table IV. Table V contains values for fluorine in a number of international standard rock and mineral samples for which few or tentative results have been published.

Mean values, standard deviations and coefficients of variation for four USGS standards are listed in Table VI. These values were obtained from individual determinations performed routinely over a period of several months. The results indicate that the proposed method is capable of providing good reproducibility on a routine basis. The mean values reported in Table VI are slightly different from the values reported in Table III, which were obtained on a single sample examined as a special project.

TABLE VI

PRECISION DATA OF PROPOSED METHOD

Sample	Mean fluorine content, %	Standard deviation, %	Number of determinations	Coefficient of variation, %
W-1	0.0248	0.0018	10	7.26
BCR-1	0.0517	0.0024	10	4.64
G-2	0.1272	0.0054	16	4.25
GSP-1	0.3592	0.0084	10	2.33

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References

1. Willard, H. H., and Winter, O. B., *Ind. Engng Chem. Analyt. Edn*, 1933, **5**, 7.
2. Ingamells, C. O., *Talanta*, 1962, **9**, 507.
3. Hollingworth, R. P., *Analyt. Chem.*, 1957, **29**, 1130.
4. Peck, L. C., and Smith, V. C., *Talanta*, 1964, **11**, 1343.
5. Huang, W. H., and Johns, W. D., *Analytica Chim. Acta*, 1967, **37**, 508.
6. Sen Gupta, J. G., *Analytica Chim. Acta*, 1968, **42**, 119.
7. Bloxom, T. W., *Chem. Geol.*, 1968, **3**, 89.
8. Clements, R. L., Sergeant, G. A., and Webb, P. J., *Analyst*, 1971, **96**, 51.
9. Frant, M. S., and Ross, J. W., Jr., *Science, N.Y.*, 1956, **154**, 1553.
10. Van Loon, J. C., *Analyt. Lett.*, 1968, **1**, 393.
11. Edmond, C. R., *Analyt. Chem.*, 1969, **41**, 1327.
12. Guth, J. L., and Wey, R., *Bull. Soc. Fr. Minér. Cristallogr.*, 1969, **92**, 105.
13. Ficklin, W. H., *Prof. Pap. U.S. Geol. Surv.*, 1970, 700-C, C-186.
14. Ingram, B. L., *Analyt. Chem.*, 1970, **42**, 1825.
15. Ingram, B. L., and May, I., *Prof. Pap. U.S. Geol. Surv.*, 1971, 750-B, B-180.
16. Peters, M. A., and Ladd, D. M., *Talanta*, 1971, **18**, 655.
17. Duff, E. J., and Stuart, J. L., *Talanta*, 1975, **22**, 823.
18. Suhr, N. H., and Ingamells, C. O., *Analyt. Chem.*, 1966, **38**, 730.
19. Medlin, J. H., Suhr, N. H., and Bodkin, J B., *Atom. Absorption Newsl.*, 1969, **8**, 25.
20. Medlin, J. H., Suhr, N. H., and Bodkin, J. B., *Chem. Geol.*, 1970, **6**, 143.
21. Bodkin, J. B., *Analyst*, 1976, **101**, 44.
22. Fleischer, M., *Geochim. Cosmochim. Acta*, 1969, **33**, 65.
23. Flanagan, F. J., *Geochim. Cosmochim. Acta*, 1973, **37**, 1189.
24. de la Roche, H., and Govindaraju, K., *Bull. Soc. Fr. Céram.*, 1973, **100**, 49.
25. de la Roche, H., and Govindaraju, K., *Méth. Phys. Analyse*, 1971, **7**, 314.
26. Abbey, S., Gillieson, A. H., and Perrault, G., Canada Center for Mineral and Energy Technology, Mineral Research Program/Mineral Sciences Laboratories Report 75–132, 1975.
27. Steele, T. W., Russell, B. G., Gouvdis, R. G., Domel, G., and Levin, J., National Institute for Metallurgy (South Africa), Report 1351, 1972.

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Determination of Total Sulphur in Naphthas

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The determination of total sulphur in naphthas and other volatile hydrocarbons is reported. The method developed involves the combustion of the sample in oxygen and conductimetric high-frequency micro-titration of the sulphate ions in the absorbing solution of hydrogen peroxide with standardised barium perchlorate solution. An aliquot is prepared by controlled evaporation to dryness (~0.5 ml) and then diluted with ethanol - water mixture (3 + 2). The method has been established for samples containing 0.4–50 p.p.m. *m/m* of sulphur. Results for precision determinations are given.

Keywords: Total sulphur determination; naphtha; petrochemicals; combustion; high-frequency titration

The emphasis placed on high-purity petrochemicals makes the accurate determination of trace amounts of sulphur in naphthas used as feedstock increasingly important to the petroleum industry. The analytical control of sulphur contents below 1 p.p.m. is covered by several methods, which afford reliable determinations with difficulty.[1–4]

Sulphur compounds that are present in naphtha may include mercaptans, disulphides, free sulphur, thiophene and polysulphides. Methods for the determination of any of these individual components are available but it is the total sulphur that is of greatest interest.

Some instrumental methods determine the total sulphur content directly (*e.g.*, X-ray fluorescence and microcoulometry)[1,5] but the methods most widely used for this determination involve combustion as a previous step. The details of the procedures depend on the kind of sample that is burnt, but all of these methods have in common the oxidation of sulphur to its oxides, which are further absorbed in a suitable solution, such as hydrogen peroxide. The sulphate ions produced can be determined either gravimetrically, colorimetrically or titrimetrically.

A method that is suitable for determining very low sulphur contents has been described by Rousseau and Lerouge.[5] In this method the sample is burnt in an oxygen - hydrogen burner (Wickbold adaptation) and the sulphate ions are titrated either colorimetrically (visual or automatic determination) or conductimetrically. The apparatus required by this method is expensive and rather complicated. Recently the European Committee for Standardization (CEN),[6] in adopting a similar combustion method, has recommended conductimetric, turbidimetric, nephelometric and visual titration finishes for different sulphur contents (1–50 p.p.m.).

Within the Compañía Española de Petróleos SA (CEPSA) laboratories, combustion methods are also preferred. Preliminary tests with well known combustion lamps, such as ASTM D 1266,[7] ASTM D 2785,[8] UOP 586[9] and that especially prepared by the International Conference of Benzole Producers (ICBP),[10] have shown that the last has the following advantages over the other methods: the apparatus is of all-glass construction, thus avoiding loss of sulphur to metals and the high cost of silica; the combustion is easily controlled; direct volumetric measurement of the sample allows the combustion conditions to be stabilised before the absorption of combustion gases begins; aromatic hydrocarbons can be burnt directly without dilution with alcohol; a high combustion rate can be attained; and there is no wick to retain sulphur compounds. However, for accurate determination of the lowest sulphur contents some technical improvements to the apparatus (the burner and the combustion chamber are of new design) and the gas purification train have been found necessary.

There are several procedures for the determination of sulphate ions. Gravimetric methods prove unsuitable for low sulphur contents, but titrimetric methods, *e.g.*, that involving the use of barium perchlorate with thorin as indicator, are still satisfactory within the range 10–100 p.p.m.[11] Below 10 p.p.m. instrumental methods, such as automatic colorimetry and conductimetry, are more suitable.[5,12] Lately, a determination of sulphate by use of flame emission inhibition titration down to 0.2 p.p.m. has been reported.[13] If the sensitivity of

the measuring instrument is adequate, high-frequency conductimetry gives good precision and rapid replicate analyses.[14,15]

From the experience gained in the determination of trace amounts of chlorine in naphtha (combustion step)[16] and in the determination of sulphate using a high-frequency conductimetric method (titration step),[15] the method described in this paper was developed. It is primarily intended for the determination of sulphur contents in the range 0.4–50 p.p.m. The volume to be burnt will depend on the anticipated sulphur content (Table I). Although most of the experimental work has been carried out with naphtha, it has been shown that the method is also applicable to non-aromatic hydrocarbons of similar boiling range, such as 2,2,4-trimethylpentane and heptane, and probably to other aromatic hydrocarbons, such as toluene and xylenes. The sample is vaporised in a current of carbon dioxide and burnt in the presence of oxygen. The products of combustion are drawn through a dilute solution of hydrogen peroxide where the oxides of sulphur are converted into sulphate ions. The sulphate ions in this absorption solution are determined by high-frequency titration.

Experimental

Apparatus

The combustion section consists of a burner and the combustion chamber with its jacket and cover (Fig. 1). An intermediate adapter flask, two absorbers in series and a condenser constitute the absorber section.

Fig. 2 shows the complete assembly, including the special burette, which is detailed in Fig. 1. During operation the suction applied through a spray-trap to the system, ensuring

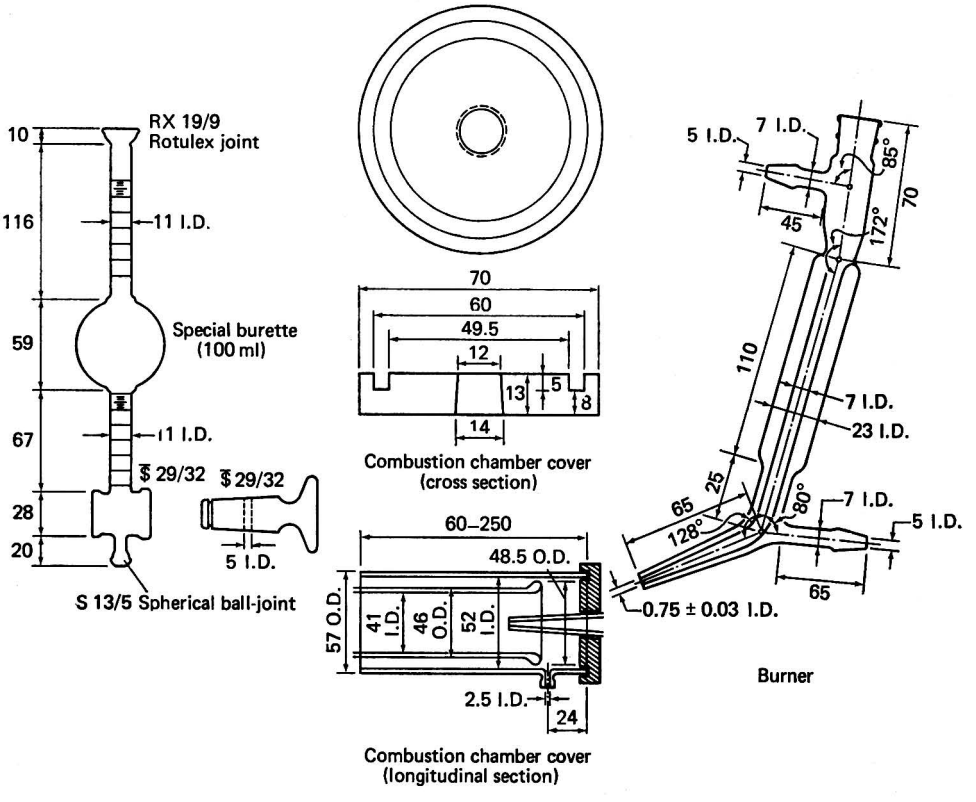

Fig. 1. Details of combustion chamber, burner and special burette construction. All dimensions in millimetres.

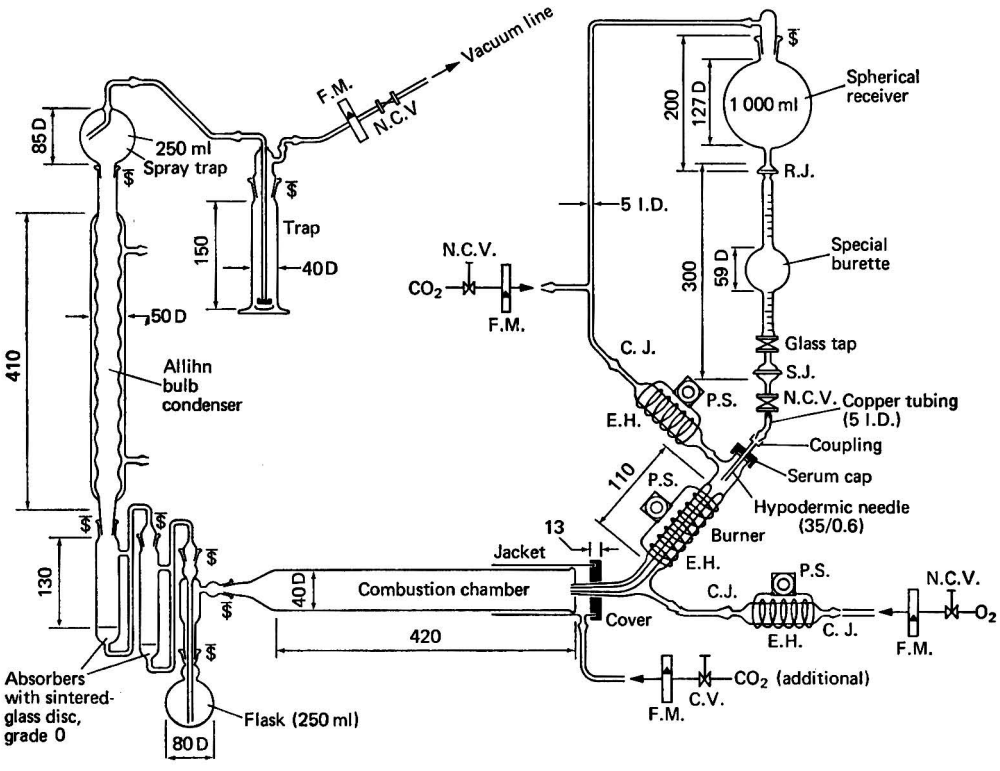

Fig. 2. Complete assembly. All dimensions in millimetres. N.C.V. = needle contro valve; F.M. = flow meter; P.S. = powerstat; E.H. = electric heater; S.J. = spherical joint; R.J. = Rotulex joint; C.J. = conventional joint; $ = conical joint (29/32).

the passage of the gases, keeps the absorbing solutions agitated. Efficient scrubbing is thus ensured.

High-frequency oscillator. This is similar to the previously reported[17] Handel Scompagnie N.V. Model HF-102A, of which the circuit diagram is shown in Fig. 3, and is equipped with a 10-ml titrimetric cell. This cell, with a working frequency of 6 MHz, permits the determination of sulphate with effective volumes of solvent as low as 6 ml.

Meter. A Hewlett - Packard digital Multimeter unit, Model 3469 B, is used as the measuring instrument. This instrument reports responses (range 1–100 μA) to an accuracy of $\pm$ 0.2%.

Burette. Agla MSO1 micrometer syringe (Burroughs Welcome and Co.) with an L-shaped glass needle.

pH Meter. Radiometer, Model PHM 28, glass - calomel combined electrode (GK 2321C).

Metal heating block. A bath in accordance with ASTM specification D381,[18] capable of being maintained at 100 $\pm$ 0.5 °C. The beaker wells are provided with aluminium rings to take 25-ml beakers (30 × 50 mm). Alternatively, the block can be replaced by a well regulated hot-plate (100 $\pm$ 5 °C) when operated by skilful operators.

Reagents

The low amounts of sulphur involved call for specialised techniques of purification and analyses such as are used in micro-analytical chemistry. The atmosphere in the room where the determinations are performed should be free from sulphur compounds. All reagents should be of the purest grade available, and especially low in sulphur as sulphate.

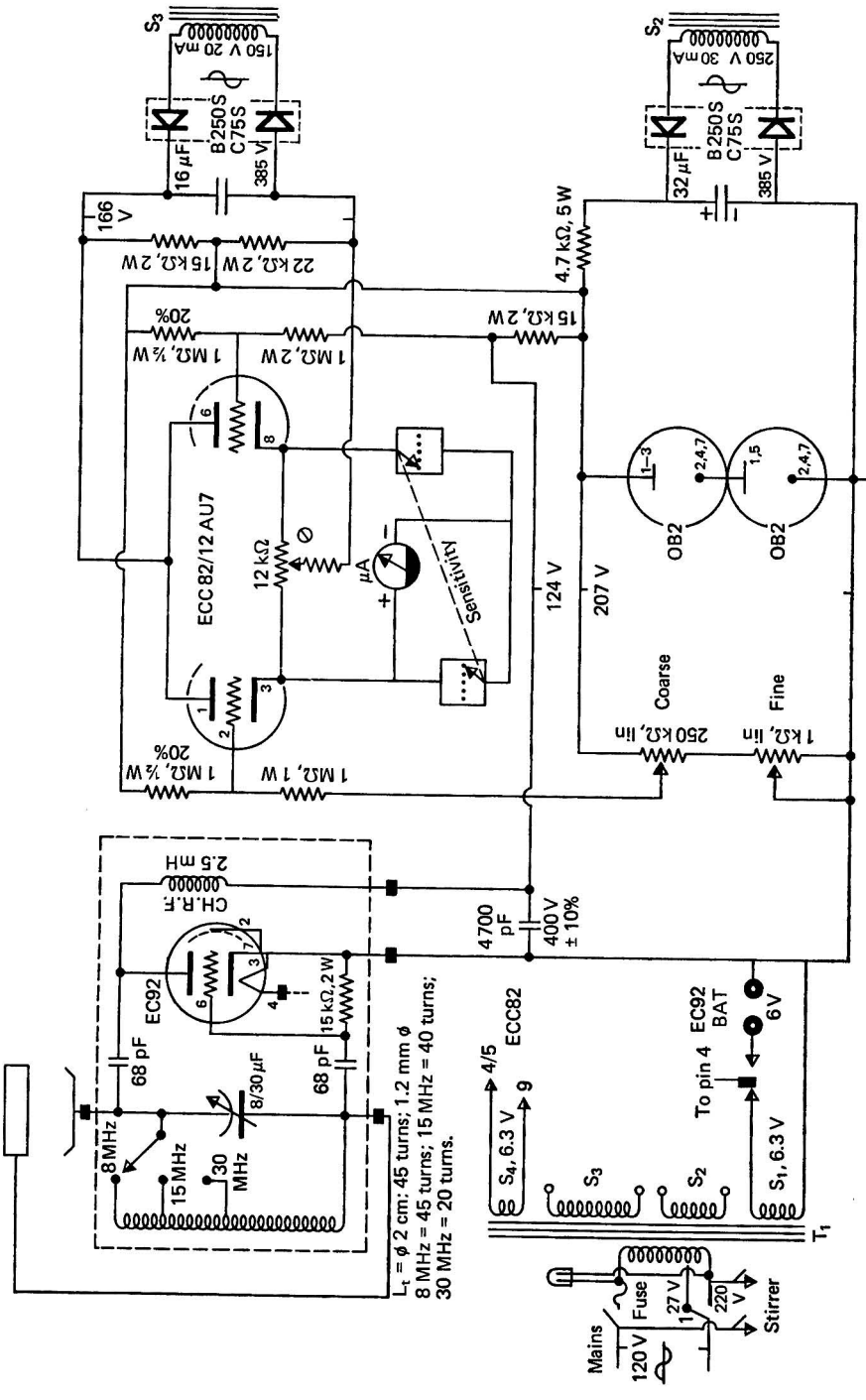

Fig. 3. Circuit diagram of high-frequency titrimeter, Model 102A.

Foreign ion free water. Water with a conductivity of about 10^{-6} Ω^{-1} cm^{-1} at 25 °C must be used throughout.

Ethanol. Ethanol containing less than 0.5 p.p.m. *m/m* of total sulphur must be used.

Hydrogen peroxide solution, 30% *m/m* (100 *volume*).

Oxygen. Compressed gas, high-purity grade (minimum purity 99.995%).

Carbon dioxide. Compressed gas, high-purity grade (minimum purity 99.995%).

Absorbing solution. Dilute 30% hydrogen peroxide solution 1 + 60 *V/V* with water.

Ethanol - water mixture. Ethanol containing 40% *V/V* of water.

Barium perchlorate solution, 10^{-2} M. Dissolve 3.90 g of barium perchlorate trihydrate in 400 ml of water and add 600 ml of ethanol. Adjust the apparent pH to about 5 with perchloric acid (10^{-2} M), using a pH meter. The titre of this solution must be expressed in micrograms of sulphur per microlitre (0.3206 μg in a 0.0100 M solution). The deviation from the average value of the titration results (titrated against a standard solution of sulphate as is described in the following paragraph) should be less than ± 0.003 μg μl^{-1} of sulphur.

Sodium sulphate standard solution, 10^{-2} M. Dissolve 1.4204 $\pm$ 0.0001 g (weighed into a closed vessel) of anhydrous sodium sulphate (previously dried for 1 h in an oven at 150 °C) in water and dilute to 1 l, again with water. As is described in the procedure under *Titration*, the 10^{-2} M barium perchlorate solution is checked by high-frequency titration by using 50 μl of this solution and 6 ml of ethanol - water mixture and adjusting the apparent pH to 5. Determine the value from the results of at least three titrations. The deviation from the average value should not be greater than 1% ($\pm 1 \times 10^{-4}$ M).

Sulphuric acid standard, 10^{-2} M. Dilute 10^{-1} M sulphuric acid with water. Check the dilution by high-frequency titration against sodium hydroxide standard solution. Take a 50–100-μl sample of the sulphuric acid and use water as the titration medium.

Sodium hydroxide standard solution, 2×10^{-2} M. Dilute sulphate- and carbonate-free 10^{-1} M sodium hydroxide standard solution with water.

Perchloric acid, approximately 10^{-2} M. Dilute the acid with ethanol - water mixture.

Methyl purple indicator solution, 0.1%. Fleisher's indicator.

Sulphur-free naphtha. Naphtha of suitable quality can be prepared from reformed naphtha (cut Arabian crude oil, boiling range 150–180 °C, d_4^{20} 0.750) following the procedure for the preparation of sulphur-free benzene described by ICBP.[10] The distillation is better carried out in a Todd column (reflux rate 10:1). Discard the first 5% of the distillate and collect the next 90% as sulphur-free naphtha.

Purified gases. The oxygen and carbon dioxide (for the use of the latter, see Fig. 2) used for the combustion were scrubbed with 10% chromic acid solution and 10% hydrogen peroxide solution and then dried with silica gel in a purification train that has already been described.[16] This train was designed to ensure thorough scrubbing of the gases at high throughputs. However, it did not prove entirely satisfactory in this instance because the solutions needed to be renewed frequently. The purification train can be discarded if gases of the highest purity available are used throughout. The assembly then gains in simplicity and the results of the tests show better repeatability. The purity of the gases (whether or not the purification train is used) must be checked by carrying out a blank determination in which 100 ml of the sulphur-free naphtha is burnt in the apparatus as though it were a sample. A result for the apparent total sulphur content of the naphtha of 0.2 p.p.m. *m/m* or less indicates that the purity of the gases is satisfactory.

Procedure

Combustion

Set up the combustion apparatus as shown in Fig. 2. Ensure that the burner unit (including the special burette) and combustion chamber are positioned so that the burner tip can be readily inserted into, or removed from, the chamber. Pour 10 ml of hydrogen peroxide absorbing solution into each of the two absorbers. Next fill the special burette with the naphtha sample to be tested and place the burette in position in the burner. The volume of sample will depend on its sulphur content (Table I); an appropriate burette (25, 50, 100 or 200 ml) (Fig. 1) must be used. Connect the electric heaters and operate their powerstat such that the bulb of the burner and the gas ducts are maintained at approximately 300 °C. Remove the tip of the burner from the combustion chamber, then open the carbon dioxide tap and the additional carbon dioxide tap and adjust the flows to about 1 and 3 l min^{-1},

respectively. Next, allow the sample to drop down the inlet tube into the burner. Place a small flame near the jet of the burner, immediately open the oxygen tap gently and adjust the oxygen flow to about 7.5 l min^{-1}. When the gases (naphtha, oxygen and carbon dioxide) have ignited, remove the small flame and adjust the flow-rate of the liquid sample to about 1 ml min^{-1} (approximately 2 drops s^{-1}). The flame should be 50–100 mm long. Insert the tip of the burner into the combustion chamber (making sure that the jacket and cover are well fitted) then open the vacuum tap quickly and adjust it to give a flow of approximately 11 l min^{-1}, also noting the level of sample in the burette. The absorbing solution should be drawn up the fritted discs of the absorbers, the passage of the gases ensuring good agitation. The gas rates quoted are suitable for light naphthas and predominantly aliphatic materials and will require modification for aromatic hydrocarbons.

TABLE I

SAMPLE SIZES

Sulphur content, p.p.m. *m/m* ..	0.4–1	1–5	5–10	10–50
Sample size/ml	200–100	100	50	20

When sufficient sample has been run in, close the burette tap and, at the same time, turn off the oxygen supply and immediately close the vacuum tap. Then turn off the carbon dioxide supply and also the additional carbon dioxide supply. Note the reading on the burette. Finally, turn off the electric heaters.

Allow the apparatus to cool. Remove the combustion chamber from the intermediate adapter flask and also remove the condenser from the absorber, raising it sufficiently to allow for the following operation. Fit the vacuum line to the free side of the intermediate adapter flask, open the tap so that absorbing solution will be drawn off into the round-bottomed flask (the lower section of the adapter) and transfer this solution into a calibrated flask (200 ml).

In order to carry out the washing stage replace the condenser and round-bottomed flask and, under vacuum as before, pour 15 ml of water into the top of condenser with the spray trap removed. Having repeated this operation two or three times, transfer the washings to the calibrated flask. Finally, dilute the contents of the flask to 200 ml with water.

Periodically, carry out a blank determination by burning a suitable volume of sulphur-free naphtha or ethanol for the same time as was taken to burn the test sample.

Evaporation

This step is required for a liquid sample, the sulphur content of which is 0.4–1 p.p.m. (Table I).

Into a 25-ml beaker (suitable to fit into the holes in the solid metal heating block; see Apparatus), pipette a 10-ml aliquot of the solution produced by the combustion step and carefully evaporate the liquid in the beaker to decompose most of the hydrogen peroxide, to remove dissolved carbon dioxide and to reduce the volume to 5–7 ml. Next cool the solution to room temperature, add a drop of methyl purple indicator solution and titrate to a faint green end-point with 2×10^{-2} M sodium hydroxide standard solution. The titration should be carried out with the Agla micrometer syringe, the tip of which is placed just below the surface of the solution in the beaker; the beaker should be subjected to hand shaking throughout the titration. Again evaporate the solution carefully to less than 0.5 ml.

Caution. Do not allow to boil dry; see Volatility of Sulphuric Acid.

For higher sulphur contents it is possible to change the volume of standard sodium sulphate solution added in the following titration step or to choose a smaller aliquot of the solution from the combustion step. In such an instance, it is advisable to dilute the aliquot in the beaker with water to about 10 ml.

Titration

Transfer the solution in the beaker into the titration cell and wash the beaker thoroughly with ethanol - water mixture, keeping in mind that the final volume in the titration cell must be 6 ml. Adjust the apparent pH of the solution to 5 ± 0.1 with 10^{-2} M perchloric acid (from a micrometer syringe) by using the combined electrode, then raise the electrode and allow it to drain well on to the inner wall of the titration cell. Next add 40 μl of 10^{-2} M

sodium sulphate standard solution and titrate the total sulphate present against the standardised 10^{-2} M barium perchlorate solution, using the high-frequency technique to detect the end-point as is described below.

The volume of sodium sulphate standard solution added to the titration cell will need to be varied according to the sulphur content of the sample in order to keep the volume of barium perchlorate solution down to about 50 μl. This recommendation will ensure that the total ionic strength is less than 10^{-3}; therefore it cannot interfere with the true inflection point and shape of the titration graph as experimentally deduced.[15] For the purposes of the calculation subtract the volume of the barium solution corresponding to the sodium sulphate solution added.

The test sample in the titration cell is diluted to 6 ml with the ethanol - water mixture and the solution is stirred continuously. The barium perchlorate standard solution is added from the Agla micrometer syringe in 10-μl increments and after 30 s readings are taken on the digital microammeter, which is re-set to zero after each reading. Equilibrium is quickly attained except when stirring is too slow. The microammeter readings are plotted on a graph against the microlitres of reagent added and the end-point deduced from the intersection of two straight lines (extrapolated if necessary). A typical titration graph is shown in Fig. 4.

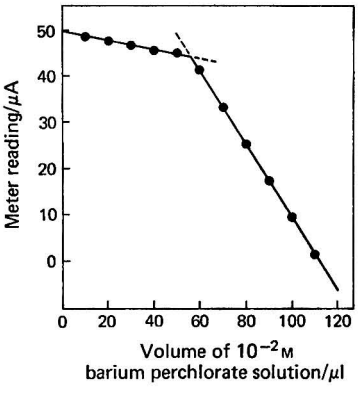

Fig. 4. Typical graph for sulphate ions corresponding to a 10-ml aliquot of the finished solution. Sample: series 2-C_2 (see Table II). Forty microlitres of sodium sulphate solution (10^{-2} M) previously added.

Calculation

The sulphur content of the sample is calculated from the equation

$$\text{Amount of sulphur}/\mu\text{g g}^{-1} = \frac{MF(T - T_b)}{VS}$$

where T is the net volume, in microlitres, of barium perchlorate solution required by an aliquot of the sample solution; T_b, the net volume, in microlitres, of barium perchlorate solution required by the same volume of the blank; M, micrograms of sulphur equivalent to 1 μl of barium perchlorate solution; F, the dilution factor, *i.e.*, the volume, in millilitres, to which the concentrated absorption solution was diluted divided by the volume, also in millilitres, of the aliquot taken (for a typical test $F = 200/10 = 20$); V, the volume, in millilitres, of sample burnt; and S, the specific gravity of the sample at the temperature of the test.

Volatility of Sulphuric Acid

Ten-millilitre aliquots of 5×10^{-5} M sulphuric acid (see Reagents) were evaporated in the metal heating block (regulated at 100 ± 0.5 °C) in three ways: firstly, until 0.5–1 ml of liquid remained; secondly, just to dryness; and thirdly, with heating in the metal block

continuing for 1 h after dryness had been reached. The residues were treated as under *Evaporation* and then titrated. The titre of the barium perchlorate solution was checked against a sulphuric acid standard that had not previously been evaporated but the pH of which had been adjusted to 5 prior to titration. Samples determined by the three different evaporation techniques yielded 95, 89 and 82%, respectively, of their known sulphate contents.

Similar experiments, in which the sulphuric acid was neutralised prior to evaporation, showed no measurable loss of sulphate content, except with acid undergoing continued heating in the metal block.

The evidence thus indicates that losses arising from the vapour pressure of sulphuric acid at 100 °C may be significant at these low levels even during short heating periods. Therefore, if free sulphuric acid solutions are involved, the acid must be previously neutralised when possible and evaporation carefully conducted so as to obtain a residue of about 0.5 ml.

These recomendations are in agreement with well established procedures[6,8,9] and with the findings of other workers.[19]

Results

In order to check the proposed method by use of the standard additions method, the results of the determination of the sulphur content in the samples, with and without the addition of extra sulphur, as indicated in Table II, were plotted on a graph (Fig. 5). This figure shows the addition graph of each sample series that was obtained by plotting the sulphur additions in parts per million (*m/m*) (see Table II, sulphur concentration added) *versus* the net volume (in microlitres) of titrant consumed (10^{-2} M sodium sulphate solution).

It should be noted that the extrapolated values agreed well with values obtained directly for the samples without added sulphur; the blank value was found by extrapolating line 2 in the figure.

