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Summaries of Papers in this Issue

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The interference of cations and anions on the absorption analytical lines of gold 242·8 nm and silver 328·1 nm has also been investigated.

T. P. MICHAILOVA and V. A. REZEPINA

Siberian Department of the USSR Academy of Sciences, The Institute of Physico-Chemical Methods of Mineral Processing, Novosibirsk, USSR.

Analyst, 1970, **95**, 769–775.

The Use of Electronically Modulated Microwave-excited Discharge Tubes in Atomic Spectroscopy

The performance of a previously described system involving electronic modulation of microwave tubes is reported, with particular reference to sensitivity in atomic-fluorescence spectroscopy. The limits attainable by using modulated and unmodulated sources with the same measuring system are compared, and the former shown to have advantages.

K. C. THOMPSON and P. C. WILDY

Southern Analytical Ltd., Frimley Road, Camberley, Surrey.

Analyst, 1970, **95**, 776–780.

Studies on the Analytical Chemistry of Hafnium and Zirconium
Part II. Fluorimetric Determination of Hafnium in the Presence of Zirconium by Using Quercetin

Hafnium (greater than 0·3 per cent.) in hafnium - zirconium mixtures can be determined by measuring the fluorescence of the hafnium complexes with quercetin or morin in 57·5 per cent. perchloric acid. The zirconium complex with quercetin does not fluoresce, and that with morin fluoresces only weakly at this acidity. Iron, titanium, vanadium, uranium and molybdenum interfere.

A mechanism for the selective fluorescence reaction is suggested.

A. BROOKES and A. TOWNSHEND

Department of Chemistry, The University of Birmingham, P.O. Box 363, Birmingham 15.

Analyst, 1970, **95**, 781–785.

The Spectrofluorimetric Determination of Sulphides

The spectrofluorimetric determination of trace amounts of sulphide with mercury(II) - 2,2′-pyridylbenzimidazole is described. The sulphide can be determined in solutions with concentrations in the range 300 pg to 300 ng ml^{-1}, with a standard deviation of 0·75 per cent. for a concentration of 60 ng ml^{-1}. Interferences can be avoided by the use of standard distillation procedures. The main advantages, apart from the extremely high sensitivity of the method, are the high stability of the reagent and of the reacted sulphide system. The over-all time of the method after liberation of the sulphide can be as little as 2 to 5 minutes, and the time lapse between the reaction and measurement of solution is not critical as up to 48 hours' delay has no significant effect.

L. S. BARK and A. RIXON

Department of Chemistry and Applied Chemistry, University of Salford, Salford, Lancs.

Analyst, 1970, **95**, 786–790.

RS FOR THE ANALYSIS OF PESTICIDES RESIDUE

CARLO ERBA HAS DEVELOPED A SERIES OF SOLVENTS WHICH POSSESS A MAXIMUM RESIDUE OF THE ORDER OF 10^{-9} % OF INTERFERING SUBSTANCES (GLC e.c.d. EXPRESSED AS ALDRIN).