TABLE II

RESULTS FROM ADDITION TEST

Sample	Concentration of sulphur, p.p.m. (*m/m*)*						
	Test number						
	1	2	3	4	Mean	Added†	Corrected value‡
Series 1—							
A_1 (Reformed naphtha) (see sulphur-free naphtha)	0.85	0.94	0.85	0.94	0.90	0.00	—
B_1 (A_1 + 0.42 p.p.m. S)	1.41	1.33	1.28	1.33	1.33	0.42	0.47
C_1 (A_1 + 0.84 p.p.m. S)	1.88	1.80	1.80	1.80	1.82	0.84	0.96
D_1 (A_1 + 1.69 p.p.m. S)	2.52	2.57	2.48	2.52	2.52	1.69	1.66
E_1 (A_1 + 3.38 p.p.m. S)	4.27	4.19	4.32	4.32	4.32	3.38	3.36
Series 2—							
A_2 (Desulphurised naphtha) (see sulphur-free naphtha)	0.17	0.26	0.34	0.26	0.26	0.00	—
B_2 (A_2 + 0.42 p.p.m. S)	0.60	0.77	0.68	0.51	0.64	0.42	0.42
C_2 (A_2 + 0.48 p.p.m. S)	0.85	1.11	0.94	0.85	0.94	0.84	0.74
D_2 (A_2 + 1.69 p.p.m. S)	1.88	1.80	1.71	1.79	1.84	1.69	1.64
E_2 (A_2 + 3.38 p.p.m. S)	3.50	3.42	3.67	3.62	3.55	3.38	3.35
Series 3—							
A_3 (Refined naphtha) (similar specification to A_1)	0.77	0.68	0.68	0.60	0.68	0.00	—
B_3 (A_3 + 0.53 p.p.m. S)	1.20	0.94	1.03	0.94	1.03	0.53	0.30
C_3 (A_3 + 1.06 p.p.m. S)	1.71	1.62	1.54	1.62	1.62	1.06	0.89
D_3 (A_3 + 2.13 p.p.m. S)	2.56	2.48	2.56	2.48	2.52	2.13	1.79
E_3 (A_3 + 4.26 p.p.m. S)	4.62	4.53	4.45	4.53	4.53	4.26	3.80

* The results shown are mean values of four titrations on every burnt sample (100 ml).

† For the first and second series sulphur was added from a standard 500 p.p.m. *m/m* sulphur solution (d_4^{20} = 0.793), which is in the form of butyl disulphide in kerosene. For the third series sulphur was added from standard 578 p.p.m. *m/m* sulphur solution (d_4^{20} = 0.865), which is in the form of thiophene in toluene.

‡ Corrected value = mean value − *x*, where *x* is the value found from the extrapolation of the respective straight lines in Fig. 1 (0.86 for series 1, 0.20 for series 2 and 0.73 for series 3). Blank, 0.20 from supposedly sulphur-free naphtha. According to lines 1 and 2, the true content in sample A_1 is 0.86 − 0.20 = 0.66 p.p.m. (*m/m*).

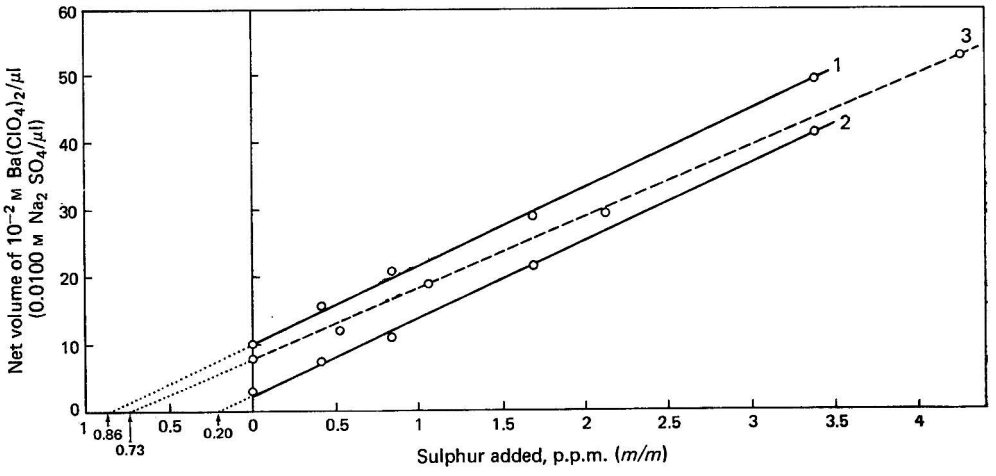

Fig. 5. Typical addition graphs for reformed naphtha (1), desulphurised naphtha (2) and refined naphtha (3). According to Table II, reformed and desulphurised samples are provided by the addition of butyl disulphide. The refined samples contain thiophene as the sulphur-containing compound.

Interferences

Some acidic residues generated during the combustion of the samples will not cause serious interferences. Moreover, almost all of these substances will be eliminated during the evaporation step. If more than 100 µl of 2×10^{-2} M sodium hydroxide solution are required to neutralise the 10-ml aliquot taken (sample 0.4–1 p.p.m. of sulphur), there is either an air leak in the apparatus or the halogen content of the samples is high. In these instances appropriate aliquots must be taken or the combustion repeated. In this way the effect of the ionic strength on the shape of the titration graph is diminished.[14,15]

Samples that contain halogens, barium, lead or appreciable amounts of ash-forming substances can be treated by use of established methods.[6,8,9,11] Sulphite, fluoride, phosphate and nitrate ions interfere while sodium, potassium, zinc and ammonium ions give low results due to co-precipitation; cations giving insoluble sulphates also interfere.[11] In the amounts in which they occur in most products these interferences are not significant but they preclude the use of this finish for most oil products containing additives if the samples have not been previously treated. The carbon dioxide and hydrogen peroxide used in this method would also interfere, but these substances are eliminated during the evaporation step. The remaining traces of hydrogen peroxide will not interfere.

Precision

All of the results shown in Table II were subjected to statistical analysis so that for each series (and for each level) the standard deviation corresponding to repeatability and relative error were calculated.[20] The maximum deviation found was less than 0.13 mg per 1 000 g, including the whole of the results for the A_1 and A_3 series. The relative errors (95% confidence) were 43% (0.2 p.p.m. of sulphur level) and 5% (4 p.p.m. of sulphur level).

The ultimate accuracy of this method is dependent on the performance of the combustion - absorption and evaporation steps, the accuracy of the sulphate titration and the accuracy of the sulphate standards. The accuracy of the titration is increased by titrating the small volume made possible by the evaporation step. The total volume titrated can thus be kept down to 6 ml, making possible the use of 10^{-2} M barium perchlorate in a high-frequency micro-titration and consequently permitting replicate titrations with a standard deviation of 0.2 µg of sulphur.[15] In accordance with calculations this deviation might be equivalent to 0.06 µg g^{-1} in typical naphthas (about 50% of the maximum deviation found) provided that the combustion - absorption and evaporation steps introduced no errors.

In order to evaluate the possible systematic errors a true value deduced according to

footnote ‡ in Table II was established. According to these considerations the method is correct and reliable except for values from samples D_3 and E_3.

Earlier tests, carried out before the method had been completely evolved, showed that the bulk of the errors were associated with the combustion rather than the evaporation and titration steps, and that the deviations did not vary significantly with the sulphur content within the range 4–50 p.p.m. It can therefore be assumed that the precisions quoted are applicable to the entire stated range.

Satisfactory tests were also carried out with ethanol, heptane and 2,2,4-trimethylpentane samples and we consider that their good burning qualities, previously observed for toluene and xylenes,[16] render them suitable for testing by use of the procedure described.

Discussion

The precision described is better than that established for standard methods, including those based on reduction with Raney nickel,[1–3] but is not as good as modified versions of Granatelli's method.[4] Nevertheless, according to Granatelli[3] olefins interfere in the reaction and compounds such as sulphoxides or sulphones are reduced with difficulty or not at all. In most instances, if such compounds are present, oxidation procedures are indicated.

The total elapsed time for a single determination in a 100-ml sample is about 3 h and it is estimated that 1 man-hour is required per sample. As the preparation and titration steps for one aliquot of the finished solution take 15–20 min, one additional man-hour allows the titration of three replicate aliquots for one burnt sample. This advantage is not possible with other procedures (*e.g.*, reduction with Raney nickel) although, for a single determination, the total time is less than that indicated.

The method described combines an easy combustion stage with an instrumental titration. The combustion apparatus is of all-glass construction and is easy to assemble and maintain. Also, it does not lead to explosions and therefore does not need the careful control required by the Wickbold system. With regard to the instrumental finish, the micro-titration applied[15] is more reliable, and a similar range can be covered with greater sensitivity than the micro-adaptations of the barium perchlorate titration method for determining sulphate of Fritz and Yamamura.[11,12] Moreover, the principle of operation is simple and most of the parts of the titrimeter are standard radio components (see Fig. 3).

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References

1. UOP Method 727–72, Universal Oil Products, Des Plaines, Ill., 1972; "1975 Book of ASTM Standards," Part 25, American Society for Testing and Materials, Philadelphia, 1975, Method D 3120–75.
2. UOP Method 357–76, Universal Oil Products, Des Plaines, Ill., 1976.
3. Granatelli, L., *Analyt. Chem.*, 1959, **31**, 434.
4. Fensom, A., Roberts, N., and Duff, G. M. S., *Analyst*, 1971, **96**, 186.
5. Rousseau, J. C., and Lerouge, C., *Rev. Inst. Fr. Pétrole*, 1974, **29**, 251.
6. European Committee for Standardization, Project prEN 41 (CEN/WG 19, N 369), Paris, 1974.
7. "1975 Book of ASTM Standards," Part 23, American Society for Testing and Materials, Philadelphia, 1975, Method D 1266–70.
8. "1975 Book of ASTM Standards," Part 24, American Society for Testing and Materials, Philadelphia, 1975, Method D 2785–70. "Institute of Petroleum Standards 1974," Part I, Institute of Petroleum, London, 1974, Method 243/70 (T).
9. UOP Method 586–71, Universal Oil Products, Des Plaines, Ill., 1971.
10. International Conference of Benzole Producers, *Analyt. Chem.*, 1964, **36**, 339.
11. Fritz, J. S., and Yamamura, S. A., *Analyt. Chem.*, 1955, **27**, 1461.
12. Persson, G. A., *Int. J. Air Wat. Pollut.*, 1966, **10**, 845.
13. Sand, J. R., and Huber, C. O., *Analyt. Chem.*, 1976, **48**, 1331.
14. Malissa, H., Kellner, R., and Pell, E., *Z. Analyt. Chem.*, 1971, **253**, 113.
15. Fernández, T., Rocha, J. M., Rufino, N., and García Luis, A., *An. Quim.*, 1977 in the press.
16. Fernández, T., Rocha, J. M., Rufino, N., and García Luis, A., *An Quim.*, 1974, **70**, 723.
17. Serrano Bergés, L., and Fernández, T., *An. Quim.*, 1966, **62B**, 793.
18. "1975 Book of ASTM Standards," Part 23, American Society for Testing and Materials, Philadelphia, 1975, Method D 381–70.
19. Teonnies, G., and Bakay, B., *Analyt. Chem.*, 1953, **25**, 160.
20. Lacroix, Y., "Analyse Chimique (Interpretation des Résultats par le Calcul Statistique)," Masson, Paris, 1962.

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Enzymatic Determination of Maltose by Amperometric Measurement of the Rate of Oxygen Depletion

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An enzymatic method for the rapid, direct kinetic measurement of maltose is described. Maltose is hydrolysed to glucose by α-glucosidase, and the glucose reacts with oxygen in the presence of glucose oxidase to form gluconic acid and hydrogen peroxide. The rate of oxygen depletion is measured with a Clark oxygen electrode. The glucose oxidase reagent contains sufficient amounts of α-glucosidase impurity for a maximum reaction rate to be obtained within 2 min after addition of maltose to the reagent. The reaction rate is obtained directly by recording the derivative of the change in amperometric current. Glucose already present in samples can be destroyed by incubation with purified glucose oxidase reagent for 10 min. Fructose, lactose and galactose do not interfere. Interference from starch (constant over a wide range of starch concentrations) can be readily corrected for by adding starch to standards, and sucrose interference can be minimised by adjustment of the pH. Excellent results were obtained for the recovery of maltose from pooled serum samples.

Keywords: *Maltose determination*; *enzymatic analysis*; *oxygen electrode*; *blood serum*

The importance of maltose in the food industry is well established. Maltose is a major constituent of corn syrup, which is widely used in processed foods, and is also added to various foods, such as modified infant milk. The determination of maltose as a clinical test has been limited to a few highly specialised cases, *e.g.*, to study maltase deficiency in infants.[1] However, it has been suggested that maltose is a good replacement for sucrose and glucose for patients requiring high carbohydrate diets, as it is less cariogenic than sucrose and less likely to induce hypoglycaemia than glucose.[2] Hence there is a need for further studies on maltose metabolism.

Few methods exist for the selective determination of maltose. Methods based on the reducing properties of maltose are subject to interference by other reducing sugars, such as glucose, lactose and galactose, which are likely to be present in test samples. Moody and Purdy[3] have described a coulometric method for determining maltose that uses maltase to hydrolyse maltose to glucose, which is then measured after a 60-min incubation period. The method is relatively selective, but is subject to glucose interference. Another method uses maltose phosphorylase coupled with glucose oxidase, but is time consuming and requires several incubation steps.[4]

The method described here is based on the amperometric measurement of the initial rate of oxygen depletion with a Clark oxygen electrode. The kinetic measurement of the reaction greatly reduces the analysis time required compared with single-point end-point methods. Glucose, produced by α-glucosidase-catalysed hydrolysis of maltose, reacts with oxygen in the presence of glucose oxidase to form gluconic acid and hydrogen peroxide. The principle of the measurement was described by Kadish *et al.*[5] for glucose determination. The glucose oxidase reagent contains sufficiently high levels of α-glucosidase impurity that additional maltase is not required. The presence of this impurity eliminates a major expense involved in an enzymatic maltose determination, as maltase is a costly enzyme. An inexpensive glucose oxidase preparation is desirable in order to ensure sufficient α-glucosidase impurity levels. The impurity level is high enough to produce a direct signal maximum within 2 min after addition of the maltose sample to the reagent. A calibration graph is prepared using maltose standards, and variations in maltose impurity concentrations are accounted for. When necessary, glucose is removed from the sample prior to analysis by incubation for 10

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min with a highly purified glucose oxidase reagent. Fructose, lactose and galactose do not interfere. Starch interference can be eliminated. Sucrose interference is negligible at relatively small concentrations, *i.e.*, 2.5 mg ml^{-1} or less of sucrose.

The sensitivity of our method is competitive with that of the other methods mentioned above, permitting the detection of maltose at concentrations of 0.5 mg ml^{-1} using a 25-μl sample, or a total of 12.5 μg of maltose per sample.

Experimental

Apparatus

A glucose analyser (Beckman Instruments, Inc.) in conjunction with a strip-chart recorder ("OmniScribe," Houston Instrument Corp.) was used to measure the rate of oxygen consumption.[6]

Reagents

Glucose oxidase reagent A. Beckman certified glucose reagent for use with the analyser was adjusted to pH 5.0 with dropwise addition of 6 M hydrochloric acid. The reagent contains 140 U ml^{-1} of glucose oxidase in a 0.2 M phosphate buffer at pH 6.0.

Glucose oxidase reagent B. Beckman research glucose reagent was used. This reagent, which was used for pre-incubation to remove glucose, contains 140 U ml^{-1} of purified glucose oxidase in 0.2 M phosphate buffer containing iodine at pH 6.0.

Benzoate buffer. Prepare a 0.02 M, pH 5.5 benzoate buffer solution by adding 2.44 g of benzoic acid to 800 ml of distilled water. Heat to boiling, cool, adjust the pH to 5.5 with 1 M sodium hydroxide solution and dilute with water to 1 l. This buffer is a good preservative for maltose and glucose.

Maltose standards. Prepare a 500 mg ml^{-1} maltose solution by dissolving 0.5 g of maltose in 100 ml of benzoate buffer. Use this solution to prepare standards containing 0.5–5.0 mg of maltose per millilitre.

Procedure

Preparation of calibration graph. Add 750 μl of glucose oxidase reagent A to the cell of the analyser. Allow the oxygen in the solution to reach equilibrium with atmospheric oxygen (indicated by a stable reading on the analyser's digital display, and by a stable base line on the recorder chart). Inject 25 μl of 5.0 mg ml^{-1} standard maltose into the cell and record the signal maximum, *i.e.*, the height of the derivative peak from the base line. Repeat for 4.0–0.5 mg ml^{-1} samples and plot the relative peak heights as a function of maltose concentration.

Measurement of unknown sample. Dilute the sample, if necessary, with benzoate buffer to bring the maltose concentration within the range 0.5–5.0 mg ml^{-1}. Inject 25 μl of sample solution into 750 μl of equilibrated reagent A in the cell and measure the signal maximum as above. Read the corresponding concentration from the calibration graph.

Removal of interfering glucose. Incubate 50 μl of sample with 50 μl of glucose oxidase reagent B for 5–10 min at room temperature and inject 25 μl of sample as above.

Results and Discussion

Electrode Response

The response of the oxygen membrane electrode changes from day to day, and the electrode must be periodically re-charged according to the supplier's directions. The signal also varies from one electrode to another, depending on the surface area of the platinum tip. The signal is maximised by employing the high-sensitivity "U-mode" of the analyser (designed for uric acid measurements), and setting the "Air Adjust" control to give the maximum response level. The calibration graph varied very little over a period of days, but for best results a new calibration graph should be prepared each day.

Effect of pH

The optimum pH for the glucose oxidase reagent A, at which maximum response to maltose occurs, was found to be 5.0 (Fig. 1). This result agrees with the findings of Moody and Purdy for their combined glucose oxidase - maltase reagent.[3]

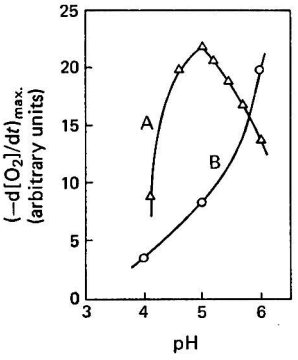

Fig. 1. Effect of pH on rate of oxygen consumption for maltose and sucrose. A, Maltose, 2.5 mg ml^{-1}; and B, sucrose, 2.5 mg ml^{-1}.

Calibration

The calibration graph is not linear over the concentration range investigated (Fig. 2). There is a short induction period, during which a small peak caused by mixing of sample and reagent occurs (see Fig. 3). The induction period varies with maltose concentration, whereas the mixing peak does not. The result is that the maltose signal approaches zero at zero maltose concentration rather than approaching a blank value which can be subtracted from the reading. At high maltose concentrations, the reaction rate becomes limited by enzyme activity and the response levels off. The 0.5–5.0 mg ml^{-1} range was therefore chosen as covering the optimum working range.

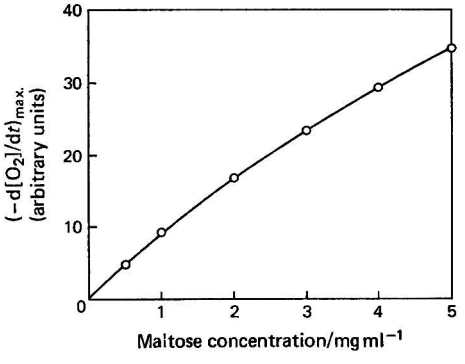

Fig. 2. Calibration graph for aqueous maltose standards (25-μl sample).

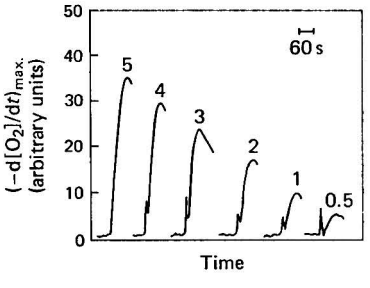

Fig. 3. Signals recorded for aqueous 25-μl maltose standards. Numbers on peaks represent concentrations of maltose standards taken in milligrams per millilitre.

Effect of Enzyme Activity

Addition of 1 U of purified maltase to 750 μl of the glucose oxidase reagent A produced no increase in signal, and in some instances decreased the signal. Addition of 100 U of glucose oxidase (the same material as that used in the preparation of glucose oxidase reagent A) caused increased signals at maltose concentrations below 0.02g ml^{-1}, and decreased signals at higher maltose concentrations (Fig. 4). The complex kinetics of enzyme-catalysed maltose hydrolysis have been described,[2] including the inhibition of maltase by glucose. There are several α-glucosidases that exhibit maltose activity,[2] and the impurity activity in the glucose reagent may be due to one or several of these.

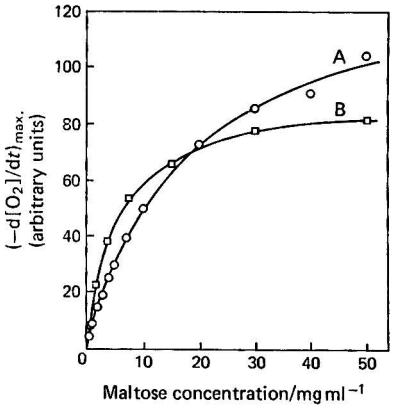

Fig. 4. Effect of glucose oxidase reagent activity on rate of oxygen consumption. A, 140 U ml^{-1}; and B, 273 U ml^{-1}.

Precision

A coefficient of variation of $\pm 2.69\%$ was calculated for 10 measurements on 2.5 mg ml^{-1} aqueous maltose solutions.

Interferences

Fructose, galactose and lactose at 5.0 mg ml^{-1} do not give signals upon injection into the glucose oxidase reagent A, and therefore do not interfere. Glucose, if present in samples, can be destroyed as described above.

Starch gives a signal caused by amylase impurity in the reagent. However, the rate of oxygen consumption for this reaction is limited by the amylase activity rather than substrate (starch) concentration. As the amount of amylase impurity is small, the signals for 0.5 mg ml^{-1} and 0.05 g ml^{-1} of starch are equal, and equivalent to that for 1.5 mg ml^{-1} of maltose. Therefore, if starch is present in the sample, the calibration graph should be prepared with maltose standards containing starch in an amount close to, but not necessarily the same as, that in the sample. The presence of appreciable amounts of starch in a sample is frequently indicated by the formation of a blue colour on addition to the glucose oxidase reagent, which contains iodine.

Sucrose also gives a signal upon addition to the reagent, probably due to sucrase impurity in the glucose oxidase preparation. However, this response is lower than the maltose response and shows a much greater pH dependence (Fig. 1). When equal amounts (2.5 mg ml^{-1}) of maltose and sucrose are present, sucrose causes a positive error of 10% if reagent adjusted to pH 5.0 is used, and 4% if the reagent is adjusted to pH 4.5. These relative errors do not correspond to the results presented in Fig. 1, owing to the time dependence of the signals. The sucrose response is slower than the maltose response, and the error due to sucrose is therefore less than would be predicted by Fig. 1. The response time also increases with decreasing pH for sucrose, and so the relative error decreases between pH 5.0 and 4.5 more than would be predicted by the relative peak heights shown. Therefore, when sucrose is present in the sample, it is preferable to work with the glucose oxidase reagent A adjusted to pH 4.5.

The above interferences would also be potential interferences in other enzymatic methods utilising glucose oxidase unless a highly purified enzyme preparation were used.

Maltose Recovery from Human Serum

The recovery of maltose from pooled serum was studied in order to test the applicability of the present method to clinical analysis. Glucose was removed from the samples prior to analysis as described above, using 50-μl samples of the serum. The pooled serum originally

contained no measurable maltose. The results are shown in Table I. Recoveries were excellent over the entire range, and the glucose in the serum did not interfere.

It is recommended that for each particular type of sample, a recovery study be made to ascertain that there are no enzyme activators or inhibitors present for this particular enzyme preparation. This would, of course, be true also for other enzymatic methods.

TABLE I

RECOVERY OF MALTOSE ADDED TO POOLED HUMAN SERUM

Maltose added/mg ml^{-1}	Maltose recovered/mg ml^{-1}	Relative error, %
5.0	4.98	0.4
4.0	4.00	0.0
3.0	3.00	0.0
2.0	2.00	0.0
1.0	1.02	2.0
0.5	0.52	4.0
		Average 1.1

It should be pointed out that maltose does not interfere in the determination of blood glucose using this instrument with the described glucose oxidase reagent. It was observed, for example, that 2.50 mg ml^{-1} of maltose did not affect the determination of 0.75 mg ml^{-1} of glucose in blood. The reason is that the maltose response is much slower than the glucose response (see above), in addition to being much smaller, and the maximum rate for the glucose reaction is recorded before appreciable reaction from the maltose occurs.

References

1. Reed, G., "Enzymes in Food Processing," Academic Press, New York, 1966, p. 391.
2. Sipple, H., *in* McNutt, K., *Editor*, "Sugars in Nutrition," Academic Press, New York, 1974, pp. 204 and 310–311.
3. Moody, J. R., and Purdy, W. C., *Analytica Chim. Acta*, 1971, **53**, 239.
4. Kamogawa, A., Yokobayashi, K., and Fukui, T., *Analyt. Biochem.*, 1974, **57**, 303.
5. Kadish, A. H., Little, R. L., and Sternberg, J. C., *Clin. Chem.*, 1968, **14**, 116.
6. Kumar, A., and Christian, G. D., *Clin. Chem.*, 1975, **21**, 325.

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Determination of Barium in Calcium Carbonate Rocks by Carbon Furnace Atomic-emission Spectrometry

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A simple and rapid method is described for the determination of barium at 0.5–30 $\mu g\ g^{-1}$ concentrations in calcium carbonate and limestone rocks. The sample is dissolved in nitric acid, caesium is added as an ionisation suppressor and the resulting solution is analysed by using carbon furnace atomic-emission spectrometry with automatic background correction using wavelength modulation. Spectral interference from CaOH band structure is low in the carbon furnace compared with flame atomisation and is removed by background correction. Other matrix interferences are negligible. The reproducibility is 5.9% at the 2.4 $\mu g\ g^{-1}$ level and the detection limit is 0.036 $\mu g\ g^{-1}$ of barium in rock samples.

Keywords: Barium determination; calcium carbonate rocks; carbon furnace atomic-emission spectrometry

Calcium causes one of the most serious interferences in the determination of barium in calcium carbonate or in rocks and minerals that contain large amounts of calcium when either atomic-emission or atomic-absorption spectrometry is used. Since Bowman and Willis[1] first reported the use of the dinitrogen oxide - acetylene flame in atomic absorption for this determination, many workers have reported and sought to overcome the interference of calcium. The most sensitive barium atomic-emission and -absorption line occurs at 553.5 nm and is overlapped by the maximum of the CaOH band in the 553–555-nm region.[2,3] In flame emission, background emission from the CaOH band due to large amounts of calcium in the sample matrix makes the detection of the normal amounts of barium in rocks impossible,[4] although Koirtyohann and Pickett[5] showed that the interference was much reduced in the dinitrogen oxide - acetylene flame.

In order to overcome this problem, Rubeška[6] recommended the use of an argon-shielded dinitrogen oxide - acetylene flame, which reduces the amount of CaOH emission, and Cresser *et al.*[7] also reduced the background interference from calcium by using a high-resolution echelle spectrometer. As the background cannot be removed completely in either instance, manual or automatic subtraction of the background using a synthetic blank solution is necessary.[8] Methods of automatic background correction using an adjacent wavelength[9] can be criticised on the grounds of possible errors due to the established variation of intensity of the CaOH band with wavelength.[2,3,10,11] The most satisfactory method of background correction is that based on wavelength modulation,[10] in which an average measurement of the background over a small band width across the operating wavelength is used. In all flame emission procedures, it should be remembered that the intense CaOH emission, even if it is background corrected, may still contribute significantly to the noise of the system and impair the detection limit for barium determinations.

In flame atomic absorption, two types of spectral interference by calcium can be identified.[4,12] The effect of the intense emission by CaOH, although reduced by modulation of the source, may increase the photomultiplier shot noise at high calcium concentrations. In addition, the radiation from the barium hollow-cathode lamp at 553.5 nm is absorbed by

the CaOH molecules[2,12] and can be used to measure the variation of the concentration of this species in flames.[13,14] Cioni *et al.*[15] have suggested that the presence of calcium may also suppress ionisation of barium to give an enhancement of the atomic-absorption signal. They have also described complex multi-element interference effects when barium is determined in a silicate rock matrix. Capacho-Delgado and Sprague[12] recommended the use of the dinitrogen oxide - acetylene flame in atomic absorption for the determination of barium in the presence of calcium while Rubeška[16] suggested the use of the nitrogen-separated dinitrogen oxide - acetylene flame. However, several workers[15,17,18] have stated that this procedure is impossible or difficult owing to interferences and recommend the separation of barium from the calcium matrix before analysis.[11,17,18]

The improved sensitivity of electrothermal atomisers in atomic absorption would appear to be attractive for the determination of barium in the presence of calcium, particularly as the concentration of CaOH in the atomiser should be much lower than in a flame because of the lower oxygen concentration present due to the inert gas atmosphere. However, CaOH molecules have recently been identified in the furnace atmosphere during the atomisation of solutions that contain high concentrations of calcium, by measurement of both absorption and emission spectra.[19] Background absorption by CaOH at the barium wavelength is much reduced compared with flames but may be significant in the determination of trace amounts of barium, and it is unfortunate that automatic background correction is not generally available for the visible region as it is for the ultraviolet region. In addition, the background signal from the graphite tube or filament reaches an apparent maximum in the visible region close to 553.5 nm and the optical alignment of the atomiser and spectrometer and the incorporation of suitable baffles in the spectrometer are critical and essential before atomic-absorption measurements can be made.[20]

Recently, Thompson and Godden[20] described a graphite rod atomisation procedure for the determination of barium in calcium carbonate in which separation of the barium from the matrix was not necessary. In this procedure, methane incorporated in the argon purge gas is said to reduce considerably the background absorption signal from the calcium matrix. This signal, however, remained small but significant even when the instrument was aligned carefully in order to reduce it further. Calcium also considerably enhanced the barium atomic-absorption signal and was incorporated in all sample and standard solutions. Cioni *et al.*[21] have suggested that it is still necessary to use a pre-separation procedure when electrothermal atomisation techniques are used for this determination. They used a graphite tube lined with titanium dioxide and found that calcium slightly enhanced barium signals in nitrate medium but caused a considerable depression in chloride medium. Separation of barium from the matrix appears to be essential as atomisation was carried out using solutions prepared in a hydrochloric acid medium.

The measurement of atomic-emission signals from graphite furnace atomisers has recently been reported[22–25] and the detection limits for barium are significantly better than those obtained by graphite furnace atomic absorption.[23] Detection limits of the order of 0.004 μg ml^{-1} were reported when commerical atomic-absorption instruments were used.[25] However, as the background signal from the graphite tube is considerable in the region of the barium atom wavelength, the incorporation of background correction significantly improves the detection limit by a factor of 50.[23] When samples of barium in a calcium matrix were atomised without background correction, it was found that a significant increase in the background emission signal was obtained at the barium wavelength owing to CaOH emission. Correction for this interference could be carried out manually by measurement of a suitable blank solution but, by using wavelength modulation, automatic correction can be carried out at the same time as correction for the tube background. The much smaller background signal from CaOH and the ease with which it can be corrected means that the separation of barium from the calcium matrix before analysis is unnecessary. Ionisation of barium during carbon furnace atomisation has been observed,[23,25] but is generally lower than in flames and can be easily suppressed.[25] In our experience, the use of solutions prepared in nitric acid medium suppresses many chemical interference effects.[24] With this freedom from matrix interferences, the use of graphite furnace atomic-emission spectrometry with automatic background correction provides a very simple, sensitive and rapid procedure for the determination of barium in the presence of large amounts of calcium. The application of this principle to the determination of barium in carbonate rocks is described in this paper.

Experimental

Apparatus

In the initial development of this procedure, a Perkin-Elmer Model 306 atomic-absorption/emission spectrometer was used and was equipped with an HGA-72 heated graphite atomiser. The spectrometer was connected to a Servoscribe RE541.20 potentiometric strip-chart recorder. The operation of this instrument for carbon furnace atomic-emission measurements has been described in detail elsewhere.[22,25] The wavelength used for barium was 553.5 nm and the spectral band width was 0.2 nm. Argon was used as the purge gas at a flow-rate of 1.5 l min^{-1} at 40 lb in^{-2} and gas flow conditions were used during the atomisation step. Aliquots of 50 μl were injected into the furnace, dried at 100 °C for 45 s and atomised at the maximum temperature, which was 2 700 °C in the HGA-72 furnace using modified tubes.[24] The tube was cleaned between injections of samples by operation of the maximum temperature button.

The analytical procedure to be described was performed on a Perkin-Elmer HGA-2100 heated graphite atomiser mounted in front of a Jarrell-Ash 0.5-m Ebert monochromator equipped with an oscillating quartz refractor plate for wavelength modulation. The experimental system was used as described previously.[23] The operating parameters are given in the procedure.