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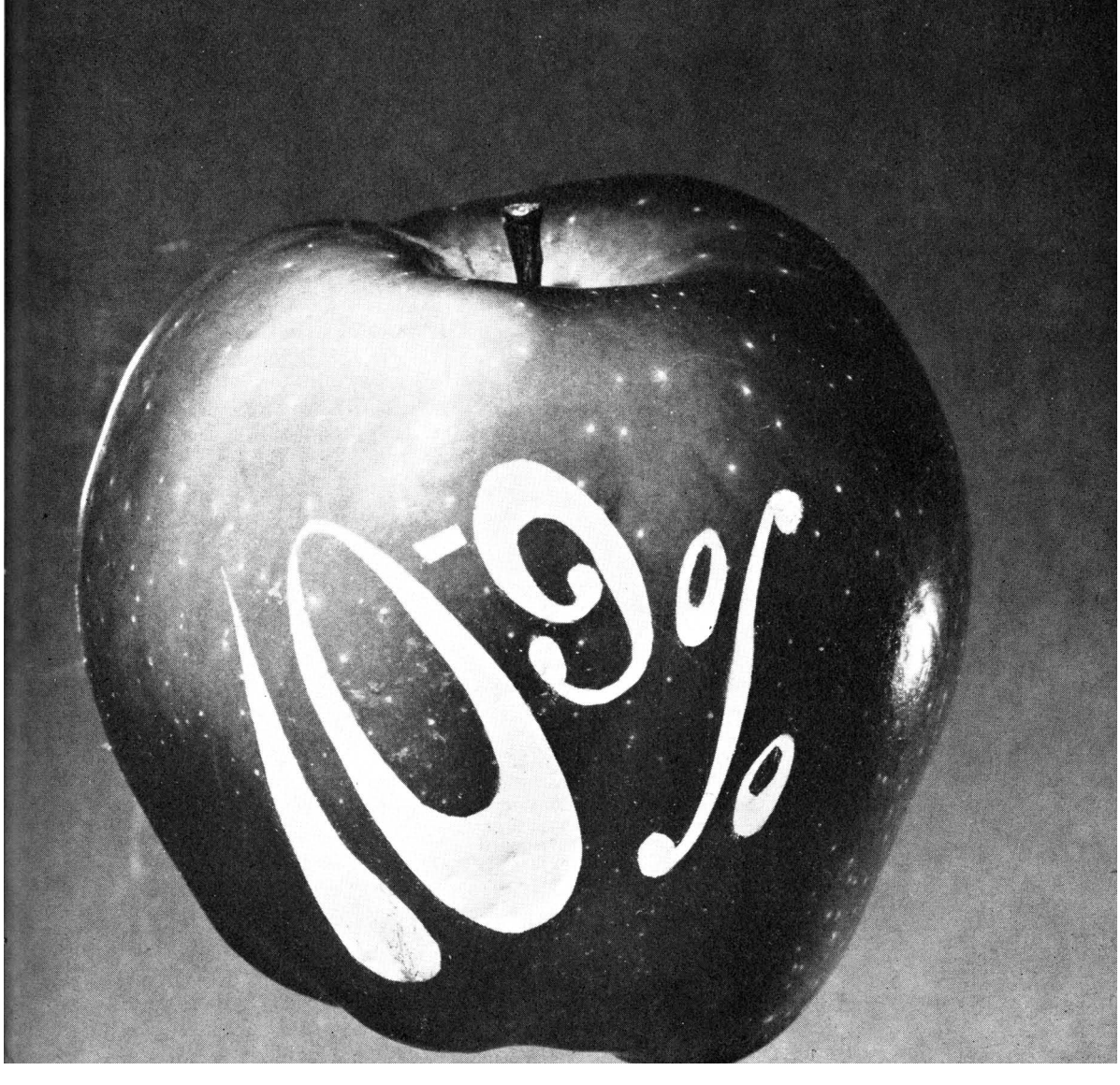

Kinetic and Analytical Investigations of the Chlorate and Iodate Oxidations of Vanadium(IV) in a Perchloric Acid Medium

Kinetic studies of the chlorate and iodate oxidations of vanadium(IV) in perchloric acid have shown that the rates of the reactions are described by two different equations. Possible mechanisms for the two reactions are described and values for the rate constants and equilibrium constants involved in the reactions are reported, together with ionic strength and temperature dependence data for the two rate constants. The feasibility of using either the chlorate or the iodate oxidation of vanadium(IV) as a titrimetric reaction is discussed in relation to both the kinetic results obtained, and to similar data reported previously for the bromate oxidation of vanadium(IV).

C. W. FULLER and J. M. OTTAWAY

Department of Pure and Applied Chemistry, University of Strathclyde, Cathedral Street, Glasgow, C.1.

Analyst, 1970, **95**, 791–796.

The Determination of Traces of Beryllium in Human and Rat Urine Samples by Gas Chromatography

The toxic nature of beryllium necessitates the use of a sensitive method for its detection. Present methods are not entirely satisfactory and the use of gas chromatography for the detection and determination of beryllium has been advocated. In the procedure described, beryllium is isolated by solvent extraction in the form of a volatile, thermally stable chelate with trifluoroacetylacetone, and is then determined by gas chromatography with an electron-capture detector. Results are presented for the determination of aqueous beryllium solutions and for beryllium contained in urine. Satisfactory results were obtained with both direct solvent extraction and solvent extraction after wet oxidation of the sample. The rate of excretion of beryllium by rats was measured by this method. The procedure is rapid, reliable and sensitive; the limit of detection is 1 ng ml^{-1}.

J. K. FOREMAN, T. A. GOUGH and E. A. WALKER

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Analyst, 1970, **95**, 797–804.

Flame-photometric Determination of Bromine in Urine

A flame-spectrophotometric method is given for the determination of bromide and other bromine-containing compounds in human urine, involving the application of the indium bromide flame technique. This method can be used, for example, to analyse excretions of bromine-containing narcotics, sleeping pills and other bromine-containing compounds, such as halothane, in a simple, quick and specific manner.

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Analyst, 1970, **95**, 805–808.

THE ANALYST

The Determination of Gold and Silver in Plant Liquors and Electrolytes by Atomic-absorption Spectrophotometry*

BY T. P. MICHAILOVA AND V. A. REZEPINA

(Siberian Department of the USSR Academy of Sciences, The Institute of Physico-Chemical Methods of Mineral Processing, Novosibirsk, USSR)

An atomic-absorption method for determining gold and silver in solutions of varying complexity and variable composition in one set of standard solutions is suggested. The sensitivity of the method is 0·05 μg ml^{-1} and the coefficient of variation is 2 to 3 per cent.

The interference of cations and anions on the absorption analytical lines of gold 242·8 nm and silver 328·1 nm has also been investigated.

THE solution to some technical problems, in particular the recovery of gold and silver from plant liquors by an electrochemical method, urgently needs to be found and new methods of analysis investigated.

Direct spectrochemical emission methods (both flame-photometric and spectrographic) for the determination of these elements are neither sensitive enough nor accurate, while chemical methods are extremely complex and ineffective.

During the last decade many papers have been published on the determination, by atomic-absorption methods, of gold in tungsten carbide, ores, lead and other materials[1 to 20] as well as of silver in ores, sulphide minerals, copper alloys, lead, zinc, gold, oil and other materials.[4 to 18, 21 to 32]

The aim of this work was to investigate and develop an atomic-absorption method for the determination of gold and silver in plant liquors and electrolytes of complex and variable composition.

EXPERIMENTAL

APPARATUS—

An atomic-absorption spectrophotometer incorporating a 3MP–3 monochromator was used. The hollow-cathode lamps used as a source of resonance radiation were operated by direct current from a high-voltage rectifier - stabiliser, BBC–1 (1000 V, 100 mA).

For atomisation of the material an acetylene - air flame from a 10 mm diameter circular burner was used and the atomisation of standard and sample solutions effected with an atomising system from a Zeiss flame photometer.

To increase the sensitivity of the determination of gold and silver a T-shaped adaptor made with a 16 mm diameter quartz tube, 250 mm in length,[28,33] was positioned at a distance of 30 mm from the top of the burner.

To illuminate the monochromator slit a system consisting of two quartz lenses with focal lengths of 150 and 75 mm was used and the light beam focused on the centre of the adaptor.

A photomultiplier (ФЭУ–18А) served as a receiver for the absorption signal, and was fed by current with a stabilised voltage from a high-voltage rectifier included in ФЭП–I. The direct current from the photomultiplier was amplified by a d.c. amplifier from ФЭП–I and the absorption signal recorded automatically by an electronic potentiometer (ПСИ–02 or ЭПП–09М2).

To choose the optimum operating conditions providing maximum sensitivity and accuracy in the determination of gold and silver by this method, the dependence of the resonance lines (gold 248·8 nm and silver 328·1 nm) absorption on the ratio of acetylene to air in the combustion mixture and the operating conditions of the hollow-cathode lamps with the width of the inlet and outlet slits of the monochromator (3MP–3) were investigated. As a result of these investigations the following optimum conditions were chosen for the analysis (Table I).

* Paper presented at the International Atomic Absorption Spectroscopy Conference, Sheffield, 1969.