Reagents

Reagents of the highest available purity were used throughout and all solutions were prepared or diluted with de-ionised water. NBS SRM 915 standard calcium carbonate was found to have the lowest barium concentration and was used in the preparation of standards.

A barium stock solution containing 100 μg ml^{-1} of barium was prepared in 1% *V*/*V* nitric acid and was diluted as required. A stock 1 mg ml^{-1} solution of caesium was also prepared in 1% *V*/*V* nitric acid for use as an ionisation suppressor.

Procedure for the Determination of Barium in Carbonate Rocks

Preparation of calibration solutions

Weigh 1.0 g of SRM 915 calcium carbonate into a 100-ml PTFE beaker and dissolve it in 10 ml of 40% *V*/*V* nitric acid. On dissolution, transfer the solution quantitatively into a 100-ml calibrated flask, add the required amount of barium standard solution and 1 ml of the caesium stock solution and dilute to the mark with water.

Preparation of sample solutions

Weigh 1.0 g of carbonate rock sample into a 100-ml PTFE beaker and dissolve it in 10 ml of 40% *V*/*V* nitric acid. Filter the solution to remove any insoluble siliceous or carbonaceous material, if necessary, through a Whatman No. 542 filter-paper into a 100-ml calibrated flask. Add 1 ml of the caesium stock solution to the flask and dilute to the mark with water.

Instrument operation

Measure the signal from samples and standard solutions during the atomisation stage using a recorder scale of 5 mV full-scale deflection and the following operating conditions:

Wavelength	553.5 nm
Slit width	0.05 mm
Drying conditions	35 s at 120 °C
Atomisation conditions	12 s at 2 800 °C
Tube-cleaning conditions	5 s at 2 800 °C
Purge gas	Argon at 15 ml min^{-1} and 20 lb in^{-2}
Sample volume	50 μl

Measure the maximum peak height for each solution as an average of three injections, and obtain the barium concentration in the samples from a calibration graph obtained from the standards.

Results and Discussion

Interference of Calcium

When atomic-emission signals were measured on the HGA-72 graphite furnace it was noticed that calcium enhanced the signal given by barium in nitric acid medium. The enhancements were not as large as reported for carbon rod atomisation[20] and, when blank solutions of calcium carbonate were atomised, signals equivalent to the enhancement were still obtained. This result could have been due to the presence of barium as an impurity in the calcium carbonate, but similar signals were obtained at other wavelengths in the same spectral region where no emission from barium solutions could be observed. The interference was identified as a spectral interference from emission of the CaOH formed in the carbon furnace. Absorption and emission spectra of this and other oxide and hydroxide species have been observed during carbon furnace atomisation.[19] The emission signal from CaOH reaches a maximum at a different time during the atomisation stage compared with barium atomic emission, as illustrated in Fig. 1, which shows that the peaks could be differentiated with time. However, it is clear from Fig. 1 that, at the time of the maximum barium atomic-emission signal, there is still a significant emission signal from CaOH. This signal would have to be subtracted manually together with the tube background from the total emission signal at 553.5 nm to obtain the net barium emission signal when samples of carbonate rocks were atomised. Accurate manual subtraction of a consistent tube background can be achieved but this is unlikely to be so for CaOH emission as it would require a knowledge of the calcium concentration. A two-line method of background correction could be used on a manual basis, but is tedious and subject to error.

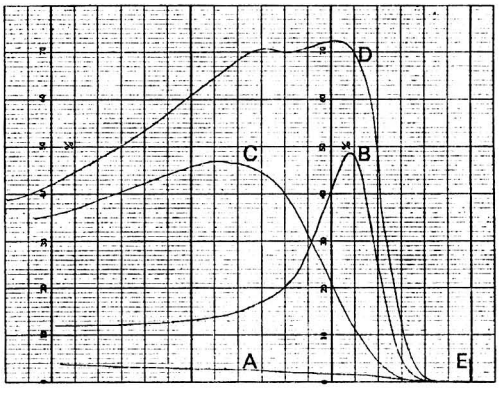

Fig. 1. Emission signals at 553.5 nm obtained during atomisation at 2700 °C using the HGA-72 furnace. A, No sample; B, 50 μl of 4 000 μg ml^{-1} calcium solution; C, 50 μl of 0.5 μg ml^{-1} barium solution; and D, 50 μl of solution containing 0.5 μg ml^{-1} of barium and 4 000 μg ml^{-1} of calcium. Atomisation starts at E. Chart speed, 2 cm s^{-1}.

Chemical interference from the calcium matrix at concentrations up to 4 000 μg ml^{-1} of calcium was found to be insignificant (Table I). The total emission signal increases with calcium concentration but, on subtraction of the CaOH background, it is found that the barium atomic-emission signal is almost constant. Ionisation of barium was shown to be minimal in a carbon furnace operated at a maximum temperature of 2 300 °C.[26] At higher temperatures, significant ionisation has been observed[23,27] and Fig. 2 shows the effect of 100 μg ml^{-1} of caesium on the barium atomic-emission signal in a furnace operated at an atomisation temperature of 2 800 °C, as used in the procedure described here. In order to overcome any effect of ionisation suppression from the constituents of the sample matrix, 100 μg ml^{-1} of caesium were incorporated in all sample and standard solutions. This concentration was sufficient to suppress the ionisation of barium completely at this temperature.

TABLE I

EFFECT OF VARYING CONCENTRATIONS OF CALCIUM ON ATOMIC-EMISSION SIGNAL OF 0.5 μg ml^{-1} OF BARIUM

Atomiser, HGA-72; wavelength, 553.5 nm; atomisation temperature, 2 700 °C.

	Signal, chart divisions		
Calcium/ μg ml^{-1}	Total emission signal (Ba + background)	Total background signal (tube + CaOH)	Barium atomic-emission signal
0	46	5	41
500	48	9	39
1 000	51	12	39
2 000	61	22	39
4 000	74	32	42

Analysis of Calcium Carbonate and Limestone Samples

The final analytical procedure was developed on the instrumentation constructed at NBS[23] in which wavelength modulation is used to correct automatically for both tube and CaOH emission background. As the CaOH emission background is corrected simultaneously, it does not matter if the concentration of calcium varies from sample to sample and similar results are obtained from standards with no added calcium. On this system, no blank signals from either the tube or the calcium matrix are obtained and the detection limit of the method is dependent on the variation of the shot noise at the photomultiplier rather than a statistical variation of the signal to background ratio.

The HGA-2100 atomiser was operated under the conditions given in the procedure. Argon at a reduced flow-rate was used as the purge gas during atomisation rather than stopped flow conditions. Although this technique had a slightly adverse effect on the detection limit, it helped to reduce memory effects. The highest available atomisation temperature was used as barium atomises at a slow rate and the emission intensity increases with increase in temperature.[25] Although it had been shown that there was no interference from calcium under these conditions (nitrate/caesium matrix and wavelength modulation), calcium was incorporated in the standards so as to ensure reproducible atomisation conditions.

The procedure was used to analyse a series of standard calcium carbonate and limestone samples provided by Dr. M. J. Russell of the Department of Applied Geology, University of

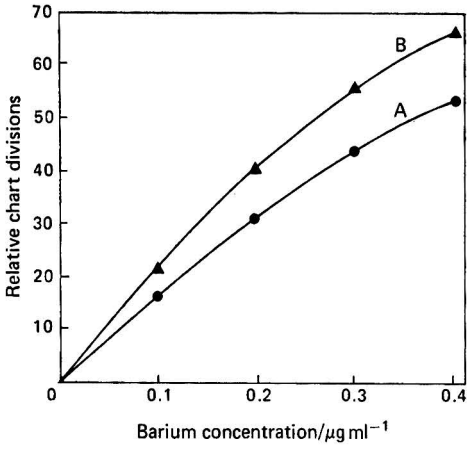

Fig. 2. Effect of 100 μg ml^{-1} of caesium on atomic-emission signal for barium at 553.5 nm. A, In the absence of caesium; and B, in the presence of caesium. Atomisation at 2 800 °C using the HGA-2100 atomiser. Recorder set at 10 mV full-scale deflection. Sample aliquots of 50 μl.

Strathclyde. Small residues that remained undissolved from the limestones were filtered off and a check on the residues showed that complete dissolution of the barium had been obtained. Calibration standards in the range 0.02–0.15 μg ml^{-1} of barium were used in most instances, but for one sample of higher barium content this range was extended to 0.05–0.3 μg ml^{-1} of barium.

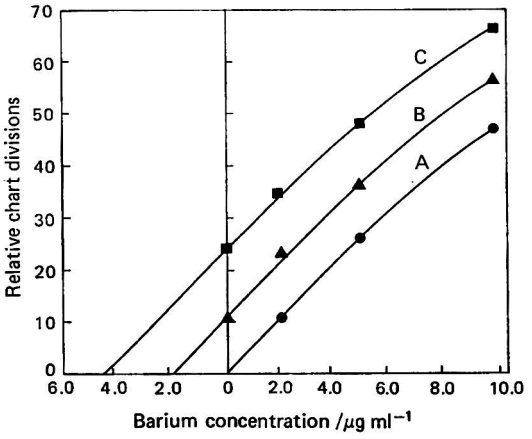

Fig. 3. Standard-additions calibration graphs for determination of barium. A, Aqueous solutions; B, BDH AnalaR calcium carbonate containing 1.9 μg g^{-1} of barium; and C, limestone sample Str. 22 containing 4.6 μg g^{-1} of barium. Atomisation at 2 800 °C using the HGA–2100 atomiser under the conditions given in the procedure.

A typical calibration graph is shown in Fig. 3. Curvature occurs at and above a concentration of 0.15 μg ml^{-1} but no serious loss of sensitivity occurs until well above 0.3 μg ml^{-1}. For these samples, no check results were available. In order to check the accuracy of the method, the results were compared with those obtained by flame emission also using wavelength modulation as described previously.[10,28] Solutions of the samples of concentration 2% were prepared in hydrochloric acid medium and analysed using a dinitrogen oxide - acetylene flame. The results obtained with the two methods are compared in Table II, and show excellent agreement. The methods are sufficiently different to provide a valid check of the accuracy of the results. Standard additions of barium to two sample solutions showed no difference in the results (Table I) and gave parallel calibration graphs (Fig. 3).

TABLE II

ANALYSIS OF BARIUM IN CALCIUM CARBONATE AND LIMESTONE SAMPLES

Sample	Barium/μg g^{-1}		
	Carbon furnace method	Flame emission	Standard additions carbon furnace emission
GPR $CaCO_3$ (M & B)	32.0, 30.0, 30.0	28.8	—
AnalaR $CaCO_3$ (BDH Chemicals)	1.6, 1.4, 1.5	1.0, 1.5	1.9
SRM 915	0.6, 0.8, 0.6	<1.0	—
SRM 1B	8.8, 8.3, 8.6	8.5	—
SRM 88A	2.4, 2.2, 2.4	2.0	—
Str. 22	4.6, 4.0, 4.3	5.0	4.6
Str. 107	1.8, 1.4, 1.8	1.8	—
Str. 221	7.0, 6.0, 7.2	6.5	—

The reproducibility of the graphite furnace atomic-emission method (11 results) was found to be 5.9% (relative standard deviation) for sample SRM 88A, which contained 2.4 $\mu g\ g^{-1}$ of barium. The detection limit of the method, expressed as one fifth of the peak to peak noise, was 0.000 36 $\mu g\ ml^{-1}$ of barium in solution or 0.036 $\mu g\ g^{-1}$ of barium in rock samples.

Conclusion

The combination of a relatively simple background correction procedure with the carbon furnace atomic-emission method has provided a simple and rapid method of analysis for the determination of barium in the presence of large amounts of calcium. Spectral interference from calcium is reduced by use of a furnace instead of a flame, and then removed by the background correction system. Preparation of sample solutions in a specifically chosen matrix has overcome other possible matrix effects such as ionisation and incomplete atomisation. The simplicity, sensitivity and accuracy of this technique would no doubt find wide application in the analysis of other types of samples.

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References

1. Bowman, J. A., and Willis, J. B., *Analyt. Chem.*, 1967, **39**, 1210.
2. Koirtyohann, S. R., and Pickett, E. E., *Analyt. Chem.*, 1966, **38**, 585.
3. Van der Hurk, J., Hollander, Tj., and Alkemade, C. Th. J., *J. Quant. Spectrosc. Radiat. Transfer*, 1973, **13**, 273.
4. Ingamells, C. O., Suhr, N. H., Tan, F. C., and Anderson, D. H., *Analytica Chim. Acta*, 1971, **53**, 345.
5. Koirtyohann, S. R., and Pickett, E. E., *Spectrochim. Acta*, 1968, **23B**, 673.
6. Rubeška, I., *Chem. Listy*, 1973, **67**, 213.
7. Cresser, M. S., Keliher, P. N., and Wohlers, C. C., *Analyt. Chem.*, 1973, **45**, 111.
8. Knapp, D. O., Omenetto, N., Hart, L. P., Plankey, F. W., and Winefordner, J. D., *Analytica Chim. Acta*, 1974, **69**, 455.
9. Boumans, P. W. J. M., and Brouwer, G., *Spectrochim. Acta*, 1972, **27B**, 247.
10. Snelleman, W., Rains, T. C., Yee, K. W., Cook, H. D., and Menis, O., *Analyt. Chem.*, 1970, **42**, 394.
11. Kawamura, H., Tanaka, G., and Ohyagi, Y., *Spectrochim. Acta*, 1973, **28B**, 309.
12. Capacho-Delgado, L., and Sprague, S., *Atom. Absorption Newsl.*, 1965, **4**, 36.
13. Harrison, A., and Ottaway, J. M., *Proc. Soc. Analyt. Chem.*, 1972, **9**, 205.
14. Harrison, A., *M.Sc. Thesis*, University of Strathclyde, 1973.
15. Cioni, R., Mazzucotelli, A., and Ottonello, G., *Analyst*, 1976, **101**, 956.
16. Rubeška, I., *Atom. Absorption Newsl.*, 1973, **12**, 33.
17. Bano, F. J., *Analyst*, 1973, **98**, 655.
18. Frache, R., and Mazzucotelli, A., *Talanta*, 1976, **23**, 389.
19. Hutton, R. C., Ottaway, J. M., Epstein, M. S., and Rains, T. C., *Analyst*, 1977, in the press.
20. Thompson, K. C., and Godden, R. G., *Analyst*, 1975, **100**, 198.
21. Cioni, R., Mazzucotelli, A., and Ottonello, G., *Analytica Chim. Acta*, 1976, **82**, 415.
22. Ottaway, J. M., and Shaw, F., *Analyst*, 1975, **100**, 438.
23. Epstein, M. S., Rains, T. C., and O'Haver, T. C., *Appl. Spectrosc.*, 1976, **30**, 324.
24. Ottaway, J. M., *Proc. Analyt. Div. Chem. Soc.*, 1976, **13**, 185.
25. Ottaway, J. M., and Shaw, F., *Appl. Spectrosc.*, 1977, **31**, 12.
26. Ottaway, J. M., and Shaw, F., *Analyst*, 1976, **101**, 582.
27. Hutton, R. C., and Ottaway, J. M., to be published.
28. Rains, T. C., and Menis, O., *Analyt. Lett.*, 1974, **7**, 715.

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Determination of Bismuth in Steels and Cast Irons by Atomic-absorption Spectrophotometry with an Induction Furnace: Direct Analysis of Solid Samples

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A graphite induction furnace has been constructed within a Perkin-Elmer 300S atomic-absorption spectrophotometer for the determination of bismuth in 2–12-mg samples of steels and irons that are dropped into the furnace. Calibration graphs of peak absorbance *versus* mass of bismuth are best constructed by use of standard alloys of roughly similar composition to the samples being analysed but, in the absence of suitable standard alloys, semi-quantitative results can be obtained if calibration graphs are prepared using small volumes of a standard bismuth nitrate solution.

Samples of alloys can be added to the furnace at 2–5-min intervals. Information is presented on the calibration graphs and on the accuracy, precision and limits of detection of the method for 40 high-purity irons and steels and for nine cast irons. With steels containing more than 0.04 $\mu g\ g^{-1}$ of bismuth, relative standard deviations of 3–10% are usually achieved. The limit of detection for bismuth in steels is 0.004 $\mu g\ g^{-1}$ when using this method.

Keywords: Bismuth determination; steel and cast iron analysis; atomic-absorption spectrophotometry; induction furnace; direct analysis of solids

An element present in very low concentrations will have no appreciable effect on the mechanical properties of a metal provided that it is uniformly distributed throughout the material. However, if certain elements are confined to dislocations or grain boundaries, they can exert profound effects on the properties of metals when present even at concentrations less than 0.001% (10 $\mu g\ g^{-1}$).[1] For example, concentrations of tin well below 1 $\mu g\ g^{-1}$ affect the rate of grain boundary migration in lead[2] and for nickel-alloy castings the SAE Aerospace Materials Specification 2280 lists maximum concentrations of certain trace elements, *viz.*, bismuth and tellurium, 0.5 $\mu g\ g^{-1}$, selenium, 3 $\mu g\ g^{-1}$, and lead and thallium, 5 $\mu g\ g^{-1}$.[3]

The metallurgist is particularly interested in knowing the concentration levels at which troublesome elements cease to impair the properties of alloys, but he is sometimes prevented from obtaining this information through research because the analyst is unable to determine such elements at the lowest concentration levels required. This inability arises because the limits of detection of the analytical methods are not good enough.

As long ago as 1961 Lynch[4] suggested that the maximum concentration of bismuth in Type 300 austenitic steel should be 10 $\mu g\ g^{-1}$ if hot embrittlement was to be avoided, and the problem of determining very low concentrations of bismuth in steels has received considerable attention since then.[5–9] The lowest reported limit of detection for bismuth in steels appears to be 0.2 $\mu g\ g^{-1}$,[5] although a limit of detection of 0.1 $\mu g\ g^{-1}$ has been reported for bismuth in nickel-base and high-temperature alloys.[10,11] However, if the metallurgist is to be given the maximum flexibility in his investigations it would be desirable to lower this limit of detection to 0.01 $\mu g\ g^{-1}$. At such low concentrations of bismuth, the amount of bismuth in a solution blank can be in excess of the amount of bismuth from a sample of steel dissolved in the solution and, therefore, a direct analysis of the metal sample without dissolution is to be preferred.

Headridge[12] described an induction furnace in conjunction with atomic-absorption spectrophotometry for the determination of trace elements in alloys. In this method solid samples of alloys were dropped into the furnace. For a 5-mg sample of cast iron, the concentration of bismuth that would produce 1% absorption was 0.4 $\mu g\ g^{-1}$. The Unicam SP90 atomic-absorption spectrophotometer used in that study was not ideally suited to the project and many improvements in the method have been made since the experiment was first described.

A re-designed furnace, used with a Perkin-Elmer 300S atomic-absorption spectrophotometer, has produced significantly better results and the limit of detection of bismuth in steels has been lowered to 0.004 $\mu g\ g^{-1}$. The new apparatus is described in this paper and results are presented for the determination of bismuth in 40 standard, high-purity irons and steels and in nine standardised cast irons.

Experimental

Materials

Standardised irons and steels. These were British Chemical Standards, alloys from the Institutet för Metallforskning, Sweden, cast irons from the British Cast Iron Research Association and four stainless steels from the British Steel Corporation.

Bismuth metal. Koch-Light Laboratories Ltd., 99.9995%.

Nitric acid. Fisons analytical reagent grade, sp. gr. 1.42.

Standard bismuth nitrate solution A (approximately 10 $\mu g\ cm^{-3}$). Weigh accurately, on a five-place balance, approximately 10 mg of bismuth. Dissolve the metal in 13 cm^3 of concentrated nitric acid and dilute the solution to 100 cm^3 in a calibrated flask. Immediately before use, dilute 10 cm^3 of this solution to 100 cm^3 in a calibrated flask with 2 M nitric acid.

Standard bismuth nitrate solution B (approximately 2.5 $\mu g\ cm^{-3}$). Immediately before use dilute 25 cm^3 of solution A to 100 cm^3 in a calibrated flask with 2 M nitric acid.

Graphite and carbon rods. The graphite rods were 525 mm long × 25 mm diameter and 900 mm long × 38 mm diameter in AGW grade graphite (British Acheson Electrodes). Specpure carbon was used to make rods 31 mm long × 6.5 mm diameter (supplied by Johnson Matthey Chemicals Ltd.).

Graphite powder. Natural graphite, 98–99%, obtained from Hopkin and Williams.

Copper tubing, 0.25 in *o.d.*

Vydaflex III sleeving, 6 mm *bore.*

Argon.

BASF catalyst R3–11. Before use regenerate the catalyst with hydrogen according to the manufacturer's instructions.

Apparatus

Atomic-absorption spectrophotometer. This was a Perkin-Elmer 300S instrument, equipped with a bismuth hollow-cathode lamp (Pye Unicam). The resonance lines at 306.8 and 227.7 nm were used with a slit-width of 0.2 nm. The output signal (absorbance) of the 300S, employed with minimum damping (position 1), was fed via a simple active noise filter to a 10-mV recorder (Honeywell Controls Ltd.). The noise filter damped minor signal fluctuations but did not affect the amplitudes of peak absorbances.

Induction generator. Radyne C50, 6-kW output, with a nominal output frequency of 450 kHz.

Optical pyrometer. Land disappearing filament pyrometer, Type DFP 2C. Temperatures could be measured with an accuracy of ± 10 °C but are not corrected for the emissivity value of graphite (0.90). The measured temperatures will be slightly lower than the true temperatures; the estimated maximum error is 35 °C at 2 500 °C.

Vibrator. Burgess Vibrotool engraver, Model No. VT62.

Flow meters. These instruments were capable of measuring 0–250 $cm^3\ min^{-1}$ [calibrated for air (Rotameter Mfg. Co. Ltd.)] and 0–10 $dm^3\ min^{-1}$ [calibrated for air (Flowbits)].

Furnace. This was constructed as is shown in Figs. 1 and 2. The central, vertical graphite rod was machined to produce a graphite well. Two holes were then drilled in the sides of the well at the correct height to accommodate the two short side-arms, which had been machined appropriately. The central graphite rod was inserted into a silica sheath of dimensions such as to allow a clearance of approximately 5 mm between the silica and the the graphite core. The graphite side-arms were then pushed through the side-arms of the silica sheath into the graphite core. They were a push-tight fit in the graphite core and a sliding fit in the silica side-arms. The copper tubing covered with Vydaflex sleeving was next wound around the silica sheath to give two turns above and four turns below the side-arms. Finally, the annular discs of graphite of push-tight fit were pushed on to the two ends of the graphite side-arm to abut against the ends of the silica. The space between the

graphite and the silica sheath was filled with graphite powder, using the vibrator to ensure an even packing.

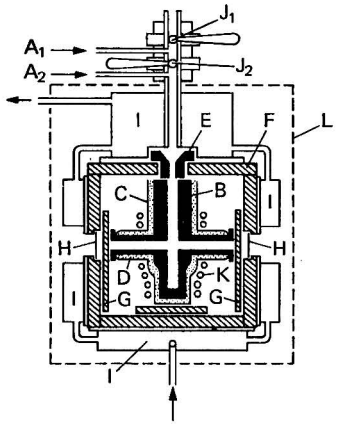

Fig. 1. Induction-heated graphite furnace for atomic-absorption spectroscopy. A_1, Argon inlet for purge gas from taps T_1 and T_2 (not shown in figure); A_2, argon inlet for stir gas; B, graphite core with side arms; C, silica sheath; D, graphite insulation; E, graphite funnel; F, Sindanyo box; G, shutter; H, silica window of diameter 40 mm; I, aluminium cooling panels through which cold water circulates (top and side panels are annular); J_1 and J_2, Econ-O-Miser ball valves, Type 44/46/T (Worcester Valve Co. Ltd.) of 13 mm nominal bore; K, induction coil; L, Faraday cage.

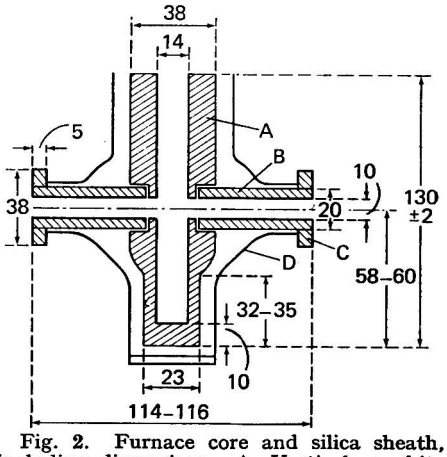

Fig. 2. Furnace core and silica sheath, including dimensions. A, Vertical graphite core; B, graphite side arm; C, annular graphite ring; D, silica sheath with thick silica base. All dimensions are in millimetres.

The Sindanyo box (a composite of asbestos and Portland cement) with water cooling panels was positioned on a shelf attached to the front of the Radyne generator such that the Perkin-Elmer 300S could be pushed into position on a trolley with the box in the large space normally occupied by the burner. The width of this space is 27.5 cm and it extends downwards from the top of the spectrophotometer for 22 cm. There is no restriction on access to this space from the front or the back of the instrument. The upper cooling panel and the valves, constructed as one unit, were removed followed by the Sindanyo lid. The silica sheath within the induction core was positioned in the Sindanyo box such that the light beam from the hollow-cathode lamp to the monochromator of the Perkin-Elmer 300S passed through the centre of the graphite side-arms. The well was checked to ensure that it was vertical. Next, the ends of the copper tubing, passing through slots in the back of the Sindanyo box, were attached to the induction generator, the Sindanyo lid was screwed back on to the box, the graphite funnel for directing samples into the vertical core was placed centrally on top of the core and the upper cooling panel, with the valves attached, was screwed into position on the Sindanyo lid.

The inlet tubes for argon gas were attached as is shown in Fig. 1. Argon destined for the inlet between the valves was passed through a simple tap T_1, through an absorption tube (40 cm long $\times$ 5 cm diameter) containing BASF catalyst in order to remove traces of oxygen, then through the 0–10 $dm^3\ min^{-1}$ flow meter and finally through a simple tap T_2 before use. Argon to be passed down the graphite core from the lower inlet tube was passed through an absorption tube (60 cm long $\times$ 5 cm diameter) containing BASF catalyst and through the 0–250 $cm^3\ min^{-1}$ flow meter before use. Argon passed into the graphite core escapes through

the side-arms into the Sindanyo box and hence through the slots at the back to the atmosphere.

When the furnace was in use, cold water from the public supply was circulated through the cooling panels in order to ensure that the temperature of the Perkin-Elmer 300S was not raised by the considerable amount of heat generated from the graphite core and also to keep the PTFE valve seats and seals cool. The furnace was also fitted with two Sindanyo shutters positioned inside the Sindanyo box. These shutters were usually pushed in, so that they lay between the side-arms and the silica windows. They were only pulled out of the light path when adjustments involving the beam were being made to the spectrophotometer and when a recording of an absorbance peak was required. Their function was to protect the hollow-cathode lamp, the lenses, the monochromator and the detector from excessive visible and infrared radiation and to minimise the deposition of very small metallic particles on the silica windows while the graphite core was being purged with argon. The furnace box was completely surrounded by a Faraday cage, which extended backwards to the front panel of the induction generator in order to ensure that the electronic components of the atomic-absorption spectrophotometer were unaffected by stray radiofrequency fields.

Method for Obtaining Absorbances for a Series of Solid Samples

Weigh 2–12 mg samples of steels or cast irons by using a five-place semi-micro balance and store them in small specimen tubes. If possible, weigh only one turning of the alloy.

Turn on the argon supplies and set the flow-rates to the upper and lower inlets at about 1 $dm^3\ min^{-1}$ and 100 $cm^3\ min^{-1}$, respectively, with the top valve closed. Turn on the induction generator according to the manufacturer's instructions and bring the graphite core up to the required temperature (see Table I) over a period of about 20 min. Allow the temperature to stabilise for a further 10 min. The temperature of the furnace is determined by opening both valves and looking down through the optical pyrometer and the open valves into the base of the furnace. Close the top valve.

TABLE I

EXPERIMENTAL CONDITIONS FOR THE DETERMINATION OF BISMUTH

Concentration range/$\mu g\ g^{-1}$	Wavelength/nm	Mass range of sample/mg	Temperature of graphite core/°C
15–200	227.6	2–10	1650–1750
1–30	306.8	2–12	1800–2000
<3	306.8	1–12	2050–2200

Pull out the shutters, select the appropriate wavelength on the atomic-absorption spectrophotometer (see Table I) and push in the shutters. This procedure is carried out only once, at the beginning of a run. Open the top valve, close the bottom valve and drop a sample into the space between the valves. Close tap T_1, wait until the reading on the flow meter reaches zero, then close tap T_2 and immediately close the top valve. Pull out the shutters and press the auto zero button to set to zero absorbance. Switch on the recorder. Open the bottom valve to allow the sample to drop on to the base of the graphite core. The alloy melts and a cloud of atomic vapour, including the bismuth in the sample, is released into the light path, thus producing an absorbance peak on the recorder. When the recorder pen has fallen to about 10% of the peak absorbance, typically in 10–20 s, switch off the recorder and push in the shutters. Open tap T_2, and then tap T_1, in order to allow argon to purge the cloud of bismuth vapour from the furnace. Allow at least 2 min before adding the next sample. Continue in this manner until all of the samples have been added to the furnace. If peak absorbances are expected to be less than 0.1, use a scale expansion of $\times 5$.

Reduce the power to the furnace from the generator gradually over a period of 15 min. Switch off the induction generator but continue to pass argon down the graphite core and water through the cooling panels until the core has reached room temperature.

Calibration Graphs

For the determination of bismuth in steels, calibration graphs of peak absorbance *versus* amount of bismuth are obtained by dropping increasing amounts of BCS 330, which can be taken to contain 3.0 $\mu g\ g^{-1}$ of bismuth, into the graphite core under conditions capable of

producing absorbances up to 1.0. For the determination of bismuth in cast irons, calibration graphs are obtained by dropping into the graphite core suitable amounts of stainless steels SS2, SS3 and SS4, containing 50, 68 and 105 $\mu g\ g^{-1}$ of bismuth, respectively. These alloys have been analysed to determine their bismuth contents in a collaborative study.[13] The amounts of alloys are selected to produce absorbances up to 1.0.

Calibration graphs can also be obtained for 1–25 and 25–300 ng of bismuth from aqueous solution using wavelengths of 306.8 and 227.7 nm, respectively, and core temperatures of approximately 2100 and 1700 °C, respectively. Suitable volumes of standard bismuth nitrate solution A or B are dispensed on to carbon discs (3 mm thick × 6.5 mm diameter) cut from Specpure carbon rod, these discs having been previously baked at 600 °C in an atmosphere of argon. The discs are dried under an infrared lamp for 30 s and then dropped into the graphite core. When the furnace is cool the used pellets are removed from the furnace well.

Analysis of Steels or Cast Irons for Bismuth

When a series of steels is being analysed suitable amounts are dropped into the graphite core over a period of 2–3 h and, during the same run, a variety of masses of BCS 330 are also added for the purpose of constructing a calibration graph, generally at the beginning of the run. At the end of the run the calibration graph is drawn and the mass of bismuth in each sample of steel is obtained from the graph. The concentrations of bismuth in the steels are then calculated. This is the best procedure to adopt because the absorbance peak for a particular mass of bismuth is temperature dependent and it is not worthwhile wasting time in bringing the temperature of the core to exactly the same value from one run to the next.

If cast irons are to be analysed, a similar procedure should be adopted.

Results

Typical calibration graphs prepared from BCS 330, from stainless steel SS2 and from bismuth nitrate solutions are shown in Figs. 3 and 4. The calibration graphs for the solutions had gradients that were about 10% different from those for the steels.

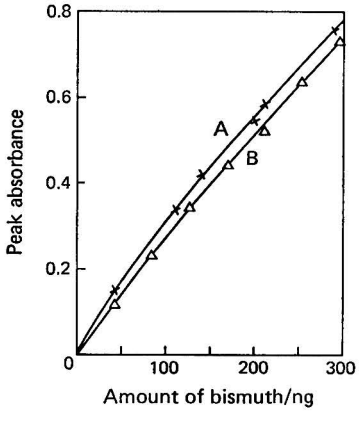

Fig. 3. Calibration graphs for bismuth in cast irons obtained with A, varying masses of stainless steel SS2, and B, varying volumes of bismuth nitrate solution A. Temperature of core, 1730 °C; resonance line, 227.7 nm.

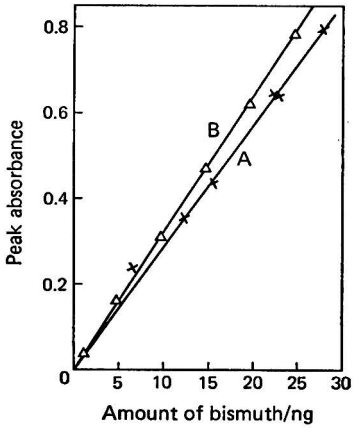

Fig. 4. Calibration graphs for bismuth in steels obtained with A, varying masses of mild steel BCS 330, and B, varying volumes of bismuth nitrate solution B. Temperature of core, 2070 °C; resonance line, 306.8 nm.

Many steels have been analysed to determine their bismuth content by dropping 1–12-mg samples into the furnace, usually at a temperature of 2100 °C. The results are shown in Table II, the reported bismuth content being the average of at least two determinations.

TABLE II

RESULTS FOR THE DETERMINATION OF BISMUTH IN STEELS

Steel	Average result/μg g^{-1}	Steel	Average result/μg g^{-1}
BCS 218/3	0.02	BCS 336	3.4
239/3	0.08	337	0.036
260/3	<0.004	338	0.052
277	0.07	339	0.05
320	0.55	340	0.04
321	0.071	341	0.05
322	0.074	342	0.6
323	0.057	451	0.20
324	0.071	452	0.093
325	0.35	453	0.087
326	0.031	454	0.10
327	0.038	455	0.086
328	0.055	456	0.014
329	26	457	0.012
330	3.0*	458	0.022
331	0.055	459	0.012
332	0.088	460	0.016
333	0.060	SS1 (Type 310)	0.010
334	0.047	JK† 1C	0.006
335	0.062	2C	0.010

*Assumed value.
†JK 1C is an ultra-pure mild steel; JK 2C is a low-alloy steel.

The limit of detection of the method is 0.004 μg g^{-1}. The limit was determined by dropping 11 samples of BCS 327, approximately 10 mg in mass, into the furnace and obtaining the standard deviations of the peak heights normalised to exactly 10 mg of each sample. The limit of detection is twice the standard deviation (0.002 μg g^{-1}).

Results for the determination of bismuth in nine cast irons are shown in Table III.

TABLE III

RESULTS FOR THE DETERMINATION OF BISMUTH IN CAST IRONS OBTAINED BY VARIOUS WORKERS

	Bismuth content/μg g^{-1}				
			Hunter *et al.*[9]		
Cast iron	BCIRA	Headridge and Richardson[14]	C furnace	C filament	Described method
D1	85	82			75
2	130	131			121
3	16	15	16	26	18
4	65	62	90	84	68
5	180	184			187
6	85	84			86
7	19	21			28
8	63	60	65	71	69
9	90	92	88	105	97

The precision of the method has been determined on numerous occasions and typical results are shown in Table IV.

TABLE IV

PRECISIONS OBTAINED WITH THE INDUCTION FURNACE METHOD

Alloy	Number of determinations	Relative standard deviation, %
BCS 327	11	5.6
BCS 330	6	2.9, 7.2, 7.7, 9.9
BCS 336	6	3.4, 8.4
Cast iron D8	6	3.0

Discussion

The results for stainless steels SS2, SS3 and SS4 were obtained as a result of a collaborative study and are likely to be fairly accurate.[13] However, SS2 contains the lowest concentration of bismuth, at 50 $\mu g\ g^{-1}$, and this is not a suitable standard for steels containing less than 5 $\mu g\ g^{-1}$ of bismuth. A search of the literature revealed that BCS 330 had been analysed to determine bismuth by Frech,[7] Fleming and Ide[8] and Hunter *et al.*,[9] who reported the concentration of bismuth to be 2.8, 3 and 3.4 $\mu g\ g^{-1}$, respectively. Therefore, in this investigation, the concentration of bismuth in BCS 330, used as the standard for steels, was taken to be 3.0 $\mu g\ g^{-1}$. However, in the absence of a solid standard it should still be possible to obtain semi-quantitative results for the determination of bismuth in steels and cast irons by using standard bismuth nitrate solutions. It can be seen from Figs. 3 and 4 that the calibration graphs for solid samples and solutions are not coincident but their slopes do not differ appreciably. Because of the different sequence of events in the production of bismuth atoms from metal samples and bismuth nitrate deposits on graphite discs it is not surprising that the calibration graphs are not coincident. In fact, only a few calibration graphs have been obtained from solutions and it would be of considerable interest to compare calibration graphs obtained from standard steels and standard bismuth nitrate solutions over a wide range of temperatures.

The shapes of the absorbance peaks at 1660 and at 2200 °C are shown in Fig. 5 for two steels. As the temperature increases the width of the peak decreases. Absorbance peaks for bismuth nitrate on graphite discs at 1730 °C were slightly broader than peaks of identical height for bismuth from steel at the same temperature. Absorbance peaks of the same height for bismuth nitrate on graphite discs and for bismuth from steel were the same shape at 2070 °C. The width at half the absorbance peak height, expressed in seconds, is greater than that usually obtained with a Massmann furnace. This is because the volume of the graphite core and side-arms is greater than the volume of a Massmann furnace and the flow-rate of stir gas is fairly low at 100 $cm^{-3}\ min^{-1}$.

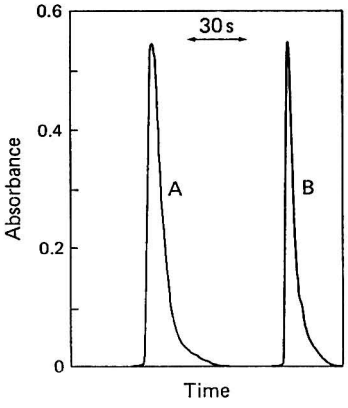

Fig. 5. Absorbance peaks for A, 181 ng of bismuth from steel SS2 at 1660 °C using the line at 227.7 nm, and B, 21.8 ng of bismuth from steel BCS 330 at 2200 °C using the line at 306.8 nm.

Information on the accuracy of the results given in Tables II and III can be obtained by comparing these results with those obtained by use of other methods. This is done in Tables III and V. Satisfactory agreement between our results and those of other workers has been obtained in most instances, and the results for BCS 329 and 336 are particularly informative. Normally, calibration at the beginning of a run is adequate for up to 3 h. If the very best accuracy that can be achieved with the furnace has to be guaranteed, then two samples of the standard alloy can be added after every ten samples to verify that no drift associated with changes in temperature or the argon flow-rate has occurred. In practice, the tem-

perature of the core and the flow-rate of argon have been found to be steady during a run.

In Table II the results for BCS 218/3, 239/3, 277, 339, 340, 341 and 342 are reported to only one significant figure because the graphite core was nearing the end of its useful life when these determinations were made. Increased scatter in the results and poorer sensitivities are found when the time for replacing a core is near.

TABLE V

RESULTS FOR THE DETERMINATION OF BISMUTH IN STEELS OBTAINED BY VARIOUS WORKERS

Steel	Bismuth content/$\mu g\ g^{-1}$ Hunter *et al.*[9]	Fleming and Ide[8]	Frech[7]	Attwell and Golden[5]	Described method
BCS 239/3		<1			0.08
320	0.8	<1			0.55
321	<0.4	<1			0.071
322		<1			0.074
323		<1			0.057
324		<1			0.071
325	0.4	<1			0.35
326	<0.4	<1			0.031
327		<1			0.038
328		<1			0.055
329	23, 34*	22			26
330	3.4	3	2.8		3.0†
334				<0.2	0.047
335				<0.2	0.062
336			3.2	4.0	3.4
337				<0.2	0.036
JK 1C			<0.5		0.006
2C			<0.5		0.010
Limit of detection	0.4	1	0.5	0.2	0.004

* Using a carbon filament, otherwise with a carbon furnace.
† Assumed value.

Leaving these seven results out of consideration, most of the other bismuth contents reported in Table II should be within 10% of the actual values provided that the bismuth content is in excess of 0.04 $\mu g\ g^{-1}$ and assuming that the bismuth content of BCS 330 is exactly 3.0 $\mu g\ g^{-1}$. The accuracy will be poorer at concentrations less than 0.04 $\mu g\ g^{-1}$. These statements are made on the assumption that other elements in the steels that have appreciable vapour pressures at 2100 °C are without effect on the bismuth peak heights.

It is likely that manganese (0.45% in the standard, BCS 330) would be the element producing most atoms in the light path at 2100 °C; manganese boils at 2150 °C. For BCS 329 and 336, the method described in this paper has produced bismuth contents of 26 and 3.4 $\mu g\ g^{-1}$, respectively, which are in good agreement with the results of other workers, namely 23 and 22 $\mu g\ g^{-1}$ and 3.2 and 4.0 $\mu g\ g^{-1}$, respectively. However, BCS 329 and BCS 336 contain 0.12 and 0.81% of manganese, respectively, which are different values from the 0.45% in BCS 330. Also, when 0.5- and 1.7-mg samples of Specpure manganese were dropped into the furnace (five times to several hundred times more manganese than in the steel samples) no detectable absorbance readings resulted, indicating that there is no scattering of light from atoms in the light path. In addition, a calibration graph was prepared at 2 250 °C using 4.7–9.4-mg samples of BCS 330 and, in the same run, 4.7 mg of BCS 330 plus 6.0 mg of Specpure manganese, and 9.7 mg of BCS 330 plus 4.0 mg of Specpure manganese, were added to the furnace. The two peak absorbances also lay on the calibration graph. Therefore, it appears that various amounts of manganese in steels and of other more volatile elements that produce atoms in the light path at much lower concentrations probably have little effect on the accuracy of the results.

Most steels contain low concentrations of sulphur, phosphorus, arsenic and antimony. If part or all of these elements is released into the gas phase when alloy samples are dropped

into the graphite core, then these elements may be present as molecules in the light path and the possibility of molecular absorption at the wavelength of the bismuth resonance lines cannot be overlooked. To test for possible molecular absorption the bismuth hollow-cathode lamp was replaced with a deuterium lamp and samples of a steel known to contain sulphur, phosphorus, arsenic and antimony were dropped into the furnace with instrumental settings of 306.8 nm and slit width 0.2 nm. In all instances the absorbance was zero, showing that molecular absorption was absent.

For the cast irons, fairly good agreement has been obtained between our results and those of others when it is considered that the only suitable metal standards available for the cast irons were the series of three stainless steels with compositions appreciably different from those of cast irons.

The precisions shown in Table IV are typical of those obtained with the induction furnace. The variations in relative standard deviation are due mainly to some inhomogeneity in the samples, and the effect of taking only six samples. For a perfectly homogeneous material the relative standard deviations are expected to be about 2–4%. Poorer precision is found as the core nears the end of its useful life, as was mentioned above. This phenomenon may be associated with a more turbulent flow of argon through a furnace core that has suffered erosion during use. If the relative standard deviation for an alloy exceeds 10%, it is advisable first to check the equipment with an alloy such as BCS 330 in order to make sure that the graphite core is vertical and properly aligned with the light beam and graphite funnel and that no deterioration in the performance of the atomic-absorption spectrophotometer has occurred. If a good precision is still obtained for BCS 330, then one can suspect that the relative standard deviation in excess of 10% for the other alloy under investigation is caused by appreciable inhomogeneity in the sample with respect to bismuth. Certainly a criticism of this very sensitive method for determining bismuth in metals is that the small samples may not be representative of the material as a whole. This is a particularly valid criticism if inhomogeneity of the material under study is to be expected. However, it is easy to test for inhomogeneity by adding, say, six samples of the material to the furnace and obtaining the relative standard deviation for the concentration of bismuth. For the steels and cast irons investigated by the author no evidence of excessive scatter due to segregation was found in the results.

The limit of detection at 0.004 $\mu g\ g^{-1}$ is 50 times better than the previous best for steels.[5] If necessary, it should be possible to improve it even further by using the most sensitive resonance line for bismuth at 223.1 nm. However, the intensity of this line from the Pye Unicam hollow-cathode lamp was rather low and, therefore, the 223.1-nm line was not used by us.

Samples can be dropped into the furnace at 2-min intervals, although it is advisable to extend the time interval between the additions of samples to 5 min if results of the highest precision are required. This increased interval is to ensure that all of the bismuth from one sample is completely removed from the furnace, particularly from the side-arms, before the next sample is added. Usually a hot core lasts for at least 50 h and no problems have been experienced with build-up of material in the bottom of the well. A core, at present being employed for the determination of lead in steels, has been in operation for approximately 45 h, is still producing satisfactory results and has had added to it 475 samples, *i.e.*, about 2.4 g of steel. In the early studies with this furnace, the annular graphite rings on the ends of the side-arms were not fitted and the life of the core was very dependent on the rate at which the graphite ends of the side-arms eroded, being finally terminated when the graphite powder insulation spilled out of the side-arms. This erosion occurred in spite of the removal of traces of oxygen from the argon gas. It may have been caused by oxidation of the graphite during the out-gassing of air as the temperature of the furnace was raised at the start of a run. Also, the flow of purge gas through the graphite core may have been insufficient to remove rapidly all of the air from the Sindanyo box, thus producing slow erosion effects on the outside of the graphite. Placing the annular graphite rings on the ends of the side-arms has prolonged the life of a graphite core very considerably. The erosion of the graphite now occurs on the periphery of the rings, where it is of little consequence, and the life of the core assembly is usually terminated by a marked deterioration in the condition of the silica sheath.

At present the furnace is being employed to investigate the determination of a wide range of troublesome elements in steels and nickel-base and copper-base alloys.

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References

1. Dillamore, I. L., and Dulieu, D., BSC Report MG/CC/Conf. Proc./617/72, British Steel Corporation, London, 1972, p. 75.
2. Aust, K. T., and Rutter, J. W., *Trans. Metall. Soc. AIME*, 1959, **215**, 820.
3. Holt, R. T., and Wallace, W., *Int. Metals Rev.*, 1976, **21**, 1.
4. Lynch, D. W. P., *Elect. Furn. Proc. AIME*, 1961, **19**, 220.
5. Attwell, M. G., and Golden, G. S., *Appl. Spectrosc.*, 1973, **27**, 464.
6. Vassilaros, G. L., *Talanta*, 1974, **21**, 803.
7. Frech, W., *Z. Analyt. Chem.*, 1975, **275**, 353.
8. Fleming, H. D., and Ide, R. G., *Analytica Chim. Acta*, 1976, **83**, 67.
9. Hunter, J. K., Ottaway, J. M., and Shaw, F., *Metallurgia Metal Form.*, 1976, **43**, 198.
10. Drinkwater, J. E., *Analyst*, 1976, **101**, 672.
11. Welcher, G. G., Kriege, O. H., and Marks, J. Y., *Analyt. Chem.*, 1974, **46**, 1227.
12. Headridge, J. B., *Lab. Pract.*, 1974, **23**, 5.
13. Statham, R. F., BSC Report MG/CC/567/72, British Steel Corporation, London, 1972.
14. Headridge, J. B., and Richardson, J., *Analyst*, 1970, **95**, 930.

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Effect of Different Sample Preparation Methods on the Atomic-absorption Spectrophotometric Determination of Calcium in Plant Material

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A study is reported showing the variability in the figures for calcium content obtained on certified standard reference plant material that had been subjected to five different methods of digestion. The results show that some methods of preparation resulted in inaccurate calcium contents. Choice of acids and methods for digesting the plant material to the stage where calcium can be extracted into dilute nitric acid, time and concentration of releasing agents (lanthanum and strontium) significantly affected the calcium results. The use of a dinitrogen oxide - acetylene flame or additional dilutions so that the calcium content of the solution was approximately 1–5 p.p.m. usually gave acceptable results.

Keywords: *Calcium determination*; *atomic-absorption spectrophotometry*; *sample digestion*; *flame oxidant*

Numerous papers have dealt with the various interferences in the determination of calcium by means of atomic-absorption spectroscopy. David[1] used additions of magnesium and sulphuric acid to both samples and standards to overcome interferences caused by phosphorus, aluminium and silicon. Amos and Willis[2] showed that calcium sensitivity can be significantly increased by use of a high-temperature flame. Manning and Capacho-Delgado[3] studied the use of the dinitrogen oxide - acetylene flame compared with the air - acetylene flame.

In this study, we used the National Bureau of Standards Standard Reference Plant Material with a certified calcium content of 2.09 $\pm$ 0.03% and determined the effects of choice of method for digesting the plant material, choice and concentration of releasing agents, the time lag between dilution with releasing agent and analysing the sample, choice of flame oxidant and the effect of additional dilution of the solution on the calcium figure obtained. Use of the term "digestion" is not intended to imply complete digestion, but digestion only to the stage where calcium can be extracted into dilute nitric acid.

Experimental

A 75-g sample of the National Bureau of Standards Standard Reference Material, 1571 Orchard Leaves, was dried as recommended at 90 °C for 24 h. Three 0.5-g samples were weighed to four decimal places for each of five methods. The same acid stock was used for each method. The acids were delivered through an automatic burette assembly (50 ml in 0.10-ml divisions; 200-ml reservoir). All samples were pre-digested overnight, with the obvious exception of those undergoing treatment by the dry-ashing method.

Three reagent blanks were run simultaneously with each set of three samples for each method. The timing of all procedures was such that the final dilutions to 50 ml could be carried out at the same time. One set of samples was diluted 1 + 9 with the releasing agents immediately after the samples had been diluted to 50 ml with distilled water. Another set was diluted 24 h later. A third set of samples that contained 0.5% of lanthanum was prepared at the same time as the first set of releasing dilutions and set aside undisturbed until the end of the study to check for any possible handling error. All analyses were standardised against their appropriate releasing standards (*e.g.*, 1% lanthanum against 1% lanthanum standards). Lanthanum and strontium releasing agents are defined by the percentage of metal in the solution. The final results were compared with the NBS certified calcium content of 2.09 $\pm$ 0.03%. This figure was obtained by using a nitric acid - perchloric acid digestion.

Both atomic-absorption spectroscopy and flame-emission spectrometry were used by the NBS to obtain the final certified calcium content.

Reagents

Perchloric acid, doubly vacuum-distilled (70%).
Nitric acid, concentrated (70.6%).
Sulphuric acid, concentrated (97.5%).
Vanadium(V) oxide, crystals.
Lanthanum oxide, powder.
Strontium chloride ($SrCl_2.6H_2O$).
The above reagents were of American Chemical Society analytical-reagent grade.

Methods

Dry-ashing methods

The 0.5-g sample was placed in a Coors 00 porcelain crucible, the crucible placed in a cool muffle furnace and the sample ashed at 550 °C overnight. Two sets of three dry ashes were prepared.

One set of three ashes was dissolved in 3 ml of concentrated nitric acid and the other set of three ashes was dissolved in 3 ml of concentrated hydrochloric acid. All solutions were then diluted to 50 ml with distilled water and finally transferred into 2-oz Nalge polypropylene bottles.[4]

Reflux wet-ashing method

The 0.5-g sample was placed in a 50-ml calibrated flask with 2 ml of perchloric acid - sulphuric acid (7 + 1) and 4 ml of nitric acid and allowed to pre-digest overnight. The flasks were then placed on a hot-plate, the contents slowly brought to boiling and the boiling was continued until the solution was clear and dense white fumes of perchloric acid were evolved. The solutions were then cooled, diluted to 50 ml and transferred into 2-oz Nalge polypropylene bottles.[1]

Bottle method

The 0.5-g sample was placed in a 2-oz Nalge polypropylene bottle with a polypropylene screw-cap. Perchloric acid (2 ml) and nitric acid (4 ml) were added, the bottle was capped and the acids were allowed to pre-digest the samples overnight. Pre-digestion is important as the bottles would burst if heated immediately. The samples, together with their three blanks, were then placed in a hot water bath at approximately 70 °C for 2–3 h. The caps were next removed, 1–2 ml of distilled water added to the bottles and the heating was continued for approximately 2 h to expel the excess of volatile acid. The bottles were then cooled and their contents diluted to 50 ml. Each sample was stored in its original digestion bottle.[5]

Vanadium(V) oxide - nitric acid method

The 0.5-g sample was placed in a micro-Kjeldahl flask with 4 ml of nitric acid and 0.1 g of vanadium(V) oxide. The sample was allowed to pre-digest overnight, placed on a Kjeldahl digestion apparatus and boiled until clear. It was then cooled, diluted to 50 ml and transferred into a 2-oz Nalge polypropylene bottle.[6]

Determination of Calcium

All samples were analysed to determine calcium on a Perkin-Elmer, Model 303, atomic-absorption spectrophotometer equipped with a Perkin-Elmer recorder readout and Model 165 recorder. The 2-in single-slot burner was aligned at right-angles to the optical axis for the air - acetylene flame and samples were diluted 1 + 999. The three-slot Boling burner was aligned along the optical axis for those samples diluted 1 + 9 999. The dinitrogen oxide - acetylene burner was aligned at right-angles to the optical axis for the samples diluted 1 + 999 and aligned along the optical axis for those samples diluted 1 + 9 999.

The samples were analysed immediately after digestion, 1 h later and at intervals during the following 16 d.

TABLE I

CALCIUM CONCENTRATIONS (%) OBTAINED ON IMMEDIATE ANALYSIS AND MEAN CONCENTRATIONS AND VARIANCES FROM SEVEN DETERMINATIONS MADE AFTER A PERIOD OF 16 d

Releasing dilution made immediately after original digest was brought to volume. Analysis with air - acetylene flame. Figures in parentheses give variances in calcium content.

		Dry-ashing methods				Wet-ashing methods					
		Nitric acid		Hydrochloric acid		Reflux		Bottle		Vanadium(V) oxide	
Time elapsed/d	Concentration of releasing metal, %	La	Sr	La	Sr	La	Sr	La	Sr	La	Sr
0	0.5	1.62	1.50	2.00*	2.20*	1.12	2.20*	1.96*	2.00*	1.46	1.86
		1.62	1.44	2.18*	2.20*	1.18	2.20*	2.08*	2.00*		
		1.62	1.44	2.18*	2.26	1.18	2.20*	2.08*	2.00*		
	1.0	1.84	1.44	2.10*	2.00*	1.26	1.94	2.00*	1.68	1.72	1.68
		1.84	1.44	2.10*	2.00*	1.26	1.94	2.00*	1.68		
		1.84	1.44	2.18*	1.94	1.26	2.00*	2.00*	1.68		
16	0.5	1.53 (0.124)	1.50 (0.126)	2.01* (0.043)	2.23 (0.123)	1.10 (0.115)	2.20* (0.086)	1.94 (0.052)	1.97* (0.134)	1.45 (0.003)	2.64 (0.161)
		1.49 (0.135)	1.40 (0.095)	2.07* (0.112)	2.25 (0.111)	1.15 (0.112)	2.20* (0.086)	1.94 (0.075)	1.96* (0.124)		
		1.50 (0.126)	1.40 (0.095)	2.07* (0.112)	2.25 (0.109)	1.13 (0.103)	2.20* (0.086)	1.95 (0.073)	1.96* (0.124)		
	1.0	1.65 (0.164)	1.50 (0.100)	2.09* (0.160)	1.98* (0.102)	1.17 (0.104)	1.95 (0.078)	1.96* (0.074)	1.70 (0.088)	1.62 (0.018)	1.71 (0.004)
		1.60 (0.171)	1.44 (0.095)	2.09* (0.160)	1.98* (0.102)	1.18 (0.096)	1.95 (0.075)	1.96* (0.058)	1.69 (0.073)		
		1.60 (0.162)	1.45 (0.097)	2.09* (0.171)	1.98* (0.102)	1.18 (0.101)	1.99* (0.059)	1.96* (0.058)	1.69 (0.073)		

* Acceptable values within ±0.06% error of NBS certified value of 2.09 ± 0.03% calcium.

TABLE II

CALCIUM CONCENTRATIONS (%) OBTAINED ON IMMEDIATE ANALYSIS AND MEAN CONCENTRATIONS AND VARIANCES FROM FIVE DETERMINATIONS AFTER A PERIOD OF 15 d

Releasing dilutions made 24 h after original digest was brought to volume. Analysis with air - acetylene flame. Figures in parentheses give variances in calcium content.

		Dry-ashing methods				Wet-ashing methods					
		Nitric acid		Hydrochloric acid		Reflux		Bottle		Vanadium(V) oxide	
Time elapsed/d	Concentration of releasing metal, %	La	Sr	La	Sr	La	Sr	La	Sr	La	Sr
0	0.5	1.24	1.44	1.90	2.22	1.24	2.22*	1.96*	1.96*	1.32	1.72
		1.28	1.44	1.96*	2.22*	1.24	2.22*	1.96*	1.96*		
		1.28	1.44	1.96*	2.22*	1.24	2.22*	1.96*	1.96*		
	1.0	1.60	1.50	2.00*	1.96*	1.20	1.96*	1.96*	1.72	1.60	1.72
		1.56	1.44	2.00*	1.96*	1.20	1.96*	1.96*	1.72		
		1.60	1.44	2.00*	1.96*	1.20	1.96*	1.96*	1.72		
15	0.5	1.22 (0.117)	1.54 (0.124)	1.94 (0.140)	2.24 (0.102)	1.16 (0.157)	2.24 (0.102)	1.94 (0.068)	1.95 (0.082)	1.39 (0.002)	1.78 (0.008)
		1.24 (0.139)	1.49 (0.115)	1.98* (0.161)	2.24 (0.102)	1.16 (0.157)	2.23 (0.076)	1.94 (0.068)	1.95 (0.082)		
		1.24 (0.129)	1.49 (0.115)	2.00* (0.137)	2.24 (0.102)	1.16 (0.157)	2.25 (0.097)	1.94 (0.079)	1.95 (0.082)		
	1.0	1.61 (0.145)	1.55 (0.107)	2.05* (0.047)	2.02* (0.081)	1.19 (0.110)	2.02* (0.069)	1.96* (0.049)	1.72 (0.064)	1.59 (0.020)	1.73 (0.002)
		1.54 (0.151)	1.49 (0.115)	2.09* (0.054)	2.02* (0.081)	1.19 (0.110)	2.01* (0.078)	1.97* (0.052)	1.72 (0.064)		
		1.56 (0.144)	1.50 (0.104)	2.08* (0.052)	2.02* (0.081)	1.19 (0.110)	2.01* (0.078)	1.99* (0.039)	1.72 (0.064)		

* Acceptable values within ±0.06% error of NBS certified value of 2.09 ± 0.03% calcium.

TABLE III

CALCIUM CONCENTRATIONS (%) OBTAINED ON IMMEDIATE ANALYSIS AND MEAN CONCENTRATIONS AND VARIANCES FROM THREE DETERMINATIONS AFTER A PERIOD OF 10 d

Releasing dilutions made immediately after original digest was brought to volume. Analysis with dinitrogen oxide - acetylene flame. Figures in parentheses give variances in calcium content.

Time elapsed/d	Concentration of releasing metal, %	Dry-ashing methods				Wet-ashing methods					
		Nitric acid		Hydrochloric acid		Reflux		Bottle		Vanadium(V) oxide	
		La	Sr	La	Sr	La	Sr	La	Sr	La	Sr
0	0.5	2.00*	2.06*	2.00*	2.06*	2.00*	2.06*	2.08*	2.12*	2.00*	2.12*
		2.00*	2.06*	2.00*	2.06*	2.16*	2.06*	2.08*	2.12*		
		2.00*	2.06*	2.00*	2.06*	2.16*	2.06*	2.08*	2.12*		
	1.0	2.16*	2.20*	2.08*	2.06*	2.08*	2.12*	2.16*	2.12*	2.16*	2.12*
		2.16*	2.12*	2.08*	2.12*	2.16*	2.12*	2.16*	2.12*		
		2.16*	2.20*	2.08*	2.12*	2.16*	2.20*	2.16*	2.12*		
10	0.5	2.09* (0.090)	2.13* (0.110)	2.09* (0.090)	2.07* (0.081)	2.06* (0.104)	2.07* (0.081)	2.11* (0.058)	2.09* (0.083)	2.11* (0.006)	2.13* (0.011)
		2.06* (0.053)	2.07* (0.081)	2.09* (0.090)	2.10* (0.053)	2.19* (0.046)	2.05* (0.041)	2.11* (0.058)	2.09* (0.083)		
		2.06* (0.053)	2.10* (0.053)	2.09* (0.090)	2.10* (0.053)	2.19* (0.046)	2.07* (0.081)	2.11* (0.058)	2.12* (0.040)		
	1.0	2.13* (0.122)	2.18* (0.094)	2.07* (0.121)	2.07* (0.081)	2.09* (0.090)	2.13* (0.130)	2.13* (0.122)	2.13* (0.130)	2.19* (0.001)	2.12* (0.001)
		2.13* (0.122)	2.12* (0.004)	2.09* (0.150)	2.12* (0.120)	2.13* (0.122)	2.09* (0.083)	2.11* (0.122)	2.13* (0.130)		
		2.13* (0.122)	2.15* (0.061)	2.09* (0.150)	2.12* (0.120)	2.13* (0.122)	2.15* (0.136)	2.11* (0.155)	2.13* (0.130)		

* Acceptable values within ±0.06% error of NBS certified value of 2.09 ± 0.03% calcium.

TABLE IV

CALCIUM CONCENTRATIONS (%) OBTAINED ON IMMEDIATE ANALYSIS AND MEAN CONCENTRATIONS AND VARIANCES FROM THREE DETERMINATIONS AFTER A PERIOD OF 10 d

Releasing dilution made 24 h after original digest was brought to volume. Analysis with dinitrogen oxide - acetylene flame. Figures in parentheses give variances in calcium content.

Time elapsed/d	Concentration of releasing metal, %	Dry-ashing methods				Wet-ashing methods					
		Nitric acid		Hydrochloric acid		Reflux		Bottle		Vanadium(V) oxide	
		La	Sr	La	Sr	La	Sr	La	Sr	La	Sr
0	0.5	2.16*	2.08*	2.16*	2.08*	2.16*	2.00*	2.16*	2.00*	2.16*	2.16*
		2.24	2.08*	2.16*	2.08*	2.08*	2.00*	2.08*	2.08*		
		2.24	2.08*	2.16*	2.00*	2.16*	2.00*	2.00*	2.08*		
	1.0	2.18*	2.00*	2.18*	2.00*	2.18*	2.08*	2.24	2.08*	2.17*	2.00*
		2.18*	2.00*	2.18*	2.08*	2.18*	2.00*	2.24	2.08*		
		2.18*	2.00*	2.18*	2.08*	2.18*	2.00*	2.18*	2.00*		
10	0.5	2.16* (0.160)	2.02* (0.178)	2.16* (0.160)	1.99* (0.223)	2.16* (0.100)	1.97* (0.212)	2.16* (0.160)	1.97* (0.212)	1.85 (0.107)	1.92* (0.029)
		2.19* (0.167)	2.02* (0.223)	2.13* (0.122)	2.02* (0.178)	2.13* (0.166)	1.97* (0.212)	2.13* (0.166)	2.05* (0.291)		
		2.19* (0.167)	1.99* (0.223)	2.16* (0.160)	1.99* (0.170)	2.16* (0.160)	1.97* (0.212)	2.09* (0.204)	2.03* (0.264)		
	1.0	2.12* (0.104)	1.99* (0.170)	2.12* (0.104)	1.97* (0.301)	2.12* (0.104)	2.05* (0.221)	2.14* (0.125)	2.08* (0.180)	2.00* (0.018)	1.85 (0.043)
		2.12* (0.104)	1.97* (0.212)	2.12* (0.104)	2.03* (0.264)	2.12* (0.104)	1.97* (0.212)	2.14* (0.125)	1.99* (0.223)		
		2.12* (0.104)	1.97* (0.212)	2.12* (0.104)	2.03* (0.264)	2.12* (0.104)	2.00* (0.260)	2.12* (0.159)	2.02* (0.131		

* Acceptable values within ±0.06% error of NBS certified value of 2.09 + 0.03% calcium.