TABLE I

OPTIMUM CONDITIONS FOR THE ANALYSIS

Element determined	Analytical lines, nm	Acetylene flow-rate, l minute^{-1}	Air flow-rate, l minute^{-1}	Slit width (3MP–3), mm		Discharge current of hollow-cathode lamp, mA	Current voltage on ФЭУ–18А, V
				Inlet	Outlet		
Gold	242·8	0·5	5	0·03	0·03	7	780
Silver	328·1	0·5	5	0·025	0·03	5	600

Under these conditions the calibration graphs are straight lines for the concentration range of 0·5 to 50 μg ml^{-1} for gold and 0·2 to 50 μg ml^{-1} for silver.

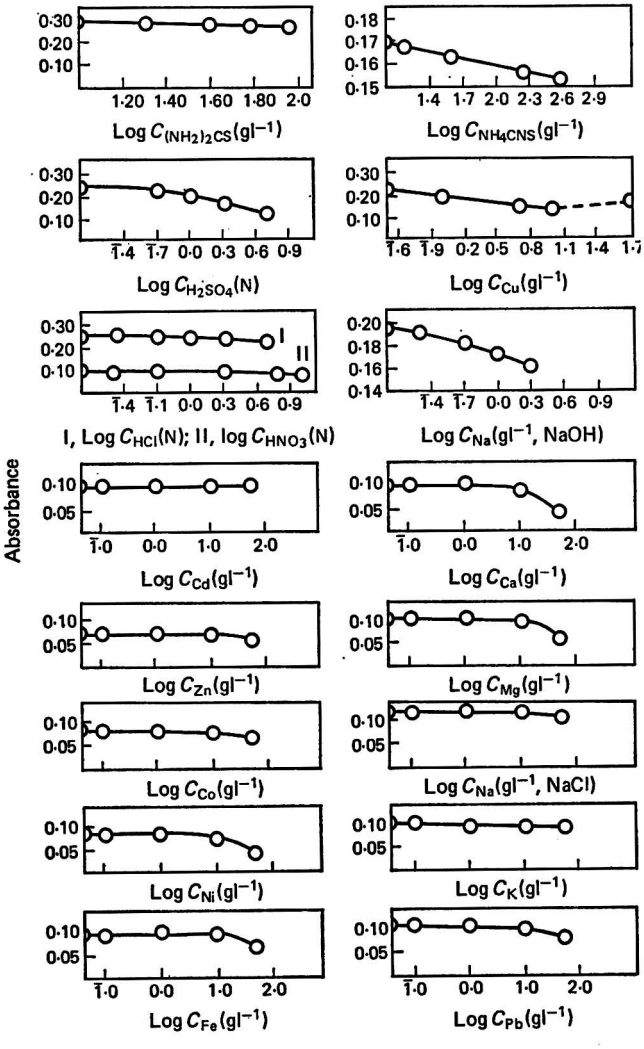

Fig. 1. Dependence of absorbance of combusted flame gases, with wavelength for gold 242·8 nm, on concentration of cations and anions in investigated solutions

The sensitivities of the gold and silver determinations are enhanced 5-fold by using the scale-expansion method of recording (IICI–02).[34] The sensitivity for silver is 0·05 μg ml^{-1} and for gold 0·1 μg ml^{-1}; the sensitivity of the latter can be increased by one order of magnitude if the gold is extracted with isopentyl alcohol.

To investigate the effect of cations and anions present in electrolytes and plant liquors on the absorption of the analytical lines of gold and silver, series of solutions containing various amounts of the cations iron, nickel, cobalt, zinc, cadmium, copper, lead, potassium, sodium, magnesium and calcium up to 50 g l^{-1}, and the anions NO_3^-, SO_4^{2-} and Cl^- up to 5 N for gold and NO_3^-, SO_4^{2-} and CH_3COO^- up to 5 N for silver, were prepared.

Experiments were carried out under the optimum operating conditions given in Table I. The dependence of the absorbance of the combusted flame gases with the wavelength for gold 242·8 nm and silver 328·1 nm (A_{Au} and A_{Ag}) on concentrations of cations and anions in investigated solutions is given in Figs. 1 and 2.