TABLE V

CALCIUM CONCENTRATIONS (%) OBTAINED ON IMMEDIATE ANALYSIS AND MEAN CONCENTRATIONS AND VARIANCES FROM THREE DETERMINATIONS AFTER A PERIOD OF 14 d

A second 1 + 9 releasing dilution was made 2 d after the initial releasing dilution. Analysis with air - acetylene flame. Figures in parentheses give variances in calcium content.

Time elapsed/d	Concentration of releasing metal, %	Dry-ashing methods				Wet-ashing methods					
		Nitric acid		Hydrochloric acid		Reflux		Bottle		Vanadium(V) oxide	
		La	Sr	La	Sr	La	Sr	La	Sr	La	Sr
0	0.5	2.10*	2.35	2.10*	2.35	2.10*	2.35	2.10*	2.25	—	—
	1.0	2.10*	2.05*	2.10*	2.05*	2.10*	2.05*	2.10*	2.05*	—	—
14	0.5	2.20* (0.005)	2.23 (0.007)	2.20* (0.005)	2.23 (0.007)	2.22* (0.007)	2.23 (0.007)	2.18* (0.007)	2.17* (0.007)	2.15* (0.000)	2.14* (0.004)
	1.0	2.22* (0.007)	2.02* (0.001)	2.17* (0.004)	2.02* (0.001)	2.18* (0.007)	2.02* (0.001)	2.18* (0.008)	2.02* (0.001)	2.20* (0.003)	2.03* (0.001)

* Acceptable values within ±0.06% error of NBS certified value of 2.09 ± 0.03% calcium.

TABLE VI

CALCIUM CONCENTRATIONS (%) OBTAINED ON IMMEDIATE ANALYSIS AND MEAN CONCENTRATIONS AND VARIANCES FROM THREE DETERMINATIONS AFTER A PERIOD OF 14 d

A second 1 + 9 releasing dilution was made 2 d after the initial releasing dilution. Analysis with dinitrogen oxide - acetylene flame. Figures in parentheses give variances in calcium content.

Time elapsed/d	Concentration of releasing metal, %	Dry-ashing methods				Wet-ashing methods					
		Nitric acid		Hydrochloric acid		Reflux		Bottle		Vanadium(V) oxide	
		La	Sr	La	Sr	La	Sr	La	Sr	La	Sr
0	0.5	2.10*	2.05*	2.10*	2.05*	2.10*	2.05*	2.10*	2.05*	—	—
	1.0	2.30	2.05*	2.20*	2.05*	2.20*	2.05*	2.20*	2.05*	—	—
14	0.5	2.17* (0.004)	2.15* (0.007)	2.15* (0.002)	2.15* (0.007)	2.15* (0.002)	2.18* (0.016)	2.20* (0.007)	2.18* (0.016)	2.18* (0.001)	2.10* (0.023)
	1.0	2.30 (0.000)	2.03* (0.034)	2.25 (0.002)	2.00* (0.022)	2.25 (0.002)	1.96* (0.037)	2.23 (0.002)	2.03* (0.103)	2.18* (0.001)	1.88 (0.076)

* Acceptable values within ±0.06% error of NBS certified value of 2.09 ± 0.03% calcium.

Results

The results are presented in Tables I–VI. In the interest of simplicity, only the calcium contents of the samples obtained on immediate analysis and the mean concentrations and variances obtained in subsequent analyses over a period of up to 16 d are presented. The results given in the tables are values after subtraction of their respective blanks; all blanks gave absorption readings of 1% or less.

Analysis of the duplicate set of 0.5% lanthanum samples, which had been prepared at the same time as the original set of 0.5% lanthanum samples and set aside undisturbed until the end of the study to evaluate any handling errors, showed that all samples except those treated by the vanadium(V) oxide method gave absorption values of less than 1%, while those subjected to the vanadium(V) oxide method gave values of less than 2%. This agreement indicates that the calcium contents obtained during the study were not influenced by repeated handling.

Table VII shows the range of absorption, dilution factors and the corresponding calcium contents in parts per million. It is an indication, in raw data form, of the extreme variability obtained by using the various analytical procedures, particularly the large absorption range when using the air - acetylene burner head at right-angles to the optical path.

TABLE VII

RANGE OF ABSORPTION READINGS, DILUTION FACTORS AND CALCULATED CALCIUM CONTENT OF SOLUTIONS

Flame or burner	Air - acetylene	Boling	Dinitrogen oxide - acetylene	Dinitrogen oxide - acetylene
Angle to optical axis	90°	180°	90°	180°
Absorption, %	21–48	14–33	32–38	19–40
Dilution factor	1 + 999	1 + 9 999	1 + 999	1 + 9 999
Calcium content, p.p.m.	9.8–22.6	2.2–3.2	17.8–20.6	1.6–2.5

Discussion

The level of error chosen as acceptable for this study was 0.06%. This restriction eliminates all calcium figures below 1.96 and above 2.22%, and corresponds to approximately ±1% absorption.

The magnitude of error between results obtained and the certified figure reached 54%. It is therefore obvious that the choice of method for calcium determination is critical and the analyst must be aware of the shortcomings of the various methods.

Dry ashing at 550 °C and dissolution of the ash in nitric acid gave results as low as 52.6% of the certified calcium content, and in no instance did it give an acceptable result with the air - acetylene flame (Tables I and II), regardless of the releasing agent used. However, it did give acceptable results with the dinitrogen oxide - acetylene flame (Tables III and IV). The second 1 + 9 dilution, resulting in a solution containing 1–5 p.p.m. of calcium, was satisfactory for both flames (Tables V and VI).

Dry ashing at 550 °C and dissolution of the ash in hydrochloric acid gave the most acceptable results regardless of time, releasing agent, dilution or flame oxidant (Tables I–VI). Strontium at 0.5% appears to have an enhancing effect compared with 1% of strontium with the air - acetylene flame (Tables I and II); this observation is also true for the wet-ashing reflux and wet pressure bottle methods.

The wet-ashing reflux method with nitric acid and perchloric and sulphuric acids (7 + 1) gave as low as 47.9% of the certified calcium content. Lanthanum consistently gave approximately 50% of the certified calcium content (Tables I and II) unless additional dilutions were made or a dinitrogen oxide - acetylene flame (Tables III and IV) was used. Strontium gave acceptable results with 50% of samples, regardless of the time lag, with the air - acetylene flame (Tables I and II). It usually gave acceptable results with the dinitrogen oxide - acetylene flame (Tables III and IV) and gave good results after additional dilution at the 1% of strontium level (Table V).

The wet pressure bottle method using 2 ml of perchloric acid and 4 ml of nitric acid gave good results if the solution was analysed within 24–36 h of the final dilution with lanthanum. Strontium gave good results at the 0.5% level, regardless of time (Tables I and II). The

dinitrogen oxide - acetylene flame, as before, reduced the various interferences well (Tables III and IV) and gave excellent results when the solution was analysed immediately. The use of additional dilution also gave satisfactory results (Tables V and VI).

The vanadium(V) oxide - nitric acid method gave very poor results (Tables I and II) except with additional dilution (Tables V and VI) or with the use of the dinitrogen oxide - acetylene flame (Tables III and IV).

The fact that the dinitrogen oxide - acetylene flame gave very good results regardless of choice and concentration of releasing agent suggested that a releasing agent might not be needed with this flame. This hypothesis was tested by comparing air - acetylene and dinitrogen oxide - acetylene flames. The results presented in Table VIII definitely show that, with few exceptions, a releasing agent is needed.

TABLE VIII

CALCIUM CONCENTRATIONS (%) OBTAINED WITHOUT USE OF A RELEASING AGENT

Comparison of air and dinitrogen oxide as oxidant for acetylene flame.

Time elapsed after digestion/d	Flame oxidant	Dilution ratio	Dry-ashing methods		Wet-ashing methods		
			Nitric acid	Hydrochloric acid	Reflux	Bottle	Vanadium(V) oxide
0	Air	1 + 999	2.36	2.36	2.36	2.42	2.42
0	Dinitrogen oxide	1 + 999	2.00*	1.92	1.86	2.36	0.70
0	Air	1 + 9 999	2.50	2.50	2.50	2.50	—
0	Dinitrogen oxide	1 + 9 999	2.45	2.45	2.45	2.45	—
2	Air	1 + 9 999	2.12*	2.22*	1.84	2.78	—
2	Dinitrogen oxide	1 + 9 999	2.64	2.56	2.64	2.64	—

* Acceptable values within ±0.06% error of NBS certified value of 2.09 ± 0.03% calcium.

Conclusions

Choice of digestion method, releasing agent and fuel oxidant affect the calcium results. Time appears to have a minimal statistical effect, but the over-all calcium results do appear to be more inconsistent with time and as the time lapse from dilution to analysis increases fewer results fall within the acceptable range of 2.09 ± 0.06% of calcium.

Ashing of the sample at 550 °C and dissolution of the ash in hydrochloric acid or the bottle pressure method are probably the two most reliable preparation methods if the analyst is unable or unwilling to use dinitrogen oxide. The use of dinitrogen oxide appears to eliminate most, if not all, of the sources of error. Additional dilution to bring the calcium content of the solution within the 1–5 p.p.m. range also helps to eliminate most errors (Tables I, II and V). The practice of placing the burner head at right-angles to the optical path, instead of additional dilution, proved to be questionable with most digestion and handling procedures unless the dinitrogen oxide - acetylene flame was utilised.

All of the results show that most of the calcium problems discussed in this paper are of flame chemistry origin. Overall, strontium at the 0.5% level appears to enhance the calcium results more than strontium at the 1.0% level.

References

1. David, D. J., *Analyst*, 1959, **84**, 536.
2. Amos, M. D., and Willis, J. B., *Spectrochim. Acta*, 1966, **22**, 1325.
3. Manning, D. C., and Capacho-Delgado, L., *Analytica Chim. Acta*, 1966, **36**, 312.
4. Heckman, M., *J. Ass. Off. Analyt. Chem.*, 1967, **50**, 46.
5. Adrian, W. J., *Analyst*, 1973, **98**, 213.
6. Bartschmid, B. R., and Skogerboe, R. K., "Atomic Absorption Spectrometry," Department of Chemistry, Colorado State University, 1975, p. 43.

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Automatic Determination of Sulphate in Water Samples and Soil Extracts Containing Large Amounts of Humic Compounds

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An automatic nephelometric method for the determination of sulphate in water samples, soil leachates and soil extracts is described. Dialysis of the samples eliminates interference from dissolved coloured humic compounds and suspended matter. The capacity of the method is 24 samples per hour and the precision is $s = 0.49$ mg l^{-1} of sulphate-sulphur in the range 1–100 mg l^{-1} of sulphate-sulphur. The method gives more reliable results for coloured samples than does the turbidimetric method with colour correction.

Keywords: *Sulphate determination*; *water*; *soil*; *automatic analysis*; *nephelometry*

The presence of dissolved humic compounds and suspended matter seriously interferes in the turbidimetric[1] and nephelometric[2] methods of sulphate determination. In our laboratory, sulphate analysis is concerned mainly either with soil or peat leachates that contain large amounts of coloured humic compounds (1–100 mg l^{-1} of organic carbon), or with soil extracts in 0.01 N calcium phosphate for the determination of soluble and adsorbed sulphate.

The large amount of calcium phosphate in the soil extracts interferes in the determination of sulphate by the recently described thorin method,[3] and the calcium and phosphate ions also interfere in the indirect determination of sulphate by atomic-absorption spectroscopy,[4,5] a method which is also relatively laborious. The removal of humic compounds by charcoal before turbidimetric determination of sulphate involves extra handling of samples.[6] As we required a single and preferably uncomplicated automatic analytical procedure, applicable to both water samples and soil extracts, we examined the possibility of removing dissolved humic compounds and suspended matter by use of dialysis. This paper describes a simple nephelometric determination of sulphate in dialysed aqueous samples.

Experimental

Apparatus

The automatic analyser consists of a Hook and Tucker A40 sampler, an Ismatic MP13 pump, a Perkin-Elmer, Model 1000, fluorescence spectrophotometer and a Perkin-Elmer, Model 56, recorder. The spectrophotometer is equipped with a 600-μl flow cell and measurements are made at 400 nm. The dialyser is made of two circular 15 cm diameter Plexiglas plates, 24 mm thick, with a 15 cm diameter Sartorius SM 11730 cellulose acetate dialysis membrane (flow path 0.10 × 100 cm). The flow diagram is shown in Fig. 1.

Samples

The water samples are preserved with concentrated hydrochloric acid (7.0 ml per litre of sample) immediately after collection in the field and stored at 4 °C until required for analysis. The soil extracts are prepared by shaking 15–30 g of air-dried peat or soil for 1 h with 125 ml of 0.01 M calcium bis(dihydrogen orthophosphate) solution, previously preserved with chloroform.[6] The slurry is centrifuged and analysed within 3 d.

Reagents

Barium chloride - Tween 80 *solution.* Barium chloride (40 g) and Tween 80 (50 ml) are dissolved in water and the solution is diluted to 1 000 ml with water.

Standard sulphate solutions. Standard sulphate solutions are prepared in 0.08 N hydrochloric acid for water samples and in 0.01 N calcium orthophosphate solution for soil extracts. Series of standards are prepared to cover the range 1–100 mg l^{-1} of sulphate-sulphur.

Supplementary sulphate solution. The solution contains 10 mg l^{-1} of sulphate-sulphur in 0.48 N hydrochloric acid, and is added so as to ensure that there are always sufficient sulphate ions present to exceed the solubility product of barium sulphate for samples low in sulphate content.

EDTA solution. EDTA, disodium salt (75 g) and sodium hydroxide (26 g) are dissolved in water and the solution is diluted to 1 000 ml with water.

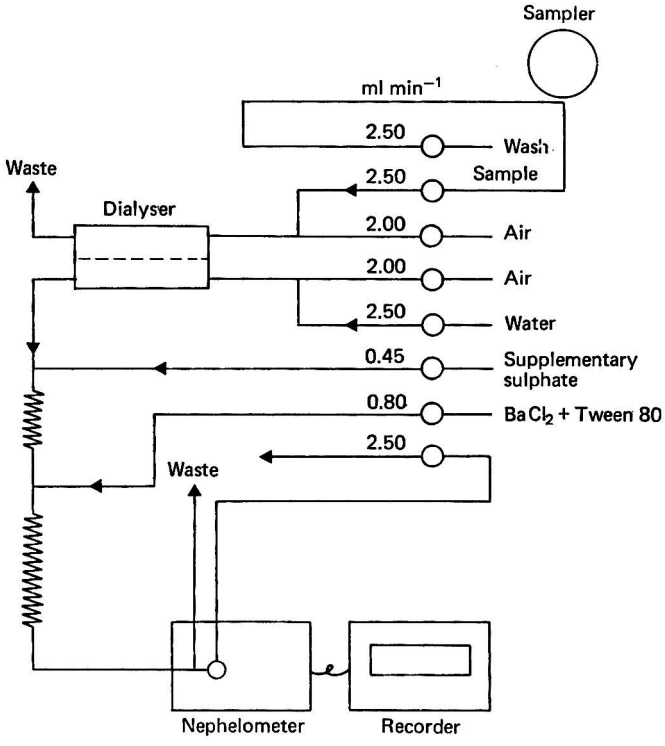

Fig. 1. Flow scheme for the automatic determination of sulphate by nephelometry.

Results and Discussion

The analytical basis of the nephelometric determination of sulphate described here is essentially the same as those for the turbidimetric[2,6,7] and nephelometric[1] techniques described earlier. The new development is the incorporation of a dialyser into the system.

The dialysis membrane has an exclusion limit for molecules with a relative molecular mass greater than 160 000 (pore size 35–20 nm). Even though the humic compounds dissolved in natural water, soil leachates and soil extracts cover a wide range of relative molecular masses, no detectable colour was found to interfere in the analysis of peat leachates after dialysis. With regard to organic carbon in these samples (20–50 mg l^{-1} of organic carbon), an average of 2.0% passed through the membrane. The membrane therefore effectively removes colour and organic carbon from the samples. For test solutions covering the range 10–100 mg l^{-1} of sulphate-sulphur, an average of 15% of the sulphate passed through the membrane. A membrane with a smaller pore size, *e.g.*, 10–5 nm, did not allow the passage of amounts of sulphate that were sufficiently large to be determined.

When changing from water samples to soil extracts, and also when starting to use a new

membrane, thorough rinsing with matrix solution is necessary in order to obtain a stable base line and reproducible results. In our laboratory the membrane lasts 4–5 weeks when used every day.

A recorder tracing indicating the sensitivity and reproducibility of the method is shown in Fig. 2. The sampling and wash times were set at 75 s. The working range is from 1 to 100 mg l^{-1} of sulphate-sulphur. The shift in the base line due to deposits of barium sulphate coating the wall of the nephelometric flow cell[6–8] can be controlled if the flow cell is rinsed with EDTA solution for 2 min after the passage of each 40 samples, and the whole manifold for 5 min at the end of each working day.

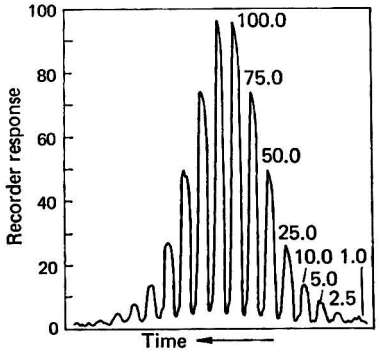

Fig. 2. Recorder trace of sulphate standards. Values on peaks represent sulphate-sulphur (mg l^{-1}).

Precision and Accuracy

In our laboratory, using coloured (200 mg l^{-1} of platinum)* peat leachates at concentrations of 7.37 and 67.8 mg l^{-1} of sulphate-sulphur, the standard deviations were 0.25 and 0.49 mg l^{-1} of sulphate-sulphur, respectively. Using soil extracts [0.01 N calcium bis(dihydrogen orthophosphate)] at concentrations of 7.60 and 52.3 mg l^{-1} of sulphate-sulphur, the standard deviations were 0.34 and 0.46 mg l^{-1} of sulphate-sulphur, respectively.

The results of recovery studies on three coloured water samples and three soils are given in Tables I and II, respectively. For the water samples the recoveries were good, the highest recovery being 103 and the lowest 97%, with a mean of 99.6%. The recoveries were also good for the soil extracts, the highest and lowest recoveries being 103 and 95%, respectively, with a mean of 99.2%.

TABLE I

SULPHATE RECOVERY STUDIES ON THREE PEAT LEACHATES MEASURED BY NEPHELOMETRY ON DIALYSED SAMPLES

Sample	Colour/ mg l^{-1} Pt	Sulphate present, p.p.m.	Sulphate added, p.p.m.	Total sulphate calculated, p.p.m.	Total sulphate recovered, p.p.m.	Recovery, %
1	280	2.0	5.0	7.0	7.0	100
			25.0	27.0	27.5	102
			50.0	52.0	51.0	98
2	240	12.5	5.0	17.5	18.0	103
			25.0	37.5	38.0	101
			50.0	62.5	63.0	101
3	90	20.5	5.0	25.5	24.5	96
			25.0	45.5	44.0	97
			50.0	70.5	69.0	98
					Mean:	99.6

Comparison with the Turbidimetric Method

Twenty-two samples of soil leachates (colour less than 100 mg l^{-1} of platinum) were analysed

*Based on a colour standard prepared from potassium hexachloroplatinate(IV) and cobalt(II) chloride.

TABLE II

SULPHATE RECOVERY STUDIES ON THREE SOIL SAMPLES MEASURED BY NEPHELOMETRY ON DIALYSED EXTRACTS

Sample	Sulphate present, p.p.m.	Sulphate added, p.p.m.	Total sulphate calculated, p.p.m.	Total sulphate recovered, p.p.m.	Recovery, %
Mineral soil	11.0	5.0	16.0	16.5	103
		25.0	36.0	34.5	96
		50.0	61.0	58.0	95
Raw humus	5.0	5.0	10.0	10.0	100
		25.0	30.0	29.0	97
		50.0	55.0	54.0	98
Peat	7.0	5.0	12.0	12.0	100
		25.0	32.0	33.0	103
		50.0	57.0	57.5	101
				Mean:	99.2

by a turbidimetric method with colour correction[9] and by the nephelometric method described here. The samples covered the range 1–56 mg l^{-1} of sulphate-sulphur. A *t*-test on the differences between results obtained by the two methods gave $t = 5.03$, which demonstrates that the two methods were significantly different ($P < 0.001$). Thirty highly coloured (150–850 mg l^{-1} of platinum) peat leachates at concentrations from 1 to 80 mg l^{-1} of sulphate-sulphur gave, by the same procedure, a *t*-value of 8.4, demonstrating the obvious difference between the two methods. For the individual samples, the nephelometric analysis performed on dialysed samples almost always gave the lower value. This difference agrees well with results obtained earlier.[10]

Interferences

Interferences in the determination of sulphate from some elements likely to be present in waters and soil extracts were examined at various concentrations. Sodium, potassium, magnesium, calcium, nitrate and phosphate did not interfere at concentrations of up to 0.001 N, the highest tested. Iron, when added at concentrations less than 15 mg l^{-1} to water samples preserved with hydrochloric acid, did not interfere in the sulphate determinations, but at concentrations of 30 and 60 mg l^{-1} of iron, the sulphate concentrations found were 5.0 and 9.0% lower, respectively, than for samples without added iron in the range 5–75 mg l^{-1} of sulphate-sulphur. This effect is only slightly related to the colour of the dissolved iron ions because at a concentration of 590 mg l^{-1} of iron in 0.08 N hydrochloric acid only 2.6% passes through the membrane.

Similarly, aluminium at concentrations less than 15 mg l^{-1} showed no significant interference in sulphate determinations, but at a concentration of 30 mg l^{-1} the results were 3% lower than for samples without aluminium.

The soil extracts that we analyse do not contain iron or aluminium in amounts large enough to give any interference. Rarely, and only for extremely acidic soil leachates or acidic soil extracts, will the iron or aluminium content be high enough to interfere in the sulphate determination as described here. In these instances the interfering ions can be precipitated prior to analysis.[10]

Conclusion

The nephelometric method for the determination of sulphate described here is simple, rapid and requires no pre-treatment of samples apart from preservation. The method can be used without modification for a large number of sample types, *e.g.*, natural water samples, soil leachates and soil extracts. The use of a dialysis membrane eliminates the problems of colour and interferences from suspended matter. The method covers the range 1–100 mg l^{-1} of sulphate-sulphur and, although the accuracy is relatively low for samples of low sulphate content, the simplicity of the method makes it useful in evaluating levels of available sulphate in water and soils, especially with regard to problems connected with plant nutrition.

References

1. American Public Health Association, American Water Works Association and Water Pollution Control Federation, "Standard Methods for the Examination of Water and Wastewater," Thirteenth Edition, American Public Health Association, New York, 1971, p. 334.
2. Toennies, G., and Bakay, B., *Analyt. Chem.*, 1961, **25**, 160.
3. Henriksen, A., and Bergmann-Paulsen, J. M., *Vatten*, 1974, **2**, 187.
4. Varley, J. A., and Poon Yew Chin, *Analyst*, 1970, **95**, 592.
5. Loeppert, R. H., and Breland, H. L., *Proc. Soil Crop Sci. Soc. Fla*, 1973, **32**, 145.
6. Sinclair, A. G., *N.Z. Jl Agric. Res.*, 1973, **16**, 287.
7. Basson, W. D., and Böhmer, R. G., *Analyst*, 1972, **97**, 266.
8. Sansum, L. L., and Robinson, J. B. D., *Commun. Soil Sci. Pl. Analysis*, 1974, **5**, 365.
9. Ogner, G., Haugen, A., Opem, M., Sjøtveit, G., and Sørlie, B., *Meddr. Norsk Inst. Skogfors.*, 1977, **33**, 84.
10. Hesse, P. R., *Editor*, "A Textbook of Soil Chemical Analysis," Murray, London, 1971, p. 301.

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Spectrophotometric Method for the Determination of Total Steroidal Sapogenin

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A specific method for the spectrophotometric determination of total steroidal sapogenin, based on colour reactions with anisaldehyde, sulphuric acid and ethyl acetate and applicable to microgram amounts, is described.

It has been shown that this determination can be carried out directly on a saponin solution and that there is virtually no interference from sugars, sterols, fatty acids and vegetable oil. The sapogenins have the same colorimetric properties whether they are in the free state, bound with sugars, esterified with acetic acid or mono- or polyhydroxylated. The method described is accurate (relative error 1.4%), rapid, easily automated and gives a chromophore with the same absorption spectrum with only one peak at 430 nm for all of the sapogenins tested: diosgenin, tigogenin, hecogenin, smilagenin, yonogenin, tokorogenin, etc. The molar absorption coefficient is approximately 49 000.

Keywords: *Steroidal sapogenin determination; spectrophotometry*

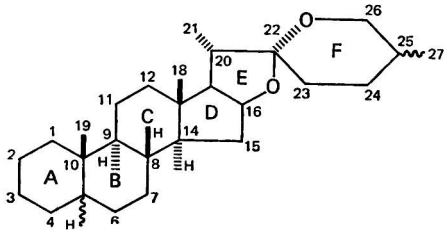
Steroidal sapogenins are raw materials that are widely used in the partial synthesis of steroidal drugs, and their economic importance is one of the reasons why much research has been carried out, especially on analytical methods. When we started our research on the possibility of using some plants growing in France to provide steroidal drugs, the previously employed methods, *viz.*, gravimetry,[1–3] infrared spectroscopy,[4,5] gas - liquid chromatography,[6–11] colorimetry,[12–23] densitometric thin-layer chromatography,[24,25] thin-layer chromatography followed by spectrophotometry[26,27] and column chromatography followed by infrared spectrophotometry,[28] were found to be unsuitable.

It appeared that spectrophotometry was the technique which would be the most useful for our purpose. However, most of the reagents used, for example, antimony(III) chloride in acidic solution or sulphuric acid and formaldehyde, were applicable only to sapogenins that have the Δ^{5-6} structure in the B-ring and a hydroxyl group in the 3-position (Table I). These characteristics also occur in phytosterols, and many sapogenins, such as tigogenin, gitogenin, hecogenin, smilagenin, yonogenin and tokorogenin, therefore cannot be determined. On the other hand, other methods determine only saturated sapogenins (reagents based on orthophosphoric acid and anisaldehyde) so that an additional method is needed for the determination of total sapogenin. Even the technique recently developed by Hiai *et al.*,[23] in which vanillin and sulphuric acid were used, is not specific for sapogenins because sterols, bile acids and triterpenoid sapogenins, which have a hydroxyl group in the 3-position, give chromophores. It is known that sapogenins treated with concentrated sulphuric acid produce characteristic chromophores, all of which, however, do not absorb at the same wavelength. In addition, the problem of interferences is particularly important in this instance. This deficiency led us to devise a method that is both more specific for sapogenins and allowed us to determine all sapogenins, whatever their structural particularities. Such properties are very important, especially when it is desirable to determine the content of sapogenins when their exact structure is not known, or when studying their physiology during certain vegetative phases. It has been shown that under these conditions there are important qualitative and quantitative differences.[18,29] Reactions involving the E and F rings of the molecules seemed to us to be a possible basis in developing a rapid, accurate and simple method.

After developing such a method, we tested it successfully on a saponin solution in order to evaluate directly the steroidal sapogenin content of the sample.

TABLE I

SAPOGENINS EXAMINED



Systematic name	Trivial name	C-25	C-5	Substituents
(25*R*)-Spirost-5-en-3β-ol	Diosgenin	*R*	Δ	3β-OH
(25*S*)-Spirost-5-en-3β-ol	Yamogenin	*S*	Δ	3β-OH
(25*R*)-5α-Spirostan-3β-ol	Tigogenin	*R*	α	3β-OH
(25*R*)-5β-Spirostan-3β-ol	Smilagenin	*R*	β	3β-OH
(25*S*)-5β-Spirostan-3β-ol	Sarsasapogenin	*S*	β	3β-OH
(25*R*)-5α-Spirostan-3β-ol-12-one	Hecogenin	*R*	α	3β-OH, 12-keto
(25*R*)-5α-Spirostane-2α,3β-diol	Gitogenin	*R*	α	2α,3β-OH
(25*R*)-5β-Spirostane-2β,3α-diol	Yonogenin	*R*	β	2β,3α-OH
(25*R*)-5β-Spirostane-1β,2β,3α-triol	Tokorogenin	*R*	β	1β,2β,3α-OH
(25*R*)-5β-Spirostane-2β,3α,4β-triol	Diotigenin	*S*	β	2β,3α,4β-OH
(25*R*)-5β-Spirostane-1β,2β,3α,5β-tetraol	Kogagenin	*R*	β	1β,2β,3α,5β-OH
(25*R*)-Spirosta-3,5-diene	Spirosta-3,5-diene	*R*	Δ	—
Δ⁵-Cholestene-3β,26-diol-16,22-dione	Kryptogenin	—	Δ	3β,26-OH

Experimental

Apparatus

Two types of spectrophotometer were used: a Jobin-Yvon instrument equipped with test-tubes with a 1-cm path length, and a Spectronic 20 instrument (Bausch and Lomb) equipped with test-tubes with a 1.27-cm path length.

Pedersen A pipettes were used for measuring microlitre volumes and Preciss A or Cornwall pipettes for millilitre volumes.

Reagents and Materials

Diosgenin, hecogenin acetate, smilagenin, tigogenin, β-sitosterol, cholesterol, lanosterol, cortisone, corticosterone, cortisol, palmitic acid, stearic acid, rhamnose, glucose, ethyl acetate, sulphuric acid and anisaldehyde were Merck products. (With some of the batches of ethyl acetate a coloured blank and a lower molar absorption coefficient were obtained. It appears that this effect is due to trace amounts of aldehydes that are difficult to eliminate.) Solasodin and tomatidin were obtained from Koch-Light Laboratories and campesterol from Applied Science Laboratories. The other sapogenins were obtained from various sources.

Method

We first considered the work of Lisboa,[30] Stahl[31] and Dawidar and Fayez,[32] who suggested the use of a reagent consisting of concentrated sulphuric acid (1.0 ml), anisaldehyde (0.5 ml) and acetic acid (5.0 ml) in order to reveal steroids, and particularly sapogenins, after their separation by thin-layer chromatography. However, this mixture is not applicable to spectrophotometry because it is unstable and rapidly develops a dark red colour. Our studies showed that the separate reagents (A) 0.5 ml of anisaldehyde plus 99.5 ml of ethyl acetate and (B) concentrated sulphuric acid were more suitable. Reagent A is stable. In order to avoid the use of concentrated sulphuric acid, it is possible to dilute it with the same volume of ethyl acetate, giving reagent C: 50 ml of concentrated sulphuric acid plus 50 ml of ethyl acetate.

Determination of sapogenin

The effects of various parameters are described under Results and Discussion, and only the standard method is described here.

Place 0–40 μg of steroid sapogenin dissolved in 2 ml of ethyl acetate in the test-tube and add 1 ml of reagent A and 1 ml of reagent C. After stirring, place the test-tubes in a water-bath maintained at 60 °C for 20 min, then allow them to cool for 10 min in a water-bath maintained at room temperature. Measure the absorbance at 430 nm.

It is possible to effect this determination without heating the tubes in the 60 °C water-bath if reagent B is used instead of reagent C.

Determination of saponin

Place in test-tubes a certain amount of saponin in an alcoholic solution (corresponding to a sapogenin content of between 0 and 40 μg). Place the tubes in a boiling water bath or in a hot air bath at 100 °C in order to remove the alcohol and, after cooling, add 2 ml of ethyl acetate and carry out the determination as for sapogenin.

Results and Discussion

In order to determine the most suitable conditions for the determination, we used diosgenin, which is the most important steroidal sapogenin for the partial synthesis of steroids.

During preliminary experiments, we tried several aromatic aldehydes (4-dimethylaminobenzaldehyde, vanillin and cinnamaldehyde) but they were less satisfactory than anisaldehyde. In addition, it was found that sulphuric acid cannot be replaced with other acids that were tested, namely nitric, hydrochloric, orthophosphoric and acetic acid.

Because of the concentration of anisaldehyde and sulphuric acid in reagents A and C, these substances limit the colour intensity. Nevertheless, it is possible to carry out this determination with smaller amounts of sulphuric acid; although the method is then less sensitive it can be of use in certain instances.

The structure of the coloured products formed during the reaction between the sapogenins, sulphuric acid and anisaldehyde has not been elucidated. However, on the basis of tests made with isomers or compounds with similar structures, it appears possible that it is more often the E and F rings that take part in condensation and other reactions. With saponins, the action of concentrated sulphuric acid causes hydrolysis and the sapogenins formed then react immediately with sulphuric acid, ethyl acetate and anisaldehyde.

Sapogenin Absorption Spectrum

The absorption spectrum (Fig. 1) was plotted by using a diosgenin concentration of 5 μg ml^{-1} and measuring the absorption between 380 and 700 nm. We observed only one peak, at 430 ± 1 nm.

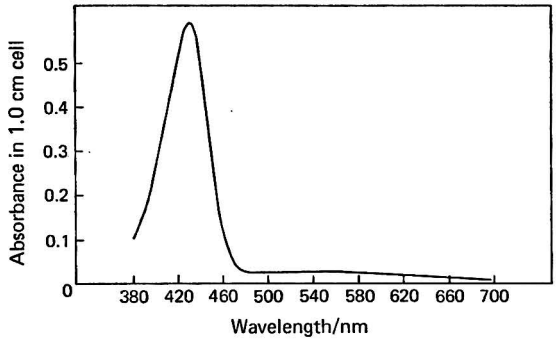


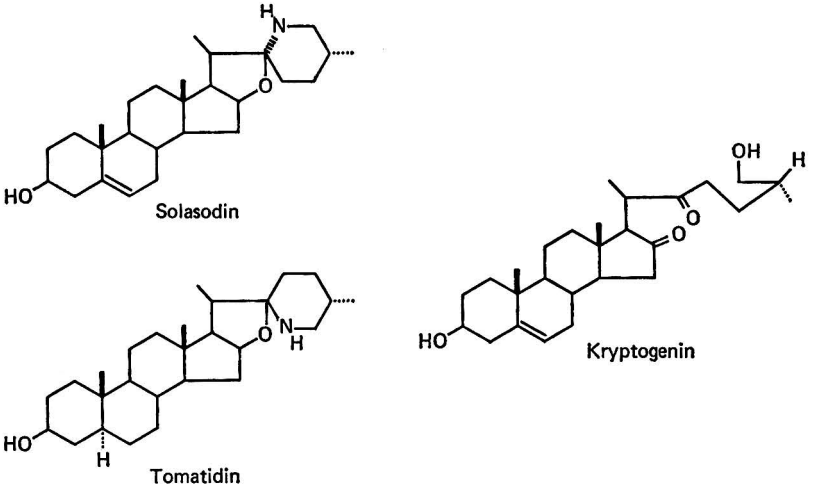
Fig. 1. Absorption spectrum of sapogenin treated with the anisaldehyde - sulphuric acid - ethyl acetate reagent.

It is interesting that yamogenin acetate, tigogenin, smilagenin, hecogenin acetate, yonogenin, tokorogenin triacetate, kogagenin and gracillin have the same maximum absorption,

which indicates that the contributions of the A and B rings and their substituents and stereochemistry do not play a major role. In fact, saturated sapogenins such as tigogenin or gitogenin react just as well as the others. Even the presence of a hydroxyl group in the 3-position is not necessary because spirosta-3,5-diene, prepared according to the method of Wall and Serota,[33] gives the same coloured chromophore. Its conversion into an ester or the presence of a glycosidic bond does not produce any changes in the spectral properties of the chromophore.

However, the sterols that were tested (sitosterol, cholesterol and lanosterol) did not give absorption peaks at 430 nm, but produced a "background" type of absorption over the full width of the spectrum.

In order to establish which parts of the molecules take part in the colour reaction, some compounds with analogous structures were tested. Solasodin and tomatidin, analogous to diosgenin but with nitrogen in the F ring, did not produce any reaction. Kryptogenin, a derivative of diosgenin in which the E and F rings have been opened, did not give the characteristic absorption spectrum. Corticosterone, cortisol and cortisone also did not produce any measurable colour.



Effect of Temperature and Reaction Time on Colour Stability

When the reagent B was used, the colour developed spontaneously owing to the increase in temperature caused by dilution of the concentrated sulphuric acid. When reagent C was

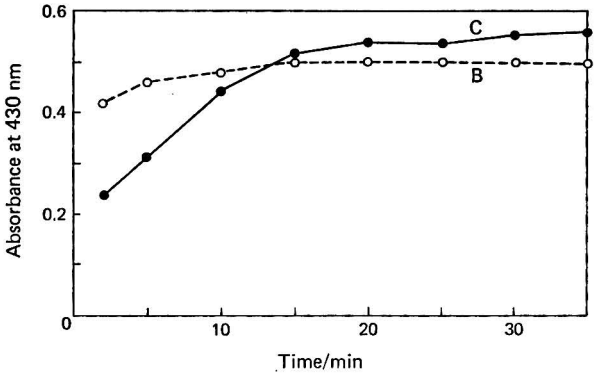


Fig. 2. Development of colour with diosgenin. B, Development at room temperature; and C, development at 60 °C.

used, heating at 60 °C for 20 min compensated for the decrease in the heat of dilution (Fig. 2). Whichever reagent was used, the maximum colour was developed in 20 min.

An absorption measurement made every half hour for 8 h showed that the absorbance did not vary.

Reproducibility, Accuracy and Sensitivity

To verify that the proposed determination is accurate and reproducible, we repeated the process with reagent C 15 times, using the same diosgenin solution, with a final concentration of 5 μg ml^{-1}. The mean absorbance reading was 0.546 $\pm$ 0.007 ($P = 0.01$), the standard deviation being 0.009 8 and the relative error 1.4%.

The calibration graphs (Fig. 3) obtained with diosgenin dissolved in 4 ml of the reaction mixture are linear between 0 and 40 μg (0–25 nmol ml^{-1}) and thus Beer's law is obeyed. The results were very close whether reagent B or C was used. The specific absorption coefficient is 111 for reagent B and 120 when reagent C is used with heating for 20 min at 60 °C. For comparison, the value obtained with the spectrophotometric method developed by Sanchez *et al.*[27] was 45.

We obtained identical calibration graphs with hecogenin acetate, smilagenin and tigogenin (Fig. 3). For these substances, the molar absorption coefficient was 49 151 l mol^{-1} cm^{-1} at 430 nm. Statistical analysis of the results according to the F-test gave a coefficient of variation of 2.24, which is lower than the Snedecor table values (4.42 for $P = 0.01$ and 2.88 for

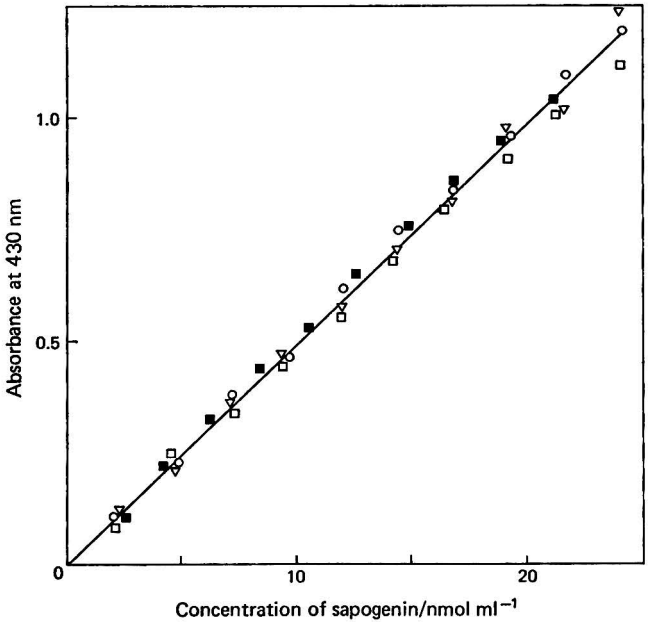


Fig. 3. Calibration graph for the determination of sapogenins. ○, Diosgenin; □, tigogenin; ▽, smilagenin; and ■, hecogenin.

$P = 0.05$). This method is easy to reproduce and, in addition, it allows one to obtain a chromophore that is the same for the four different sapogenins and whose molar absorption coefficient is 49 151 $\pm$ 890 l mol^{-1} cm^{-1} ($P = 0.01$).

As discussed above, the other sapogenins demonstrate the same spectral characteristics and the same sensitivity. Because of the small amounts of the substances available, further statistical analysis could not be carried out.

The sensitivity of the method is especially good, as the formation of the chromophore can be detected without difficulty for a sapogenin concentration as low as 0.5 μg ml^{-1}. It is possible to measure the colour obtained with 2 μg of diosgenin dissolved in 4 ml of the reaction

mixture. It is evident that this limit could easily be improved by using test-tubes with a longer optical path or a recording system more sophisticated than the simple reading screen on the spectrophotometer.

Whichever reagent is used, maximum colour is developed after a relatively short period (20 min), compared with other techniques in which 50, 80 or even 120 min are required before a stable colour is obtained.

It must be taken into account that, in general, steroidal sapogenins in plants are not in the free state but in the form of glycosides (saponins), which must be hydrolysed by either acid or enzymes. Purification of the aglycone requires several steps: filtration, drying, extraction and final recovery. This procedure can be avoided by the direct determination of the total sapogenin in the saponin solution.

In order to carry out this determination, and beginning with gracillin, 3-*O*-{[β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glycopyranosyl}diosgenin, we were able to construct a calibration graph between 2.5 and 20 μg ml^{-1} of gracillin, corresponding to 1.12–9 μg ml^{-1} of diosgenin. We obtained a linear relationship between the absorbance and the concentration of gracillin, with a molar absorption coefficient identical with that for diosgenin. This coincidence indicates that the saponin colour is due only to the aglycone part of the saponin and is not affected by the glycosidic part.

Interferences

The above results with gracillin led us to believe that sugar molecules (glucose and rhamnose) bonded with diosgenin have no influence on the colour reaction. Using our method, the analysis of a mixture of diosgenin, glucose and rhamnose gave an absorption spectrum and a molar absorption coefficient that were identical for diosgenin alone, and for diosgenin in admixture with the sugars.

Hecogenin acetate and tokorogenin triacetate gave the same spectrophotometric responses as the free genins. The addition of palmitic or stearic acid did not modify the absorption of the chromophore.

TABLE II

DETERMINATION OF DIOSGENIN IN MIXTURES USING THE PROPOSED METHOD

A Spectronic 20 instrument was used.

Compound or mixture	Amount/μg ml^{-1}	Absorbance at 430 nm	Recovery of diosgenin, %
Diosgenin	5	0.55	—
Diosgenin + rhamnose + glucose	5 + 4.4 + 2.2	0.56	102
Diosgenin + palmitic acid	5 + 5	0.55	100
Diosgenin + palmitic acid	5 + 50	0.55	100
Diosgenin + stearic acid	5 + 5	0.55	100
Diosgenin + stearic acid	5 + 50	0.55	100
Diosgenin + cholesterol	5 + 5	0.55	100
Diosgenin + cholesterol	5 + 15	0.55	100
Diosgenin + campesterol	5 + 5	0.55	100
Diosgenin + campesterol	5 + 15	0.55	100
Diosgenin + β-sitosterol	5 + 5	0.545	99
Diosgenin + β-sitosterol	5 + 15	0.60	109
Diosgenin + lanosterol	5 + 5	0.56	102
Diosgenin + lanosterol	5 + 15	0.58	105
Diosgenin + fixed oil from fenugreek seed	5 + 5	0.545	99
	5 + 10	0.54	98
	5 + 15	0.54	98
	5 + 20	0.54	98
	5 + 25	0.53	96
	5 + 50	0.54	98
		Mean:	100.21
		Standard deviation:	2.8

Interferences due to sterols and fixed oil from fenugreek seed were also tested, but none were found (Table II). Even oil concentrations that were 10 times higher than that of diosgenin did not affect the colour intensity. In another method in which interferences were investigated,[21] groundnut oil at a concentration four times higher than that of diosgenin caused a 25% decrease in the absorption. Tests have shown that one must use 50 times more oil than diosgenin (1 000 μg of oil and 20 μg of diosgenin in 4 ml of reaction mixture) in order to observe a 24% over-estimation in the measurements.

Whereas interferences virtually never occur with our method, in infrared spectrophotometry they are a particular problem. This problem is also found in the spectrophotometric method described by Hiai *et al.*,[23] in which interferences from sterols and sugars, especially rhamnose, are particularly intense. Even when the colour reaction is effected directly with the saponin, sterols such as steryl glucosides and acylated steryl glucosides, which occur in higher plants and dissolve in polar solvents, are not removed.

Conclusion

The method described here for determining steroidal sapogenins is simple, rapid, specific and sensitive. This technique is more efficient than other spectrophotometric methods, especially with respect to the possibility of determining all of the steroidal sapogenins independent of their structural particularities such as unsaturation, presence of a keto group or additional hydroxyl groups and different A/B conformations. Most of the methods based on antimony(III) chloride in acidic solution[14,21,22] or on sulphuric acid mixed with formaldehyde[16,20] are capable of determining only those sapogenins that have a Δ^{5-6} structure. The Okanishi and Togami[26] method, developed especially for saturated sapogenins, does not give a convincing chromophore with diosgenin. To our knowledge there is no general and specific method that gives comparable results with all steroidal sapogenins with no interference problems.

In addition, the sensitivity of this method is noteworthy, being 7–8 times greater than that of Paseshnichenko *et al.*[20] and 5–6 times greater than that of Sofowora and Hardman.[21] Apart from the sensitivity, other advantages are the simplicity of the operations and the possibility of carrying them out with common laboratory equipment.

It is also possible to combine this spectrophotometric method with chromatographic techniques (thin-layer or column chromatography) in order to determine the different sapogenins after their separation.

The possibility of determining total steroidal sapogenin content directly in a saponin solution represents considerable savings in time and a great simplification, which is useful in experiments such as selection of plant variety or in a programme for improved plant yields.

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References

1. Rothrock, J. W., Hammes, P. A., and McAller, W. J., *Ind. Engng Chem.*, 1957, **49**, 186.
2. Morris, M. P., Roark, B. A., and Cancel, B., *J. Agric. Fd Chem.*, 1958, **6**, 856.
3. Chakravarti, R. N., Dash, S. N., and Chakravarti, D., *J. Proc. Instn Chem. India*, 1970, **42**, 165.
4. Wall, M. E., Eddy, R. C., McLennan, M. I., and Klumpp, M. E., *Analyt. Chem.*, 1952, **24**, 1337.
5. Brain, K. R., Fazli, F. R. Y., Hardman, R., and Wood, A. B., *Phytochemistry*, 1968, **7**, 1815.
6. VandenHeuvel, W. J. A., and Horning, E. C., *J. Org. Chem.*, 1961, **26**, 634.
7. Gonzalez, A. G., and Barrera, J. B., *An. Fis. Quim.*, 1962, **58**, 559.
8. Lopez, G. S., Medina, A. J. C., and Valle, O. R., *Ciencia, Mex.*, 1968, **26**, 167.

9. Tomita, Y., Uomori, A., and Minato, H., *Phytochemistry*, 1970, **9**, 111.
10. Cooke, B. K., *Analyst*, 1970, **95**, 95.
11. Rozanski, A., *Analyst*, 1972, **97**, 968.
12. Diaz, G., Zaffaroni, A., Rosenkranz, G., and Djerassi, C., *J. Org. Chem.*, 1952, **17**, 747.
13. Walens, H. A., Turner, A., Jr., and Wall, M. E., *Analyt. Chem.*, 1954, **26**, 325.
14. Yamagishi, M., and Nakamura, I., *Chem. Pharm. Bull., Tokyo*, 1958, **6**, 421.
15. Slack, S. C., and Mader, W. J., *Analyt. Chem.*, 1961, **33**, 625.
16. Panina, V. V., and Loshkarev, P. M., *Medskaya Prom. SSSR*, 1963, **17**, 45; *Chem. Abstr.*, 1963, **59**, 11871c.
17. Wang, M. C., and Chou, T. H., *Yao Hsueh Hsueh Pao*, 1964, **11**, 235; *Chem. Abstr.*, 1964, **61**, 7344a.
18. Akahori, A., Yasuda, F., Okuno, I., Togami, M., and Okanishi, T., *Phytochemistry*, 1969, **8**, 45.
19. Kartnig, Th., Danhofer-Nöhammer, R., and Wegschaider, O., *Arch. Pharm., Berl.*, 1972, **305**, 515.
20. Paseshnichenko, V. A., Shittabe, K., and Guseva, A. R., *Prikl. Biokhim. Mikrobiol.*, 1972, **8**, 92; *Chem. Abstr.*, 1972, **76**, 137725.
21. Sofowora, E. A., and Hardman, R., *Planta Med.*, 1974, **26**, 385.
22. Rishi, A. K., Kapoor, R., and Vakhlu, V. K., *Curr. Sci.*, 1976, **45**, 327.
23. Hiai, S., Oura, H., and Nakajima, T., *Planta Med.*, 1976, **29**, 116.
24. Blunden, G., Hardman, R., and Morrison, J. C., *J. Pharm. Sci.*, 1967, **56**, 948.
25. Lockwood, G. B., Brain, K. R., and Turner, T. D., *J. Chromat.*, 1974, **95**, 250.
26. Okanishi, T. and Togami, M., *Chem. Pharm. Bull., Tokyo*, 1969, **17**, 315.
27. Sánchez, G. L., Acevedo, J. C. M., and Soto, R. R., *Analyst*, 1972, **97**, 973.
28. Jefferies, T. M., and Hardman, R., *Analyst*, 1976, **101**, 122.
29. Marker, R. E., Wagner, R. B., Ulshafer, P. R., Wittbecker, E. L., Goldsmith, D. P. J., and Ruof, C. H., *J. Am. Chem. Soc.*, 1947, **69**, 2167.
30. Lisboa, B. P., *J. Chromat.*, 1964, **16**, 136.
31. Stahl, E., *Editor*, "Thin-Layer Chromatography," Second Edition, Springer-Verlag, Berlin, Heidelberg, New York, 1969, p. 857.
32. Dawidar, A. M., and Fayez, M. B. E., *Z. Analyt. Chem.*, 1972, **259**, 283.
33. Wall, M. E., and Serota, S., *J. Am. Chem. Soc.*, 1956, **78**, 1747.

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Applications of Particle Size Analysis in the Pharmaceutical Industry

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Recent innovations in equipment for particle size analysis have enabled complete particle size distributions of fine powders to be obtained both cheaply and rapidly. This development has enabled applications, which previously would have been of only academic interest, to be developed as practical methods for routine use in the pharmaceutical industry. Four applications of particle size analysis based on a Model T_A Coulter counter are described: (1) the detection of fluctuations in the process variables of a batch crystallisation process; (2) close control of pharmaceutical grinding; (3) evaluation of the particle size distribution of the disintegration products of tablets; and (4) the assay of the percentage of amorphous insulin in Insulin Zinc Suspension.

Keywords: *Particle size analysis*; *pharmaceuticals*; *crystallisation*; *grinding*; *amorphous insulin*

In the past, fine particle size analysis has been used primarily to provide information on the size of particles for quality control or research purposes rather than as a general tool in the pharmaceutical industry. A possible exception has been the measurement of the dissolution rate of sparingly soluble drugs by monitoring the decrease in both the size and number of particles with time.

One reason for the apparent lack of interest in possible applications of particle size analysis has been the relatively high cost and time involved in determining the size distribution of a fine powder. Recent innovations in the apparatus available have enabled a full particle size distribution for many systems to be obtained both quickly and relatively cheaply.

The purpose of this paper is to describe some applications of particle size analysis in the pharmaceutical industry.

A Model T_A Coulter counter (Coulter Electronics Ltd.) was used for the work described here. The Coulter counter,[1,2] introduced in 1953, is an apparatus that detects and counts individual particles and measures their size by the change in electrical resistance that occurs when the particle passes through a small orifice. The counting speed is of the order of 4 000 particles per second, large numbers thus contributing to precision of the method. Until recently, in order to obtain a full size distribution on a powder a lengthy laboratory procedure was necessary, followed by a cumbersome calculation. For example, with relatively simple powders a skilled person could complete a maximum of ten analyses per day, whereas for complex powders this rate could decrease to two or three per day. The Model T_A Coulter counter permits a throughput of 50 or more analyses per day.

In this work, the standard procedure was used with a Model T_A Coulter counter. Unless otherwise indicated, the electrolyte was a 0.9% m/V saline solution saturated with the powder being examined and filtered through a 0.45-μm Millipore filter before use.

Monitoring of a Crystallisation Process

The crystallisation of a compound under ideal conditions would result in the physical characteristics of the crystals being reproducible between batches. In practice this reproducibility does not occur as many of the conditions are subject to variation. Small fluctuations in the crystal characteristics often have little effect on the subsequent processing, but when a low-dose, sparingly soluble drug is made into tablets problems associated with uniformity of content and dissolution rate may arise.

The drug examined had a low aqueous solubility (0.004% m/V) and the proportion of drug in the tablet was only 0.2% m/m. This investigation was initiated because of variations in uniformity of tablet content and dissolution rate.

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The batch process being used for crystallisation gave crystals of two types, the proportions of which varied between batches. Thin plate crystals with a high ratio of surface area to volume and much larger and thicker crystals with a lower ratio of surface area to volume were found in most batches. It would have been very difficult to quantify the variations between batches using only microscopic examination. Therefore, each batch was examined for size distribution using the Coulter counter, and the results were compared with the assessment using a microscope in order to see if they followed the pattern shown by the microscope description.

The powder obtained from the crystallisation process contained aggregates of crystals, which were reduced to their component crystals during the pharmaceutical processing. Before a useful particle size distribution could be obtained, it was necessary to reduce the aggregates to their component crystals. The method of dispersion chosen was the use of an ultrasonic probe.

Experimental

The conditions used to achieve ultrasonic dispersion were carefully controlled. At intervals, small samples of the suspension were removed and inspected microscopically for evidence of crystal fracture and/or the presence of aggregates. This procedure was carried out for a number of samples that were representative of the drug produced over the last 82 batches. The results showed that there was no evidence of crystal fracture after 15 s in any sample although there was a varying degree of aggregation in all samples. After $7\frac{1}{2}$ min no batch showed aggregation, although there was a varying degree of crystal fracture. It was decided to count with a Coulter counter after both 15 s and $7\frac{1}{2}$ min of ultrasonic dispersion in the supposition that the actual crystal size lay somewhere between the results for the two counts.

A 280 or 140 μm aperture tube was used for all of the determinations. After ultrasonic dispersion, an aliquot of the concentrated suspension was transferred to the electrolyte in the Coulter beaker. Duplicate runs were performed and the mean results recorded.

Results and Discussion

The weight median diameter (WMD) for both levels of ultrasonic dispersion are shown for each batch in Fig. 1. To determine if there were any significant changes in the particle size during the run of 82 batches, the results were examined using the cumulative sum technique.[3]

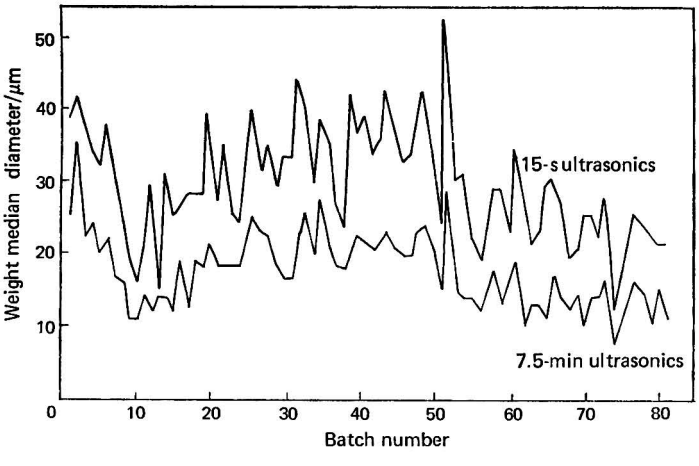

Fig. 1. Monitoring of a production crystallisation process.

Significant changes were indicated at batches 8, 14 and 55. The batches immediately before and after each change were examined microscopically and it was confirmed that a change in crystal size and shape had occurred. The yield of product for each batch was also subjected to analysis by the cumulative sum technique and significant changes were indicated at the same batch numbers.

An investigation into the history of this batch crystallisation process revealed that the differences were due to process variation associated with different workers operating the process. These differences were eliminated and a standard product was then produced in subsequent batches. The decrease in the variation of particle size achieved has resulted in a decrease in the variation of the dissolution rate and uniformity of content of tablets.

Fine-scale Control of Pharmaceutical Grinding

Close particle size control of many drugs is fundamental to successful formulation and manufacture. When using a continuous grinding process to obtain a specified particle size, a problem that often occurs is fluctuation in the particle size distribution of the final product. A number of factors may cause this variation, including changes in the size of the feedstock, variation in the feed rate, mechanical wear on the mill and the operating procedure itself. The close monitoring of continuous grinding processes for finely ground powders had not been possible in the past owing to the lengthy analysis and consequent delay in receiving the results.

A drug was being ground for the first time in a chemical production unit and the grinding of 2 000 kg of powder was monitored for particle size.

Experimental

A 70-μm aperture tube was used for all determinations. A concentrated suspension of powder in electrolyte was prepared on a watch-glass, agglomerates being dispersed with a camel-hair brush. Sufficient of this suspension was added to the electrolyte in the Coulter beaker. Duplicate runs were performed and the mean results recorded.

Results and Discussion

The feed rate of the grinding mill was approximately 25 kg h^{-1} and the particle size results are shown in Fig. 2, the three graphs showing the 95% undersize, the 50% undersize (*i.e.*, the WMD) and the 5% undersize. The dotted line indicates the point at which the responsibility for operating the plant was transferred from the commissioning engineer to normal production personnel; the results in Fig. 2 show that a significant change in the particle size of the ground powder had occurred at this point. Although the problem of obtaining a standardised product from a grinding process is not new, owing to the number of analyses necessary data of the type shown in Fig. 2 are seldom, if ever, available. The advantage of an instrument such as the Model T_A Coulter counter is that these data are rapidly and easily obtained. The data obtained, although treated circumspectly owing to problems associated with sampling, can serve as a critical process parameter. For example, the powdered drug in question was being specially ground to enable a formulation containing a very high proportion of drug to be produced. Powder to the left of the dotted line in Fig. 2 gave satisfactory results when tabletted, whereas powder to the right of the dotted line was borderline, in that it was not possible to obtain granules with suitable compression characteristics.

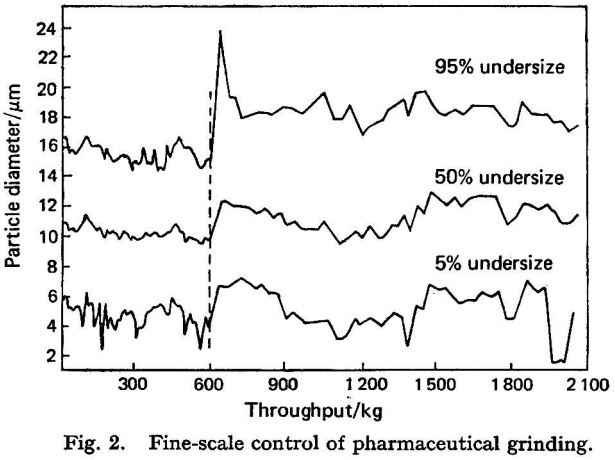

Fig. 2. Fine-scale control of pharmaceutical grinding.

Evaluation of the Particle Size Distribution of a Disintegrated Tablet

In general, a pharmaceutical tablet should disintegrate on being swallowed by a patient, and the type and degree of disintegration are often critical to the subsequent dissolution of the drug in the gastrointestinal juices. It has been recognised that while the official disintegration test of the British Pharmacopoeia[4] gives a minimum standard for disintegration, the results themselves, in many instances, are of little value as a guide to successful formulation. In essence, the British Pharmacopoeial test measures the time taken for a tablet to disintegrate in water under specified conditions of agitation so that all of the particles pass through a 1700 μm aperture mesh. No information is obtained from this test on the particle size distribution of the sub-1700-μm material. Shotton and Leonard[5] described a method based on a combination of wet sieving and Coulter counting for determination of the size distribution of the sub-1700-μm material, and used this method to study the effect of various formulation parameters on the disintegration of tablets. The disadvantage of this work was that the Model T_A Coulter counter was not then available and, owing to the slowness of the technique, it was largely of academic interest. The purpose of this paper is to illustrate how, by using the Model T_A instrument, Shotton and Leonard's method may be of practical use in both formulation studies and in-house quality control procedures.

Experimental

One tablet was weighed and disintegrated in 200 ml of 0.9% *m/V* saline solution, stirring slowly for 1 min with a glass rod. The suspension formed was passed through a 250-μm sieve, washing gently with saline solution, and the retained particles were dried and weighed. The suspension was counted on the Model T_A Coulter counter using a 400 μm aperture tube. No additional dispersion was used, the results being expressed as a percentage undersize of disintegrated tablet.

Results and Discussion

The results for two tablets are shown in Fig. 3. Both tablets were similar formulations of a product containing a high percentage of drug: tablet I was from a production batch whereas tablet II was from an experimental batch for which the dispersion characteristics were considered to be optimum for this product. The results in Fig. 3 differentiate clearly between the two formulations. It is considered that the simple test described here gives a useful assessment of dispersion.

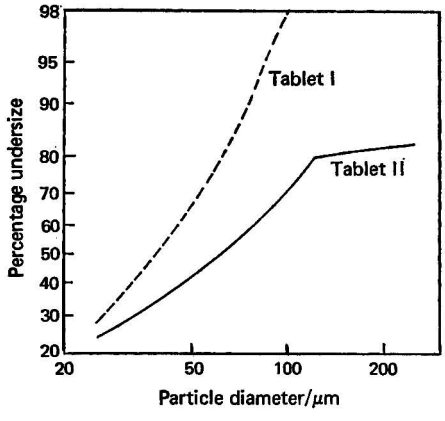

Fig. 3. Particle size distribution of disintegrated tablets.

In pharmaceutical formulations in which the particle size of the drug critically affects parameters such as dissolution rate and uniformity of content, it would be desirable to record the particle size of the drug in the final product in addition to the particle size of the powdered

drug prior to processing. A tablet containing 1% *m/m* or less of a sparingly soluble drug would be an example of a formulation for which a knowledge of the particle size of the drug in the final product would be most reliable. Attempts have been made to achieve this by using an extension of the above method, and the most successful approach so far has been as follows. The tablet is allowed to disintegrate completely in an aqueous electrolyte previously saturated with drug and an initial count is then recorded. Using a suitable solvent, the drug particles are dissolved and a second count is then obtained. Although some progress has been made, the results so far have been disappointing owing to poor reproducibility.

Quantitative Assay of Amorphous Insulin in Insulin Zinc Suspension

Insulin Zinc Suspension (IZS) is produced in three forms: Semilente, Lente and Ultralente. The Semilente contains amorphous insulin, the Ultralente is predominantly crystalline insulin but can contain up to 15% of amorphous insulin and the Lente is a mixture of Ultralente and Semilente forms. The ratio of amorphous to crystalline insulin in any preparation is important as it affects the duration of action of the drug. The British Pharmacopoeial assay for the percentage of amorphous insulin in IZS involves extraction of the amorphous insulin with a buffered acetone solution followed by a lengthy assay procedure. A simpler assay, based on the different size distribution of the amorphous and crystalline fractions of insulin, was desired.

Experimental

The standard procedure was used for all determinations.

Results and Discussion

The size distributions of the amorphous Semilente and the predominantly crystalline Ultralente are shown in Fig. 4. The size distribution of the Ultralente is bimodal, the smaller peak being taken to represent the small amount of amorphous insulin present in the Ultralente.