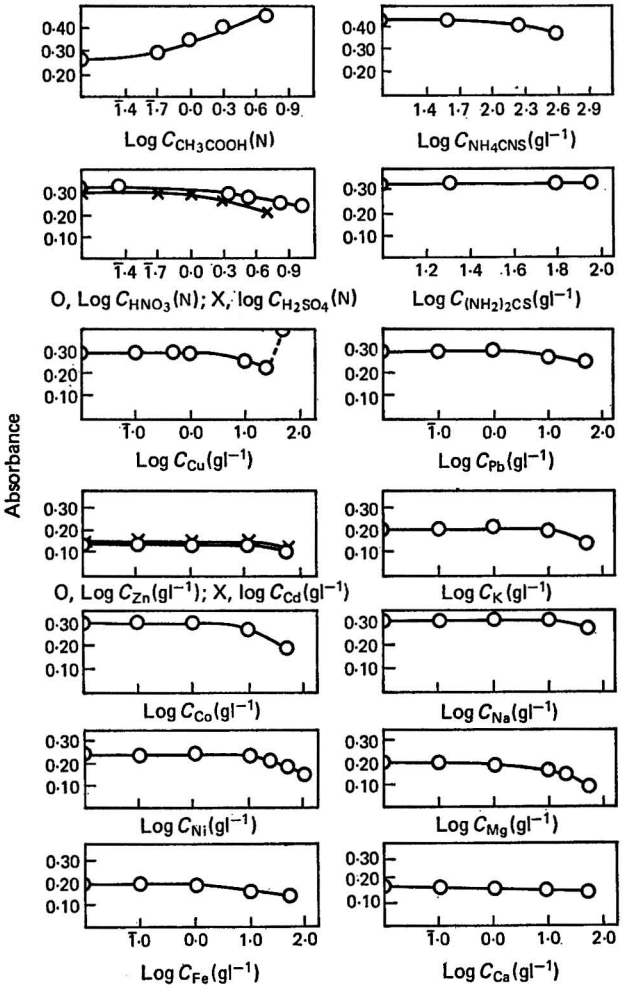

Fig. 2. Dependence of absorbance of combusted flame gases, with wavelength for silver 328·1 nm, on concentration of cations and anions in investigated solutions

The limiting concentrations of the elements present with gold and silver in plant liquors, and also the chemical combinations present in electrolytes, which do not interfere in the determination of gold and silver, are given in Tables II and III.

TABLE II

LIMITING CONCENTRATIONS OF CATIONS AND ANIONS THAT DO NOT INTERFERE IN THE GOLD DETERMINATION

Chemical compound	Cation or anion	Limiting concentrations of cations (g l^{-1}), and anions (N, per cent.) not interfering in the determination	Maximum concentrations of cations (g l^{-1}), and anions (N, per cent.)	Percentage interference at maximum concentration
NH_4SCN	SCN^-	29	290	−23
$(NH_2)_2CS$	CS^{2-}	46	52	−22
NaOH	OH^-	0·2	2·6	−58
$CuCl_2$	Cu^{2+}	0·1	50	−38
$Ni(NO_3)_2$	Ni^{2+}	1·0	50	−65
$NaNO_3$	Na^+	10	50	−10
$MgCl_2$	Mg^{2+}	10	50	−39
$CaCl_2$	Ca^{2+}	10	50	−51
$Zn(NO_3)_2$	Zn^{2+}	10	50	−11
$Pb(NO_3)_2$	Pb^{2+}	10	50	−22
$FeCl_3$	Fe^{3+}	10	50	−33
$CoSO_4$	Co^{2+}	10	50	−21
KNO_3	K^+	50	50	−4
$Cd(NO_3)_2$	Cd^{2+}	50	50	+4
H_2SO_4	SO_4^{2-}	0·25	5	−49
HCl	Cl^-	0·5	5	−14
HNO_3	NO_3^-	0·5	5	−30