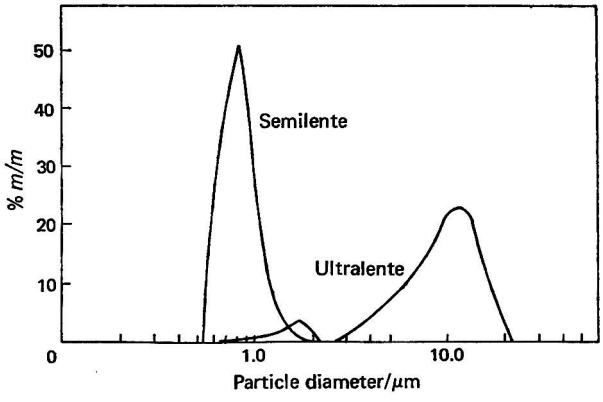

Fig. 4. Particle size distribution of the amorphous Semilente insulin and the predominantly crystalline Ultralente insulin.

The boundary between the two peaks is approximately 2.5 μm and this value was confirmed by microscopic examination. Samples from a batch of Ultralente (crystalline) insulin were mixed with 1, 2, 3, 4 and 5 parts of Semilente (amorphous) insulin. These mixtures were counted with a Coulter counter and the results are shown in Fig. 5 together with those for the original Ultralente and Semilente insulins. An inflection in the size distribution curve occurs at approximately 2.5 μm for each mixture. The percentage of amorphous insulin was calculated from the particle size distribution for each mixture, and Fig. 6 shows the correlation between the percentage of amorphous insulin by Coulter counting and the parts of amorphous insulin

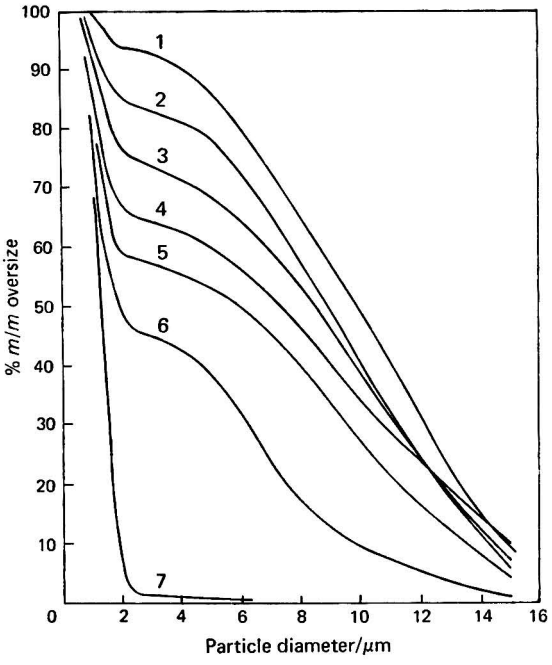

Fig. 5. Particle size distribution of mixtures of amorphous and crystalline insulin. Proportions of Ultralente (crystalline) insulin and Semilente (amorphous) insulin: 1, pure Ultralente; 2, 9 + 1; 3, 8 + 2; 4, 7 + 3; 5, 6 + 4; 6, 5 + 5; 7, pure Semilente.

added. The Ultralente insulin contained 6.5% of amorphous insulin and the correlation obtained was good.

Finally, five samples of IZS containing unknown amounts of amorphous insulin were supplied by a separate laboratory. These samples were analysed for their content of amorphous insulin by using the Coulter counter technique and the results compared with those provided by the supplying laboratory and based on the British Pharmacopoeial assay. The two sets of results are given in Table I.

The results show the two methods to be comparable. The reason for the discrepancy between the results from the BP method and the Coulter counter for sample 5 is believed to be

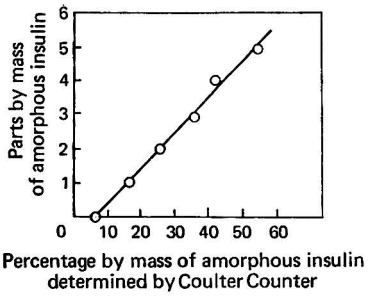

Fig. 6. Correlation between percentage by mass of amorphous insulin determined by Coulter counting and the amount of amorphous insulin added.

the poor reproducibility of the BP assay. A complete comparison of the reproducibilities, accuracies and sensitivities of the two methods is to be the subject of further work.

TABLE I

PERCENTAGE OF AMORPHOUS INSULIN AS MEASURED BY COULTER COUNTING AND THE BP METHOD

Sample	1	2	3	4	5
Coulter counter	27.5	28.0	10.0	8.5	11.0
BP method	27.9	27.9	10.2	8.8	9.4

General Discussion

The importance of particle size in the pharmaceutical and chemical industries is not unrecognised but the extent of the problem involved in controlling particle size is often great. All too often small but significant changes in the particle size of raw materials remain undetected owing to insufficiently rigorous quality control of particle size. These small changes may critically affect a number of parameters that should be kept constant to ensure a satisfactory final product. The investigations on the crystallisation and grinding processes outlined above illustrate how the production process with raw materials can be controlled more efficiently if particle size data are systematically recorded. The value of the Model T_A Coulter counter lies in its ability to produce results easily, rapidly and cheaply. The use of the cumulative sum technique enables a significant change in the particle size of a batch process to be recognised quickly, thus allowing remedial action to be taken. It must be emphasised that in the use of the Coulter counter for this type of application the technique of dispersion must be carefully standardised and it is advisable always to check the dispersion microscopically before counting takes place.

The assay of the percentage of amorphous insulin in Insulin Zinc Suspension based on differences in particle size is not only of importance in providing an accurate, cheap and rapid alternative to the pharmacopoeial procedure, but could also be important as a general method of analysis when there is a particular component or impurity that is difficult to assay by conventional means.

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References

1. Bean, H. S., Beckett, A. H., and Carless, J. E., *Editors*, "Advances in Pharmaceutical Sciences," Volume 2, Academic Press, London, 1967, p. 167.
2. Coulter, W. H., *U.S. Pat.*, No. 2 656 508, 1953.
3. Woodward, R. H., and Goldsmith, P. L., "Cumulative Sum Techniques," ICI Monograph No. 3, Imperial Chemical Industries, London, 1967.
4. "British Pharmacopoeia 1973," HM Stationery Office, London, 1973.
5. Shotton, E., and Leonard, G. S., *J. Pharm. Sci.*, 1976, 65, 1170.

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SHORT PAPERS

Determination of Nitrogen-15 by Emission Spectrometry: Procedure for Use with Small Amounts of Nitrogen

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Keywords: Nitrogen-15 determination; plant material analysis; emission spectrometry

A previous paper[1] described a sample preparation procedure for the determination of nitrogen-15 in plant material that is suitable for samples containing 200–500 μg of nitrogen, such as occur in conventional fertiliser experiments. This procedure has been improved to enable it to be applied to samples that contain only 20 μg of nitrogen, and the time taken for the precipitation stage has been reduced. This nitrogen-15 technique can now be used for more detailed analysis of plant materials, *e.g.*, for extracts that have been fractionated by chromatography. Other published procedures for the determination of small amounts of nitrogen employ more elaborate methods,[2] apply only to a limited range of compounds,[3] or do not permit the determination of total nitrogen in the sample.[4]

In our procedure, after a Kjeldahl digestion the ammonium ion is precipitated with Nessler reagent, converted into nitrogen gas by Dumas combustion, and the enrichment measured by emission spectrometry.

The total nitrogen content of a sample must be determined both for the determination of nitrogen-15 and for the interpretation of the values obtained. In the range 200–500 μg of nitrogen, any standard analytical method can be used. At about 20 μg of nitrogen, however, this determination should consume the absolute minimum aliquot and hence we use an ammonium ion-selective electrode.

With the extension of the nitrogen-15 technique to small amounts of nitrogen, contamination by natural nitrogen during the analytical procedures can give rise to incorrect results. The isotope dilution technique is used to determine such contamination by reagents and filter-papers. Results are given to illustrate the effect that correcting for reagent contamination has on atom per cent. excess values.

We have always used a Kjeldahl digestion mixture containing a selenium catalyst. Should copper or mercury catalysts be preferred, they should be removed after the digestion to prevent interference with both the ion-selective electrode and the Nessler precipitation of the ammonium ion. A simple method is described in which the metals are precipitated and removed as sulphides.

Procedure for Samples Containing 20–500 μg of Nitrogen

Reagents

Digestion acid A. A 0.5% *m/V* solution of selenium in concentrated sulphuric acid.

Digestion acid B. A 5% *m/V* solution of salicylic acid in concentrated sulphuric acid.

Sodium sulphide solution. A 5% *m/V* solution of sodium sulphide ($Na_2S.9H_2O$) in water.

Sodium hydroxide solutions, 10 and 1.0 M.

Nessler reagent. A solution of 1.35 g of mercury(II) iodide plus 1.2 g of potassium iodide in 100 ml of water.

Hydrochloric acid, ethanolic. 1 M hydrochloric acid containing 10% *V/V* of ethanol.

Ion-selective electrode filling solution. A solution of 4.5 g of potassium sulphate (anhydrous) in 100 ml of 0.1 M ammonium chloride solution.

Water. Purified by passing through a mixed-bed ion-exchange column and distilling from acidic permanganate solution.

Digestion of Sample

Weigh up to 150 mg of sample into a Kjeldahl flask and add 2 ml of digestion acid A; heat gently at first to evaporate water, if present, and then more strongly until the solution clears, and continue digestion for a further 1 h. Allow the digest to cool, dilute it to 20 ml with water and mix thoroughly. Samples containing nitrate can be subjected to an overnight treatment with 2 ml of sulphuric acid containing salicylic acid (digestion acid B).[5] If copper or mercury catalysts have been used add a slight excess of sodium sulphide solution, mix thoroughly and heat in a water-bath at 80 °C for about 30 min. Remove the precipitate by centrifugation at 2 000 rev min^{-1} for 5 min and then proceed with the analysis of the digest. Should it be necessary to filter the digest in order to obtain a clear solution, use a Whatman No. 1 filter-paper.

Determination of Total Nitrogen by Use of an Ion-selective Electrode

For measurement by use of an ion-selective electrode take a 2-ml aliquot from the diluted digest, add 9 ml of 1.0 M sodium hydroxide solution, mix thoroughly and immediately circulate the solution through the flow cell of an EIL ammonium ion-selective electrode, Model 8002–2. In order to eliminate drift caused by concentration effects across the electrode membrane, fill the EIL probe with a solution of 4.5 g of potassium sulphate in 100 ml of 0.1 M ammonium chloride solution. Maintain the probe at constant ambient temperature and circulate the solutions for analysis through the probe flow cell at about 4 ml min^{-1} by a Schuco mini-pump, Model 111. Measure the electrode potential with a Radiometer research meter, Model PHM 64, within 10 min, as soon as a steady value is obtained.[6] When the samples have comparable ammoniacal nitrogen contents they can follow one another through the flow cell, about 2 ml of a new sample being pumped to waste before starting continuous circulation. Should successive samples decrease in concentration by more than 10-fold, a solution of diluted digestion acid A (1 + 9, *V*/*V*) made alkaline with the appropriate amount of 1.0 M sodium hydroxide solution is first pumped through the flow cell to overcome the hysteresis that occurs owing to the diffusion of ammonia from the internal electrode solution into the low-concentration sample.[6]

A calibration graph is prepared by plotting the logarithm of the concentration of nitrogen (mg l^{-1}) against the differences in potential (mV) of a range of standard solutions with respect to the potential of a reference solution of known concentration.[7] This graph is essentially linear over the range 0.1–50 mg l^{-1}. Samples are similarly measured against the reference standard and the ammonium concentrations read from the calibration graph. Typical values for recovery of nitrogen from diluted digests are given in Table I.

TABLE I

RESULTS FOR NITROGEN OBTAINED WITH AN AMMONIUM ION-SELECTIVE ELECTRODE ON STANDARD SOLUTIONS OF AMMONIUM SULPHATE DISSOLVED IN DILUTE KJELDAHL DIGESTION ACID

Nitrogen content of 20 ml of solution/μg	Nitrogen content of 20 ml of solution, from ion-selective electrode measurements on 2-ml aliquots/μg		
	Duplicate determinations		Mean
20	20.0	20.2	20.1
40	40.6	39.4	40.0
60	59.4	60.6	60.0
80	79.4	80.0	79.7
100	101.2	99.0	100.1
140	144.0	139.8	141.9

Precipitation of Ammonium Ion and Preparation of Discharge Tubes

Neutralise the remainder of the dilute digest in a 50-ml centrifuge tube with 10 M sodium hydroxide solution using an external indicator, then adjust the alkalinity to 0.4 M with respect to hydroxide, add 4 ml of Nessler reagent, mix and heat the solution in a water-bath at 80 °C

for 30 min. Centrifuge the tube and contents at 2 000 rev min^{-1} for 10–15 min, reject the supernatant liquid, invert the tube and allow it to drain for 5 min. Dissociate the precipitate into ammonium chloride and mercury(II) iodide by adding sufficient ethanolic hydrochloric acid to give a final nitrogen concentration of about 250 μg ml^{-1}. Mix the acidic solution thoroughly, allow it to stand for 10–15 min and centrifuge at 2 000 rev min^{-1} for 10–15 min. Decant the ammonium chloride solution into a watch-glass and fill two short lengths of glass tubing (o.d. 3 mm; i.d. 2.5 mm) by capillary action. The length of tube should be such that the volume of solution that it holds will contain about 10 μg of nitrogen. From this stage the remainder of the procedure, namely warming the tube to leave a deposit of ammonium chloride, preparation of the discharge tube, Dumas combustion and measurement of nitrogen-15 with an emission spectrometer, is as described previously.[1]

Measurement of Reagent Contamination

When the total amount of nitrogen in a sample is of the order of 20–30 μg even small amounts of natural nitrogen acquired during sample processing can seriously depress the measurement of nitrogen-15 enrichment. The degree of contamination introduced by the various procedures to which the samples are subjected and the levels occurring in different batches of reagents should be measured so that if necessary corrections can be applied to the nitrogen-15 values obtained. The extent of contamination is readily determined by using the isotope dilution technique in which an aliquot of a nitrogen compound of known nitrogen-15 enrichment is processed using the reagents under investigation.

The results in Table II show that while the complete digestion and Nessler procedure added only 2–3 μg of nitrogen to the sample, filtration of the digest through an acid-washed paper added another 30 μg of nitrogen. Further tests showed that 25–40 μg of nitrogen could be eluted from acid-washed Whatman filter-papers, Nos. 50, 540 and 541, by 20 ml of diluted acid digests; the corresponding value for No. 1 filter-paper was about 3 μg.

TABLE II

DETERMINATION BY ISOTOPE DILUTION OF NITROGEN CONTAMINATION ACQUIRED DURING PROCESSING

Each analysis carried out on 100 μg of nitrogen, as ammonium chloride, containing 3.94% of nitrogen-15.

Method	Replicate sample No.	Nitrogen-15, % Replicate tubes			Mean from replicate tubes	Over-all mean	Standard error	Calculated contamination nitrogen/ μg	Standard error
Initial analysis by direct Dumas combustion	—	3.966	3.932	3.931	3.943		±0.012	—	—
Precipitation by Nessler reagent followed by Dumas combustion	1	3.976	3.933	3.939	3.950	3.965	±0.009	—	—
	2	3.939	3.972	4.014	3.975				
	3	3.979	3.962	3.972	3.971				
Standard digestion: 2 ml of digestion acid, neutralised, precipitation by Nessler reagent followed by Dumas combustion	1	3.852	3.797	3.806	3.819	3.850	±0.014	2.7	±0.5
	2	3.924	3.851	3.817	3.864				
	3	3.825	3.924	3.849	3.866				
As above but diluted digest filtered through acid-washed filter-paper, Whatman 540	1	3.029	3.065	3.063	3.052	3.060	±0.036	32.8	±1.4
	2	2.881	3.011	3.021	2.971				
	3	3.200	3.134	3.139	3.158				

Some typical values for total nitrogen and for atom per cent. excess of nitrogen-15 obtained by using the procedures described above on experimental samples are given in Table III. The enrichment values have been corrected for the 2.7 μg of natural nitrogen contamination of the reagents used in the standard digestion procedure.

Redesigned Sample Tube

The sample tubes described previously[1] have been simplified to eliminate the glass sockets, thus reducing the time and labour involved in manufacture. The tubes now consist of 200-mm lengths of Pyrex glass, 5 mm i.d., sealed at one end and drawn to 1 mm i.d. over 25 mm

TABLE III

DETERMINATION OF ATOM PER CENT. EXCESS OF NITROGEN-15 ON SAMPLES CONTAINING SMALL AMOUNTS OF NITROGEN

Values corrected for the nitrogen contamination in the reagents.

Sample code No.	Total nitrogen in digest/μg	Atom per cent. excess of nitrogen-15			
		Observed values			
		Replicate tubes		Mean	Corrected values*
C40	27.2	2.774	2.847	2.81	3.12
C52	29.0	1.410	1.413	1.41	1.56
E60	20.3	0.156	0.157	0.16	0.18
E127	27.6	1.120	1.128	1.12	1.25
E127	28.7	0.452	0.445	0.45	0.50
T32	25.5	1.198	1.192	1.19	1.34

* Corrected for 2.7 μg of nitrogen introduced by reagent contamination.

about 50 mm from the closed end. The second constriction is omitted, as experienced operators can judge the correct place to seal the tubes. The cone and sockets are replaced by glass screw joints (A) using silicone rubber discs (B) and nylon nuts (C) (Fig. 1). These joints have been found to give satisfactory seals when the tubes are evacuated on the vacuum manifold.

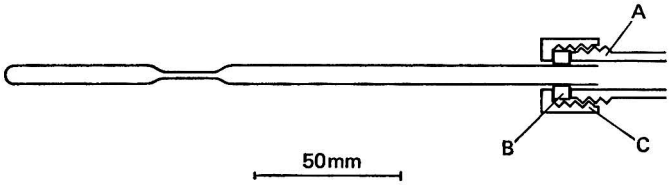

Fig. 1. Sample tube attached to manifold. For key to lettering see text.

References

1. Lloyd-Jones, C. P., Hudd, G. A., and Hill-Cottingham, D. G., *Analyst*, 1974, **99**, 580.
2. Keeney, D. R., and Tedesco, M. J., *Analytica Chim. Acta*, 1973, **65**, 19.
3. Yoneyama, T., and Kumagawa, K., *Analyt. Biochem.*, 1975, **67**, 327.
4. Goleb, J. A., and Middelboe, V., *Analytica Chim. Acta*, 1968, **43**, 229.
5. Horwitz, W., *Editor*, "Official Methods of Analysis of the Association of Official Analytical Chemists," Twelfth Edition, Association of Official Analytical Chemists, Washington, DC, 1975, p. 16.
6. Evans, W. H., and Partridge, B. F., *Analyst*, 1974, **99**, 367.
7. Midgley, D., and Torrance, K., *Analyst*, 1972, **97**, 626.

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Elimination of Sulphite Interference in the Spectrophotometric Determination of Nitrite

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Keywords: Nitrite determination; sulphite interference; spectrophotometry

The presence of nitrite in beet sugar factory juices has been attributed to the reduction of nitrate by thermophilic bacteria.[1] Recently, Oldfield *et al.*[2] have suggested that the amount

of nitrite present in diffusion juice could be used as an indicator for the control of thermophilic bacterial infection in the diffuser. As simple and sensitive spectrophotometric methods are available for the determination of nitrite, the thermophilic bacterial activity of various factory streams can be monitored routinely.

A photometric method developed by Shinn,[3] which had been applied for determining nitrite in soil[4] and in beet sugar factory juices,[2] was employed in this investigation. The method is based on the reaction of nitrites with sulphanilamide in acidic solution and coupling the diazotised sulphanilamide with *N*-(1-naphthyl)ethylenediamine dihydrochloride. The product is a reddish purple dye that has its maximum absorption near 540 nm.

Occurrences of non-reproducible results were often experienced by our analysts and subsequent investigation showed that the erratic results were caused by the presence of sulphite in the sample. The interference of sulphite was eliminated by the use of formaldehyde and changing the order of addition of the reagents, as described in this paper.

Experimental

Apparatus

Absorbance measurements were made with a Beckman Model DU spectrophotometer using 1-cm silica cells.

Reagents

Formaldehyde solution, 5% *m/V*.

Sulphanilamide solution, 0.2% *m/V*.

Hydrochloric acid, 6 M.

N-(1-*Naphthyl)ethylenediamine dihydrochloride*, 0.2% *m/V*. This solution is stable for a month when stored in a refrigerator.

Standard nitrite solution, 1000 mg l^{-1} *of nitrite*. A 1.500-g portion of sodium nitrite was dissolved in distilled water and the solution diluted to 1 l. A pellet of sodium hydroxide and 1 ml of chloroform were added in order to prevent liberation of nitrous acid and to inhibit bacterial growth. Portions of this solution were diluted and used for preparation of a calibration graph.

Procedure

Highly coloured samples, such as diffusion juice, thick juice and molasses, are clarified with Horne's dry lead[5] and filtered. Nitrite is determined in the filtrate.

A known amount of sample containing 4–30 μg of nitrite is transferred into a 50-ml calibrated flask. Sufficient distilled water is added to bring the volume of the test solution to about 20 ml, then 2 ml of 5% formaldehyde solution, 5 ml of 0.2% sulphanilamide solution and 2 ml of 6 M hydrochloric acid are added, mixing the contents after each addition. After allowing the flask to stand for 3 min, 1 ml of 0.2% *N*-(1-naphthyl)ethylenediamine dihydrochloride solution is added and the contents are mixed. The solution is then diluted to the mark and mixed thoroughly. A sample blank containing the sample and reagents as described above, except for the *N*-(1-naphthyl)ethylenediamine dihydrochloride, is prepared in a similar manner. The absorbance at 540 nm *versus* the sample blank is measured after 2 min.

A reagent blank and three standards containing 10.0, 20.0 and 30.0 μg of nitrite are prepared in a similar manner and the absorbance at 540 nm *versus* a reagent blank is measured after 2 min.

Results and Discussion

Interference of Sulphite

Reaction of nitrite with sulphite in beet sugar factory juices to yield imidodisulphonate had been reported.[1,2] Previous investigations conducted in our laboratory indicated that the rate of the nitrite - sulphite reaction increases as the hydrogen-ion concentration of the medium increases. As the photometric method described by Shinn[3] recommended the addition of hydrochloric acid to the test solution before the addition of other reagents, sulphite ions, if present in the sample, would react with nitrite ions, thus decreasing the nitrite value.

The results in Table I, obtained by using Shinn's method, confirm that sulphite interferes, and the error increases with increasing concentration of sulphite in the test solution. In

addition, the interference of sulphite was time dependent, as the longer the nitrite - sulphite reaction was allowed to proceed before addition of sulphanilamide, the greater was the interference. The elapsed time was measured from completion of addition of hydrochloric acid to the addition of sulphanilamide.

TABLE I

INTERFERENCE OF SULPHITE IN THE DETERMINATION OF NITRITE

Nitrite concentration, 0.400 mg l^{-1}.

Sulphite concentration/ mg l^{-1}	Absorbance after various elapsed times		
	0.2 min	1.0 min	3.0 min
0	0.451	0.452	0.452
0.4	0.450	0.442	0.430
1	0.445	0.420	0.402
2	0.408	0.375	0.322
10	0.324	0.262	0.193
20	0.160	0.103	0.036

Order of Addition of Reagents

As the nitrite - sulphite reaction is time dependent, the diazotisation reaction may be more rapid than the nitrite - sulphite reaction. It was surmised that the interference of sulphite might be circumvented by changing the order of addition of the reagents, *e.g.*, by addition of sulphanilamide before the addition of hydrochloric acid.

The results in Table II indicate that the interference of sulphite is substantially reduced when sulphanilamide is added to the test solution before hydrochloric acid. As much as 10 mg l^{-1} of sulphite causes no interference when 0.4 mg l^{-1} of nitrite is present. However, if the sulphite concentration is 20 mg l^{-1} or higher, the interference is significant.

TABLE II

RESULTS OF ADDITION OF SULPHANILAMIDE FOLLOWED BY HYDROCHLORIC ACID

Nitrite concentration, 0.400 mg l^{-1}.

Sulphite concentration/ mg l^{-1}	Absorbance	Error, %
0	0.452	—
2	0.450	−0.4
10	0.454	+0.4
20	0.410	−9
40	0.386	−15
60	0.331	−27

Elimination of Sulphite Interference

The amount of nitrite in some factory juices is often low, and consequently a large volume of sample will be required for the nitrite determination. The concentration of sulphite in the test solution may be much greater than 10 mg l^{-1} and, in order to avoid a serious error in the determination of nitrite, it would be necessary to eliminate or render inert the sulphite in the test solution.

Formaldehyde reacts with sulphite to form a stable formaldehyde addition product and this reaction should render the sulphite inactive towards nitrite ions.

Table III reveals that up to 3 ml of 5% formaldehyde solution had no effect on the colour intensity of the azo dye. A gradual decrease in intensity was noted when more than 3 ml of the formaldehyde solution was used and all subsequent tests were therefore performed with the addition of not more than 3 ml of the solution. The order of addition of the various reagents used to obtain the results appearing in Table III was as described above.

The results in Table IV show that the interference of sulphite in the photometric determination of nitrite can be effectively eliminated with the use of formaldehyde and a change in the order of addition of the reagents. Two millilitres of 5% formaldehyde solution were added to each sample. Sulphite ion concentrations greater than 200 mg l^{-1} were not investigated.

TABLE III

EFFECT OF FORMALDEHYDE ON NITRITE ABSORBANCE

Nitrite concentration, 0.400 mg l^{-1}.

5% formaldehyde solution/ml	0	1.0	2.0	3.0	4.0	5.0
Absorbance	0.451	0.452	0.452	0.450	0.433	0.405

In the presence of formaldehyde, the intensity of the azo dye reaches a maximum after 2 min and then remains constant for at least 2 h. A graph of absorbance *versus* nitrite concentration indicated that Beer's law is obeyed in the range 0.02–0.8 mg l^{-1}.

TABLE IV

ELIMINATION OF SULPHITE INTERFERENCE WITH FORMALDEHYDE

Nitrite concentration, 0.400 mg l^{-1}.

Sulphite concentration/mg l^{-1}	0	2	20	100	200
Absorbance	0.452	0.451	0.452	0.451	0.452

To evaluate the accuracy of the proposed method for determining nitrite, different amounts of nitrite were added to equal volumes of a sample of factory juice and the nitrite concentrations in the original factory juice and samples with added nitrite were determined. For comparison purposes, similar tests were performed with the same sample of factory juice using Shinn's method.[3] Each millilitre of sugar beet juice employed in this experiment contained 65 μg of sulphite; the sulphite content was determined iodimetrically as described previously.[6]

TABLE V

COMPARISON OF METHODS FOR RECOVERY OF NITRITE ADDED TO FACTORY JUICE

Method	Nitrite added/μg	Absorbance	Nitrite found/μg	Nitrite recovered/μg	Recovery, %
This work	0	0.098	4.4	—	—
	10.0	0.325	14.4	10.0	100
	20.0	0.552	24.6	20.2	101
	30.0	0.762	34.2	29.8	99
Shinn's method[3]	0	0.023	1.0	—	—
	10.0	0.092	4.1	3.1	31
	20.0	0.183	8.2	7.2	36
	30.0	0.583	26.0	25.0	83

The results in Table V demonstrate that the proposed method gives a quantitative recovery of nitrite in the presence of sulphite, while the results obtained using Shinn's method are unacceptably low. The proposed method is rapid and suitable for routine determinations.

References

1. Carruthers, A., Gallagher, P. J., and Oldfield, J. F. T., Paper presented at 11th Annual Technical Conference of the British Sugar Corp., 1958.
2. Oldfield, J. F. T., Dutton, J. V., and Shore, M., *Int. Sug. J.*, 1974, **910**, 301.
3. Shinn, M. B., *Ind. Engng Chem., Analyt. Edn*, 1941, **13**, 33.
4. Buckett, J., Duffield, W. D., and Milton, R. F., *Analyst*, 1955, **80**, 141.
5. Bates, F. J., *Circ. U.S. Natn. Bur. Stand.*, 1942, No. C440, 123.
6. Lew, R. B., *J. Am. Soc. Sug. Beet Technol.*, 1975, **18**, 252.

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Determination of Water in Iodine by Infrared Spectroscopy

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Keywords: *Water determination*; *iodine*; *infrared spectroscopy*; *bromine solvent*

The Analytical Standards Sub-Committee of the Analytical Methods Committee of the Analytical Division of The Chemical Society considered that high-purity iodine could, with arsenic trioxide, form a suitable oxidimetric system to investigate, provided that information on the trace impurities in the iodine, particularly moisture, was available.[1] A highly purified sample of iodine was referred to silver[2] and gave assay figures in excess of 99.99%, and an examination with a mass spectrograph indicated that the detectable impurities were present in amounts not exceeding 100 $\mu g\ g^{-1}$. The determination of trace amounts of water in iodine is also important if the iodine is intended for use in the manufacture of quartz - iodine lamps.

Infrared spectroscopy can be used to determine trace amounts of water in bromine[3–6] and this paper describes the application of this method to iodine, using bromine as solvent. The absorbance of the water peak at about 3 665 cm^{-1} is measured and the method gives a coefficient of variation of 4.3% at the 54 $\mu g\ g^{-1}$ level.

Experimental and Results

Initial tests showed that the solubility of iodine in bromine is high, allowing the preparation of solutions containing up to 170% *m/V* of iodine. Solutions of water in bromine show absorption peaks at 3 665, 3 582 and 1 607 cm^{-1}, but the last wavenumber is beyond the cut-off point for Infrasil cells. The peak at 3 665 cm^{-1} was chosen and base-line correction was applied by subtracting the absorbance at 4 000 cm^{-1}. Some shift in this peak occurs in the presence of iodine.

Bromine, after drying with molecular sieves, has an absorbance of approximately 0.005 for a 10-mm path length at 4 000 cm^{-1} and, except for small water peaks, the spectrum is flat to at least 3 000 cm^{-1}. Ten determinations of the absorbance for the water peak in the blank, using 40-mm cells, gave a mean of 0.009 with a standard deviation of 0.002.

Standards containing 10–100 $\mu g\ cm^{-3}$ of water in bromine were prepared and the absorbances measured in 10-mm cells, giving a rectilinear calibration graph with a slope of 0.004 21. Standards prepared in solutions of iodine in bromine gave identical calibration data. Further work showed that the slope is proportional to path length for 10–40-mm cells.

Bromine rapidly absorbs water from the atmosphere so that it is necessary to fill the cells in an atmosphere of dry air. It is most convenient to prepare solutions of iodine in the spectrophotometer cell on a gravimetric basis. Experiments with added water showed that a shaking time of at least 50 min is required in order to dissolve the water.

The spectrum obtained with a sample of iodine containing 6.1 $\mu g\ g^{-1}$ of water is shown in Fig. 1. Most commercial iodine contains more water than this level, usually in the range

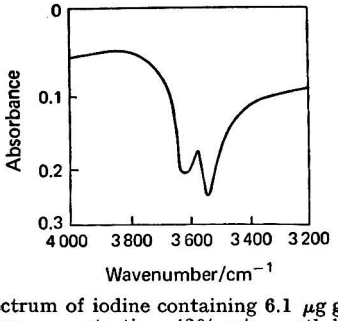

Fig. 1. Spectrum of iodine containing 6.1 $\mu g\ g^{-1}$ of water in bromine. Iodine concentration, 42% *m/m*; path length, 40 mm.

100–2 000 μg g^{-1}. The distribution of water within a sample is often highly heterogeneous and appears to be related to lump size. If the sample is ground, much of the water is lost and the precision improves considerably (Table I).

TABLE I

PRECISION OF RESULTS FOR THE DETERMINATION OF WATER

Sample	Water found/μg g^{-1}	Mean/μg g^{-1}	Standard deviation/μg g^{-1}
Purchased commercial sample ..	2 100, 700, 3 400, 2 700, 150, 2 500, 130, 2 100	1 720	1 240
Same sample after grinding ..	57, 53, 56, 53, 55, 54, 57, 52, 51, 51	54	2.3
High-purity sample	6.4, 6.1, 6.0, 6.6	6.3	0.3

Method

Apparatus

A Perkin-Elmer 457 infrared spectrophotometer was used in the slow scan mode with a slit setting of 7. Stoppered Infrasil cells of path lengths 10, 20 and 40 mm were obtained from Hellma (England) Ltd. and Thermal Syndicate Ltd.

Reagent

Bromine. Add about 50 g of molecular sieve type 4A to 500 ml of AnalaR-grade bromine and allow the mixture to stand overnight. Pass the bromine through a 200 × 25 mm column of molecular sieve supported on a sintered-glass disc. Arrange the column so that it will discharge into the cell in an atmosphere of dry air.

Procedure

Weigh the iodine sample in a dry, stoppered Infrasil cell, taking up to 19 g in a 40-mm cell or proportionately less in smaller cells. Flush the cell with dry air, fill with dry bromine and weigh. Shake the cell for 1 h and place it in the spectrophotometer. Use a beam attenuator in the reference beam to set the pen at zero absorbance at a wavenumber of 4 000 cm^{-1} and then scan over the range 3 700–3 600 cm^{-1} to record the peak absorbance. Repeat the procedure with a blank of bromine in the same cell, measuring the absorbance at the same frequency as for the sample. Calculate the water content from the equation

$$\text{Water}\,(\mu\text{g g}^{-1}) = \frac{\{A_1 - A_2\,[1 - 0.022\,9\,(M_2 - M_3)]\} \times [32.06\,(M_1 - M_2) + 22.9\,(M_2 - M_3)]}{(M_2 - M_3)F}$$

where A_1 = sample absorbance, A_2 = blank absorbance, M_1 g = mass of cell plus iodine plus bromine, M_2 g = mass of cell plus iodine, M_3 g = mass of cell and F = 100 × slope of the calibration graph. In this work, for 20-mm cells, F = 0.842.

Calibration

Prepare standard solutions by dissolving 0.2–1.0 μl of water, dispensed with a calibrated 1-μl syringe, in 10 cm^3 of bromine in sealed ampoules. Determine the absorbance as in the procedure above.

References

1. Report of the Analytical Standards Sub-Committee, *Analyst*, in the press.
2. Strouts, C. R. N., Wilson, H. N., and Parry-Jones, R. T., *Editors*, "Chemical Analysis," Volume 1, Clarendon Press, Oxford, 1962, p. 203.
3. Wagner, H., *Z. Anorg. Allg. Chem.*, 1965, **337**, 54.
4. Duvall, R. B., and Kiley, L. R., *Analyt. Chem.*, 1958, **30**, 549.
5. Hahn, L. H., and Pauling, L. E., *Analyt. Chem.*, 1968, **40**, 1283.
6. Matauschek, K., *Z. Anorg. Allg. Chem.*, 1965, **337**, 48.

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Book Reviews

NMR. Basic Principles and Progress. Volume 13. Introductory Essays. Edited by M. M. Pintar. *Lectures to the International Summer School on Nuclear Magnetic Resonance, University of Waterloo, June* 23–28, 1975. Pp. xii + 154. Berlin, Heidelberg and New York: Springer-Verlag. 1976. Price DM72; $29.60.

The eleven lectures by physicists from Belgium, Canada, Switzerland, the UK, the USA and Yugoslavia reprinted in this volume discuss relaxation theory, the thermodynamics of spin systems in solids, coherent averaging and double resonance in solids, macroscopic dipole coherence phenomena, nuclear spins and non-resonant electromagnetic phenomena, nuclear spin relaxation in molecular gases, spin-lattice relaxation in nematic liquid crystals, molecular tunnelling and its effect on NMR absorption and relaxation and the construction of a Fourier transform NMR spectrometer. Each essay has a short bibliography covering the literature up to 1975.

The lectures provide interesting introductions to their specialised subjects, but the theoretical approach makes the volume of more value to physicists and chemical physicists than to analytical chemists.

J. E. Page

Struktur und Absorptionsspektroskopie Organischer Naturstoffe. By Manfred Kraft. *Wissenschaftliche Forschungsberichte. Reihe* 1: *Grundlagenforschung und Grundlegende Methodik. Abteilung A: Chemie und Physik. Band* 76. Pp. xii + 321. Darmstadt: Dr. Dietrich Steinkopff Verlag. 1976. Price DM98.

In this book, the author provides a basic review of spectroscopic structural analysis for students and laboratory workers. The organic natural products are grouped in chapters under proteins, nucleic acids, carbohydrates, lignins, lipids, vitamins, steroids, alkaloids, pigments and antibiotics. The author has covered these large fields of endeavour in an acceptable length by concentrating on the key features that have, and are capable of, revealing structure and conformation. The opening chapter discusses the basic principles of NMR, IR, Raman and UV spectroscopy and optical rotatory dispersion and circular dichroism, but does not mention mass spectrometry. This is an unfortunate omission in any account of the structure of steroids and alkaloids, and when mass spectrometry is used widely in conjunction with these other methods.

By grouping the applications of these methods to each field in turn, the author contrasts the types of information given and thereby guides the reader to the procedures most likely to be of benefit in a particular situation. Sufficient basic description of structure and conformation is given. A large number of diagrams and line drawings clarify the text so that only a little ability with the German language is needed to use this book. There are a few NMR scale errors, *viz.*, with ^{1}H NMR scales in Figs. 3.9 and 4.6, and with the sign of ^{13}C NMR scales in Figs. 4.9, 9.17, 9.19, 9.20 and 9.30.

Only a small proportion of the 1 000 or so references are after 1971. In this respect, and the way ^{13}C NMR is presented in the introductory chapter (one page with no references), this book appears to have been prepared in 1971–2 and then additional items inserted. Consequently, some of the chapters seem unbalanced for a 1976 book. Despite this shortcoming, the author can be said to have assembled an instructive source-book of spectroscopic structural work, which should be of value to analysts.

I. K. O'Neill

Electron Spin Double Resonance Spectroscopy. By Larry Kevan and Lowell D. Kispert. Pp. x + 427. New York, London, Sydney and Toronto: John Wiley & Sons. 1976. Price £18; $30.70.

This book is intended to introduce resonance non-experts to ENDOR and ELDOR. While these techniques are not general purpose in the same sense as ESR and NMR, ENDOR in particular could undoubtedly be applied far more widely than at present if it were not for the extremely high cost of commercial equipment. This book is to be welcomed in making the power of these techniques more widely known, but many readers who may wish to begin using these methods will have difficulty in gaining access to instrumentation.

A good introduction covers the various mechanisms of ENDOR and ELDOR with a minimum of formal treatment. A clear discussion of resolution enhancement is given, together with useful summary tables to relate the effects to the different relaxation conditions that may occur. The

second chapter is allocated to experimental techniques, including a brief summary of commercially available spectrometers. Unlike ESR, for which broadly similar sample and cavity arrangements are offered by all manufacturers, ENDOR spectrometers demonstrate a wide divergence of designs and it is disappointing that the opportunity was not used to make a critical comparison between them. Specification of radiofrequency levels in watts is meaningless unless the transmitter coil details are given; both in this chapter and elsewhere it would have been preferable to express radiofrequency levels in terms of the magnetic field generated. It is also surprising that sample temperature control systems were not described, in view of the critical importance of temperature. Success or failure can often depend on having a continuous coverage from helium to room temperature, such as is provided by some types of liquid helium transfer systems. A survey of these systems would have been a valuable addition as temperature requirements may have to take precedence over microwave and radiofrequency arrangements.

Liquid- and solid-phase ENDOR are covered separately in two chapters with a wide range of examples and supporting theory, and also included are matrix ENDOR and some less common techniques (dispersion mode, double ENDOR and ENDOR-induced ESR). Tables are provided that classify the literature up to 1974. Liquid-phase, single-crystal and powder ELDOR are treated in two further chapters, and a final chapter on biochemical applications emphasises the application of ENDOR and ELDOR to proteins. Curiously, although the ELDOR of nitroxide spin labels is described, there is no discussion of ENDOR on this important class of molecules.

The book is well written and produced, but extremely expensive by current standards.

BARRY A. COLES

MICROSCALE MANIPULATIONS IN CHEMISTRY. By T. S. MA and V. HORAK. *Chemical Analysis, Volume* 44. Pp. xvi + 488. New York, London, Sydney and Toronto: John Wiley & Sons. 1976. Price £19.40; $33.

In these days of high technology (and one wonders sometimes whether the word "high" is being used in its pejorative American sense), it is a great relief to come across a book such as this, and realise that not everyone is anxious to have a button to press, a knob to twiddle or a digital display to read, and that man's ingenuity can produce elegance and simplicity as well as ugliness and complexity. Every chemist who has worked "with his hands" will have a favourite piece of apparatus that he remembers with pleasure, and he will have a very good chance indeed of finding it mentioned in this book (alas, I could not find the Schilow burette, but then one cannot expect everything). Of course, one would not deny *some* value to the modern black boxes; after all, button pressing may lead to an understanding that there is a finite time between initiation and completion of a movement and that lack of synchronisation can cause error, and knob twiddling may introduce the notion that back-lash may be important, or even reveal that grub-screws can work loose. As for digital displays—they do illustrate rather well what is meant by statistical fluctuation. Best of all, perhaps, the black boxes may give the chemist enough time to read such books as this, which will almost certainly seduce him away from automation to do-it-yourself chemistry, which is really the most satisfying kind of all. In modern jargon, a good read and a good buy.

R. A. CHALMERS

PROCEEDINGS OF THE INTERNATIONAL NUCLEAR AND ATOMIC ACTIVATION ANALYSIS CONFERENCE AND 19TH ANNUAL MEETING ON ANALYTICAL CHEMISTRY IN NUCLEAR TECHNOLOGY, GATLINBURG, TENNESSEE, USA, OCTOBER 14–16, 1975. Edited by W. S. LYON, T. BRAUN and E. BUJDOSÓ. Reprinted from the *Journal of Radioanalytical Chemistry*. Pp. 242. Budapest: Akadémiai Kiadó. 1976. Price £16.10.

This book is a reprint of 20 papers from the *Journal of Radioanalytical Chemistry* for 1976. The analytical techniques used are mostly instrumental neutron-activation analysis, but include charged-particle and photon activation, prompt gamma-emission, proton- and gamma-induced X-ray fluorescence, ion microprobe work and atomic-absorption spectroscopy. The materials analysed include aluminium, silicon, steel, platinum - rhodium alloys, cement, coal, fly ash, dusts, natural waters, teeth, human tissues, diets and excreta, as well as tree rings. More than half of the contributions are concerned with environmental and medical applications.

The work will be useful to analysts specialising in the fields mentioned. Forty-three elements can now be determined simultaneously in natural waters and aerosols (L. C. Bate *et al.*), while

chlorine, fluorine and lead can be determined in a matter of seconds by neutron activation of isotopes with short half-lives (D. A. Miller and V. P. Guinn). The determination of trace elements in tree rings appears to have useful potential in historical studies of pollution, and perhaps also in absolute dating (K. K. S. Pillay).

This book is not suitable as an introductory text to any of the techniques described, and few workers will be really interested in more than a few of the papers in it. It has received the minimum of editing, with an author index but no subject index, the quality of the printing is mediocre, and the binding of my copy was defective. The price of about 7p per page is much too high.

H. J. M. Bowen

Modern Methods of Chemical Analysis. Second Edition. By R. L. Pecsok, L. Donald Shields, Thomas Cairns and Ian G. McWilliam. Pp. xxii + 573. New York, Santa Barbara, London, Sydney and Toronto: John Wiley. 1976. Price £13.50; $21.50.

Despite its title, the book is mainly concerned with instrumental methods of analysis and little attention is given to chemical aspects of sample treatment, chromogenic reagents or chelate extractants.

The selection of techniques included by the authors is difficult to understand as many important to undergraduate courses have been omitted and others are dealt with so briefly as to be of little assistance as instructional or ancilliary reading material. The omissions are disappointing in view of the excellent treatment of certain topics, particularly those concerned with the elucidation of molecular structure. Topics omitted include thermal methods, laser Raman, electron-emission and resonance spectroscopy and emission, direct-reading and spectrographic methods. Relatively brief sections (number of pages in parentheses) include high-performance liquid chromatography (2), thin-layer chromatography (1), modern polarography (½) and fluorescence and phosphorescence (2¼). Despite the reservations above, separation methods are given considerable emphasis.

The chapters on ultraviolet, infrared, mass and nuclear magnetic resonance spectroscopy are outstanding and for these alone the book should be in the personal library of all those who teach analytical chemistry at the tertiary level. The section on spark-source mass spectroscopy is especially welcome in view of the possible increase in interest in this technique and its omission from most undergraduate-level text-books.

The principles of a wide variety of automatic analysers, process analysers and feedback process control systems are given and provide most useful surveys of and introductions to these areas, as do those on data processing and computerised instrumental systems. The associated chapter on the statistical treatment of data could well have included comparison of means and other methods of detection of systematic errors.

There has been a regrettable loss of very useful material from the first edition (see *Analyst*, 1969, **94**, 336), notably the individual chapters on the role of the solvent in acid - base chemistry, effects of molecular structure on acidity and kinetics.

The general layout, diagrams and illustrations are of a very high standard and the annotated references most helpful.

This is an interesting and individual text which is highly recommended for individual and institutional purchase.

D. Thorburn Burns

Cours de Chimie Analytique Générale. Tome IV. Exercices. By M. Machtinger and R. Rossett. Pp. vi + 284. Paris, New York, Barcelona and Milan: Masson. 1976. Price Fr75.

Like the third volume, this one contains, under four chapter headings, a minimum of explanatory introduction followed by a variable number of exercises, many of which are taken from examination papers. Acid - base, complexation, oxidation - reduction and precipitation titrations are grouped in Chapter I, while Chapter II deals with non-aqueous media. Chapter III deals with current - voltage curves in three parts, coulometry and conductimetry, and the final chapter covers photometric titrimetry. This part of the book occupies 88 pages. The remainder of the book, covering 190 pages, provides not just numerical answers, but complete worked-out solutions

to the exercises together with pithy comment. The most rudimentary knowledge of French is adequate for grasping the gist of exercise and answer. The treatment is at undergraduate level and the exercises are suitable for tutorial or examination work. E. BISHOP

WILSON & WILSON'S COMPREHENSIVE ANALYTICAL CHEMISTRY. Volume III. ELEMENTAL ANALYSIS WITH MINUTE SAMPLES. STANDARDS AND STANDARDISATION. SEPARATIONS BY LIQUID AMALGAMS. VACUUM FUSION ANALYSIS OF GASES IN METALS. ELECTROANALYSIS IN MOLTEN SALTS. Edited by G. SVEHLA. Pp. xvi + 322. Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company. 1976. Price Dfl125; $49.95.

Volume III contains five diverse monographs. The first, by Günther Tölg, is on Elemental Analysis with Minute Samples, in 138 pages with 1421 references, and is a joy to read for anyone who has worked on the micro-scale. It is Benedetti - Pichler up-to-date, and includes instrumental methods in brief summaries adequate for the selection of such a method for further reading. The greater part of the chapter is concerned with gravimetry, titrimetry and the more classical optical and electrochemical methods, together with some admirably lucid philosophy of perspective and appraisal. In the second chapter, on Standards and Standardisation (34 pp., 114 references), R. A. Chalmers has pertinent and penetrating points to make in his inimitable style. This section is worthy of careful, considered and meditative reading by all practising chemists. A neglected technique in which interest is stirring again, Separation by Liquid Amalgams, is authoritatively brought to attention by Kozlowski and Songina (31 pp., 53 references) in the next monograph. Considerable attention is given to the theory and practice of cementation. The fourth monograph is a tightly written account of the Determination of Gases in Metals by Vacuum and Inert-gas Fusion by W. T. Elwell and D. F. Wood (40 pp., 204 references). Methods for the determination of hydrogen, nitrogen and oxygen in ferrous materials and individual non-ferrous metals and alloys thereof are critically discussed. The inert gas methods are treated more briefly. The final monograph on Electroanalytical Chemistry in Molten Salts (56 pp., 302 references) is by Fung and Mamantov, and carries discussions of melt purification, reference electrodes, cell design and the techniques of potentiometry, voltammetry, chronoelectrometry and coulometry. Investigations are summarised in 20 pages of tables, fully documented. This monograph perhaps suffers most from limitation of space.

This is, therefore, a very heterogeneous collection. It is unlikely that any individual will be working in, or seek to inform himself with a view to working in, more than a couple of the fields covered; indeed, few institutions will be supporting work on such a variety of topics. All the monographs suffer in varying degree from brevity, although all of the authors carry great distinction in their respective fields. The policy of publishing volumes of miscellaneous contents whenever sufficient material comes to hand may have been forced on the Editor by the intermittent flow of manuscripts, and so must be accepted, but the mix of contents so produced does not commend itself to the consumer. Together with the price, this would seem to restrict sales to reference libraries. This is a pity, because there is a great deal of good material in this volume.

E. BISHOP

MÉTHODES D'ANALYSE AUTOMATIQUE DES EAUX. By PIERRE WARZEE. *Notes de Recherche*, 5. Pp. 63. Arlon (Belgium): Fondation Universitaire Luxembourgeoise. 1975.

This 63-page booklet contains a series of methods for the routine automatic analysis of Luxembourg waters. It does not claim to present any original material or statistical data relevant to the methods, but is simply a clear working manual (in French) for local use., The methods are based on Technicon recommended procedures and Technicon flow diagrams are shown for each procedure described.

The levels of concentration dealt with and the determinands included are those normally encountered in Luxembourg. The determinands can be readily summarised: some nitrogen, phosphorus and sulphur compounds, silicates, chloride, hardness, calcium, sodium, potassium and finally rhodamine.

The instructions are clearly set out, but there is probably little to be gained by buying the book outside Luxembourg. STELLA J. PATTERSON

WATER. A COMPREHENSIVE TREATISE. Volume 4. AQUEOUS SOLUTIONS OF AMPHIPHILES AND MACROMOLECULES. Edited by FELIX FRANKS. Pp. xxii + 839. New York and London: Plenum Press. 1975. Price $45.

This book is Volume 4 of a five-volume series, produced with the objective of reviewing the scientific roles of water. Other volumes in the series deal with the physics and physical chemistry of water, with aqueous solutions of simple non-electrolytes and of electrolytes and with water in disperse systems; their contents are listed in the preface to this volume.

Volume 4 describes the role of water in biological and technological situations, in chapters written by individual specialists in their field; for example, there are, amongst others, chapters dealing with surfactants, with dyestuffs, with synthetic polymers and with peptides and proteins. There is a comprehensive bibliography containing 1 600 references.

Much of the material presented is still the subject of active research, giving the impression that it could rapidly become out-dated. If the reader is not led to the book by way of an abstract, the main title, "Water, a Comprehensive Treatise," could be misleading. It is not a book for the "water industry" but, as with other volumes in the series, is essentially a book for the "academic" reader. It may be of value on the bookshelf of the analyst working in one of the fields covered but it contains no analytical chemistry as such. STELLA J. PATTERSON

STRATEGIES FOR MARINE POLLUTION MONITORING. Edited by E. D. GOLDBERG. Pp. x + 310. New York, London, Sydney and Toronto: John Wiley. 1976. Price £16.50; $27.50.

The growing awareness of the possible ecological consequences of the increasing use of the sea as a receptacle for man's waste products has led in recent years to a change in the emphasis of the work of the marine chemist. Because of the importance of pollution monitoring, and also because of the lack of any practical manual on this subject, the reviewer opened this book with a sense of anticipation. However, he was disappointed to find that instead of the strategies pretentiously promised by the title, he was presented only with tactics, and incomplete and rather poor ones at that! The treatments of the individual chapters are very non-uniform; some are review articles, some are research papers, others describe in detail the determination of one or more pollutants, while others make suggestions about the design of monitoring programmes.

The book is divided into four sections. The first covers the analysis of sea water, and in some instances sediments and marine organisms, for organic species including petroleum hydrocarbons, chlorinated aliphatic and aromatic hydrocarbons and phthalates. The second section, which deals with inorganic components, is very restricted in its scope, and no reference to electrochemical methods is made. It commences with an excellent review chapter on the application of neutron-activation analysis to sea water, marine organisms and atmospheric particulates. Then follows a sketchy chapter on atomic-absorption spectrophotometry. Unfortunately, most of the procedures that are described are far too insensitive for the analysis of sea water. Although the use of heated graphite furnaces is advocated, the need for background correction is not mentioned. In the field of radionuclides the coverage is particularly disappointing as only the determination of plutonium is described.

The third section, which is entitled "Analytical Aids," is concerned with a variety of topics such as inter-calibration, the avoidance of contamination in sampling and the use of ion-exchange resins for pre-concentration. The development of sampling and monitoring programmes is discussed in a general fashion in the final section.

It is difficult to know for whom this book has been compiled. Certainly, anyone wanting an over-all view of the problems of marine pollution monitoring will be disappointed, as also in general will those requiring details of the determination of a particular pollutant. Because of the incompleteness of its coverage and the poor quality of many of its chapters, the reviewer does not feel that he can recommend this book. J. P. RILEY

IMMOBILIZED ENZYME TECHNOLOGY. RESEARCH AND APPLICATIONS. Edited by HOWARD H. WEETALL and SHUICHI SUZUKI. Pp. x + 321. New York and London: Plenum Press. 1975. Price $30.

The potential of immobilised enzymes is being widely investigated in many branches of science and industry, including analytical chemistry. The present text, which consists of 19 papers

presented at a US - Japanese seminar in Tokyo in November, 1974, is concerned with the various methods of immobilising enzymes, of measuring their properties, and of the chemical engineering and industrial aspects of the subject. For those interested in these areas, the coverage is widespread and the treatment clear and informative.

The general analytical chemist, however, will find little to interest him, although the use of a silica - alumina absorbent to which thiol groups have been covalently attached via silane intermediates as a means of removing heavy metals from flowing aqueous systems (R. P. Chambers *et al.*, Tulane University, New Orleans) was particularly interesting, and has far wider implications than in protecting enzymes from heavy metal inhibitors. ALAN TOWNSHEND

LABORATORY SAFETY HANDBOOK. Pp. 16. Thornaby, Cleveland: Sanderson Chemical Consultants Ltd. 1976. Price £0.60 per copy for 50 or more copies.

This booklet provides a brief introduction to safe practice in chemical laboratories, and can be supplied to companies for distribution to their personnel, especially new employees. The cover is blank and can be overprinted with a company's name and other details, and p. 3, listing mandatory rules, can be replaced by a company's own particular requirements.

The booklet is divided into four sections: Introduction; Laboratory Working Practice (covering general procedures, accidents, fire and eye protection); Laboratory Operations (use of glass apparatus, distillations, vessels under pressure or vacuum, refrigerants and waste disposal); and Chemicals. The pages of the Handbook are clearly laid out, with important rules emphasised in bold type, and it provides a useful guide to and reminder of safe working practice. P. C. WESTON

PROCEEDINGS OF THE FIRST EUROPEAN SYMPOSIUM ON THERMAL ANALYSIS. ESTA 1. *University of Salford, U.K., 20–24 September* 1976. *Organised by the Thermal Methods Group of the Analytical Division of the Chemical Society.* Edited by DAVID DOLLIMORE. Pp. xxxvi + 458. London and Rheine: Heyden. 1976. Price $23; £11.50; DM73.50.

This book is a useful collection of, in most instances, comprehensive abstracts of the 146 papers presented at the Symposium. The abstracts average about 3 pages each and many include diagrams of apparatus and results expressed graphically or in tables. The papers are fairly evenly grouped into six sections, as follows: Instrumentation and Technique, Physical Chemistry, Organic Chemistry and Polymers, Inorganic Chemistry, Earth Sciences and Associated Industries, and Applied Sciences.

TG, DTA and DSC are the principal techniques considered, but there are papers on adsorption, mass spectroscopy combined with thermal analysis, enthalpimetric and thermometric methods and hot-stage microscopy. The wide range of compounds and materials examined by the thermal methods includes oxides, hydroxides, carbonates, nitrates, sulphides, carboxylates, polymers, clays, metals and alloys, glasses and textiles.

The emphasis is rather more on inorganic than on organic materials. Pharmaceuticals rate two papers only, and no biochemical investigations are reported.

The book will be helpful to specialists in the field of thermal analysis because it gives an indication of much of the work currently being undertaken by research groups throughout Europe and at some non-European centres (30 of the 288 contributors are from other continents). It is difficult to select any one paper for special mention. The three longest abstracts, in terms of pages, are concerned with the analysis of polyimides, co-precipitated lead and barium carbonates, and dolomite. One of the titles in the contents list, "A Thermobalance for Work to 15 000 °C," may intrigue materials scientists; unfortunately the last 0 is an error!

E. J. GREENHOW

ASSAY OF DRUGS AND OTHER TRACE COMPOUNDS IN BIOLOGICAL FLUIDS. Edited by ERIC REID. *Methodological Developments in Biochemistry, Volume* 5. Pp. x + 254. Amsterdam, New York and Oxford: North-Holland. 1976. Price Dfl65; $24.95.

This slim volume is the fifth in a series, "Methodological Developments in Biochemistry," largely concerned with separation techniques. The text constitutes a lightly edited record of a

"Techniques Forum" held in 1975 at the University of Surrey. The 44 contributors include 33 from academic and clinical establishments and the remainder from six industrial concerns. The papers are arbitrarily grouped into four sections, themselves arranged in a not obviously logical sequence.

For determining drug species in physiological samples, publications frequently describe end-method strategy without revealing or exploring the tactics in the choice of, or factors affecting, prior separation; this volume attempts both objects. In its first section, five principal papers deal with the instrumental finish. Stationary phases and detectors for GC are reviewed with special reference to wall-coated (capillary), and fine-support, open-tubular columns; in general, the latter are less effective but permit higher loading. Comments by other contributors mainly refer to particular GC - MS experience but a useful note rehearses a number of derivatisation reactions suitable for ECD quantitation. The second main contribution describes polarographic applications and includes a table of amenable functional groups: despite being in the "end-game" section of the book, separation stages are stressed. Widely used fluorescence, and more limited phosphorescence, analyses are well reviewed, with some attention given to sensitivity limitations arising from natural ("biological") luminescence. The limitations and reproducibility of semi-quantitative TLC, by elution or densitometry, are contrasted with its inherent flexibility and prospect of added sensitivity and selectivity, including the novel cyclic technique of programmed multiple development. Finally, factors important in detection and resolution by HPLC are considered in the particular context of porphyrin ester assay.

The section on general analytical strategy, curiously placed in the middle of the book, offers some strategic options and a little perspective on earlier and subsequent contributions. One article is directed to the state of chromatographic art, particularly bonded-phase packings for HPLC and general calibration procedures; the "catechism" of assay principles should be read carefully. The second paper reviews immunoassay and protein-binding techniques, both as rationale and for synthesis of the conjugates, and the third deals with factors affecting ion-pair extraction into organic solvents for a variety of charged or chargable species. The sample preparation section contains six papers and the following discussion. Solvent extraction is, naturally, well represented: a logical approach and key factors are canvassed. Freeze-drying conditions, *in situ* masking with suitable reagents, pre-column techniques in HPLC and anion exchange all receive a mention. The final, and largest, section consists of 12 case studies with particular examples of corticoids, adrenergics, neuroleptics, anticonvulsants, an anti-inflammatory, a chlorophenolic, cephem and aminoglycoside antibiotics, some metabolites (variously in blood or urine) and a pertinent study of a prophylactic in animal feeds.

The format of the contributed articles and reports of detailed discussion gives a somewhat disorganised impression. The commendable objective of rapid (but, unhappily, not inexpensive) publication is achieved by photo-reduction of typescript. This expedient is not universally welcome; some readers will be distracted by the multiplicity of "golf-ball" type-faces, the frequent columnar or tabular layout and the lack of margin justification. The style varies from simple lecture script to full academic tutorial; not surprisingly, it does not achieve an agreeable narrative style. The index is neither systematic nor rigorous but, taken with the list of individual papers, it is adequate for instructional use. Whether the book itself achieves its subsidiary aim, *viz.*, becoming a desk guide for pharmaceutical bioanalysts, is more speculative.

G. F. PHILLIPS

Effect of Different Sample Preparation Methods on the Atomic-Absorption Spectrophotometric Determination of Calcium in Plant Material

A study is reported showing the variability in the figures for calcium content obtained on certified standard reference plant material that had been subjected to five different methods of digestion. The results show that some methods of preparation resulted in inaccurate calcium contents. Choice of acids and methods for digesting the plant material to the stage where calcium can be extracted into dilute nitric acid, time and concentration of releasing agents (lanthanum and strontium) significantly affected the calcium results. The use of a dinitrogen oxide - acetylene flame or additional dilutions so that the calcium content of the solution was approximately 1–5 p.p.m. usually gave acceptable results.

Keywords: *Calcium determination*; *atomic-absorption spectrophotometry*; *sample digestion*; *flame oxidant*

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Analyst, 1977, **102**, 446–452.

Automatic Determination of Sulphate in Water Samples and Soil Extracts Containing Large Amounts of Humic Compounds

An automatic nephelometric method for the determination of sulphate in water samples, soil leachates and soil extracts is described. Dialysis of the samples eliminates interference from dissolved coloured humic compounds and suspended matter. The capacity of the method is 24 samples per hour and the precision is $s = 0.49$ mg l^{-1} of sulphate-sulphur in the range 1–100 mg l^{-1} of sulphate-sulphur. The method gives more reliable results for coloured samples than does the turbidimetric method with colour correction.

Keywords: *Sulphate determination*; *water*; *soil*; *automatic analysis*; *nephelometry*

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Analyst, 1977, **102**, 453–457.

Spectrophotometric Method for the Determination of Total Steroidal Sapogenin

A specific method for the spectrophotometric determination of total steroidal sapogenin, based on colour reactions with anisaldehyde, sulphuric acid and ethyl acetate and applicable to microgram amounts, is described.

It has been shown that this determination can be carried out directly on a saponin solution and that there is virtually no interference from sugars, sterols, fatty acids and vegetable oil. The sapogenins have the same colorimetric properties whether they are in the free state, bound with sugars, esterified with acetic acid or mono- or polyhydroxylated. The method described is accurate (relative error 1.4%), rapid, easily automated and gives a chromophore with the same absorption spectrum with only one peak at 430 nm for all of the sapogenins tested: diosgenin, tigogenin, hecogenin, smilagenin, yonogenin, tokorogenin, etc. The molar absorption coefficient is approximately 49 000.

Keywords: *Steroidal sapogenin determination*; *spectrophotometry*

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Analyst, 1977, **102**, 458–465.

Applications of Particle Size Analysis in the Pharmaceutical Industry

Recent innovations in equipment for particle size analysis have enabled complete particle size distributions of fine powders to be obtained both cheaply and rapidly. This development has enabled applications, which previously would have been of only academic interest, to be developed as practical methods for routine use in the pharmaceutical industry. Four applications of particle size analysis based on a Model T_A Coulter counter are described: (1) the detection of fluctuations in the process variables of a batch crystallisation process; (2) close control of pharmaceutical grinding; (3) evaluation of the particle size distribution of the disintegration products of tablets; and (4) the assay of the percentage of amorphous insulin in Insulin Zinc Suspension.

Keywords: *Particle size analysis*; *pharmaceuticals*; *crystallisation*; *grinding*; *amorphous insulin*

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Analyst, 1977, **102**, 466–472.

Determination of Nitrogen-15 by Emission Spectrometry: Procedure for Use with Small Amounts of Nitrogen

Short Paper

Keywords: *Nitrogen-15 determination*; *plant material analysis*; *emission spectrometry*

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Analyst, 1977, **102**, 473–476.

Elimination of Sulphite Interference in the Spectrophotometric Determination of Nitrite

Short Paper

Keywords: *Nitrite determination*; *sulphite interference*; *spectrophotometry*

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Analyst, 1977, **102**, 476–479.

Determination of Water in Iodine by Infrared Spectroscopy

Short Paper

Keywords: *Water determination*; *iodine*; *infrared spectroscopy*; *bromine solvent*

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