TABLE III

LIMITING CONCENTRATIONS OF CATIONS AND ANIONS THAT DO NOT INTERFERE IN THE SILVER DETERMINATION

Chemical compound	Cation or anion	Limiting concentrations of cations (g l^{-1}), and (anions N, per cent.) not interfering in the determination	Maximum concentrations of cations (g l^{-1}), and anions (N, per cent.)	Percentage interference at maximum concentration
NH_4SCN	SCN^-	68	290	−17
$(NH_2)_2CS$	CS^{2-}	52	52	+3
$MgSO_4.7H_2O$	Mg^{2+}	1·0	50	−56
$Ca(NO_3)_2.4H_2O$	Ca^{2+}	1·0	50	−10
CH_3COOCu	Cu^+	1·0	25	−21
$FeSO_4.7H_2O$	Fe^{2+}	1·0	50	−27
KNO_3	K^+	10	50	−22
$Zn(NO_3)_2$	Zn^{2+}	10	50	−21
$Cd(NO_3)_2.4H_2O$	Cd^{2+}	10	50	−26
$Pb(NO_3)_2$	Pb^{2+}	10	50	−12
$CoSO_4$	Co^{2+}	10	50	−36
$Ni(NO_3)_2$	Ni^{2+}	10	50	−49
$NaNO_3$	Na^+	50	50	−3
CH_3COOH	CH_3COO^-	0·1	5	+70
HNO_3	NO_3^-	0·2	13	−26
H_2SO_4	SO_4^{2-}	0·5	5	−32

In view of the need to determine macro amounts of gold and silver, from 0·05 to 2 g l^{-1}, in electrolytes the solutions to be analysed were diluted 10 to 100-fold. With such dilute solutions the effects of sulphuric acid, thiourea, ammonium thiocyanate and sodium hydroxide on the absorption of the analytical lines of gold and silver can be neglected.

Thus, according to the above experiments, it can be concluded that it is possible to determine gold and silver in electrolytes by using one series of standards. Moreover, by investigating the effect of the elements present together with gold and silver it can also be concluded that copper, iron, magnesium, calcium, nickel, cobalt, lead, zinc, cadmium, sodium and potassium (at their maximum concentrations in the solutions) do not interfere in the determination of gold and silver in plant liquors. Consequently, their determination in both plant liquors and electrolytes can be carried out with sufficient accuracy by using one series of standards, which is especially important when carrying out routine determinations of gold and silver in solutions of variable and complex composition.

PREPARATION OF STANDARDS—

A stock standard solution of gold was prepared by weighing 2·090 g of chloroauric acid ($HAuCl_4.4H_2O$) (1 g of gold) into a 1-litre measuring flask and adding distilled water to the mark. A series of dilute standard solutions (0·05 to 50 μg ml^{-1}) for the determination of gold in electrolytes and plant liquors was prepared by diluting the stock standard solution with 20 per cent. solution of aqua regia (Note 1).

NOTES—

1. Twenty per cent. solution of aqua regia is used for the dilution because on long standing the gold precipitates from an aqueous standard solution.

2. Standard solutions of silver with a mercury content of 0·3 g l^{-1} can be stored in darkened bottles for several months without loss of silver concentration.

A stock standard solution of silver was prepared as follows. One gram of metallic silver was placed in a flask and dissolved by heating with nitric acid on an electric hot-plate. After dissolution and evaporating nearly to dryness the residue was dissolved in 1 to 2 drops of concentrated nitric acid followed by a little distilled water, and the solution then transferred into a 1-litre measuring flask and diluted to the mark with distilled water. A series of dilute standard solutions was prepared by diluting the stock standard solution (1000 μg ml^{-1}) with distilled water, with the addition of mercury nitrate (0·3 g l^{-1}) (C_{Hg}).[22] Mercury (Note 2) is added to the standard solutions of silver to prevent the formation of silver chloride, which is readily precipitated on the walls of atomising and mixing chambers and may lead to a decrease in absorption.

RECOMMENDED PROCEDURE—

One to two millilitres of the sample solution of electrolytes or plant liquors were placed in a measuring flask of 10 to 100-ml capacity (for the determination of gold 2 to 20 ml of aqua regia are added, and for silver 0·3 g l^{-1} of mercury) and made up to the mark with distilled water. After thoroughly mixing the solution, the determination was carried out by the method of comparison in one series of standards.

The absorbances A_1, A_2 and A_x of the combusted flame gases in the adaptor, with the wavelengths of gold 242·8 nm and silver 328·1 nm, on atomising the standard solutions with

TABLE IV

RESULTS FOR THE ATOMIC-ABSORPTION DETERMINATION OF GOLD IN SYNTHETIC SOLUTIONS OF SIMILAR CHEMICAL COMPOSITION TO ELECTROLYTES

Gold present, μg ml^{-1}	Gold found, μg ml^{-1}	Error, per cent.
15·0	15·0	0·0
10·0	10·2	2·0
1·0	0·9	10·0
3·0	2·8	6·0
5·0	4·8	4·0
10·0	9·8	2·0
15·0	15·0	0·0
20·0	20·6	0·3
25·0	25·0	0·0
30·0	30·3	1·0
40·0	40·2	2·0
3·0	3·0	0·0
5·0	5·2	4·0

the concentration of the element $C_1 < C_x$ and $C_2 > C_x$, and the sample solution with concentration of C_x, are calculated from the recorded values of the absorption signal. Then, from A_x, the unknown concentration of gold or silver (C_x) is determined either from the calibration graph or by calculation according to the formula given elsewhere.[35]

To check the accuracy of the atomic-absorption method for the determination of gold and silver in electrolytes of variable chemical composition, synthetic solutions with compositions similar to electrolytes containing known amounts of gold and silver were prepared. The results obtained for gold and silver in these solutions, by using the comparison method, with one series of standards, are summarised in Tables IV and V.

TABLE V

RESULTS FOR THE ATOMIC-ABSORPTION DETERMINATION OF SILVER IN SYNTHETIC SOLUTIONS OF SIMILAR CHEMICAL COMPOSITION TO ELECTROLYTES

Silver present, μg ml^{-1}	Silver found, μg ml^{-1}	Error, per cent.
10·0	10·14	1·4
10·0	9·7	3·0
10·0	9·7	3·0
10·0	9·8	2·0
10·0	9·94	0·6
10·0	9·9	1·0
10·0	10·3	3·0
10·0	10·1	1·0
10·0	10·16	1·6
10·0	10·1	1·0
10·0	10·0	0·0
10·0	10·3	3·0
10·0	9·87	1·3
10·0	10·27	2·7
10·0	9·8	2·0

The results given in Tables IV and V show that by using the atomic-absorption method no systematic errors occur from the effects of variable chemical composition of electrolytes.

The accuracy of the atomic-absorption determination of gold and silver in plant liquors by the comparison method was checked by the method of addition.

To evaluate the coefficient of variation characterising the reproducibility of the method, the determination of gold and silver was carried out on twenty-five samples (two parallel determinations for each sample in a series of analyses).

Statistical processing of the results shows that the coefficient of variation of the determination of gold and silver in the concentration range 1 to 40 μg ml^{-1} is 2 to 3 per cent., and at the sensitivity limit is 0·05 μg ml^{-1} (ratio of signal to noise = 2) $>$ 20 per cent.

The developed atomic-absorption method for the determination of gold and silver has found practical application at the Institute of Physico-Chemical Methods of Mineral Processing in the Siberian Department of the USSR Academy of Sciences for the routine determination of gold and silver in electrolytes and plant liquors of complex and variable chemical composition.

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The Use of Electronically Modulated Microwave-excited Discharge Tubes in Atomic Spectroscopy

By K. C. THOMPSON and P. C. WILDY
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The performance of a previously described system involving electronic modulation of microwave tubes is reported, with particular reference to sensitivity in atomic-fluorescence spectroscopy. The limits attainable by using modulated and unmodulated sources with the same measuring system are compared, and the former shown to have advantages.

The operation of microwave-excited discharge tubes with modulation effected by electronic means, as opposed to mechanical "choppers," has already been described,[1] and these tubes have now been investigated for use as primary light sources in atomic-fluorescence spectroscopy. The reasons for modulation and those for using electronic modulation have already been discussed, and will not be dealt with further here; the advantages of microwave tubes as sources for this type of work have been described by Dagnall and West[2] and their use with mechanical chopping by Browner, Dagnall and West.[3]

Experimental

The modified Southern Analytical A3000 atomic-absorption spectrophotometer used in the earlier work was fitted with a burner giving a rectangular cross-section flame suitable for fluorescence measurements. This burner consisted of the normal type of burner body and head, with the slotted top-plate replaced by one with a series of forty-five holes arranged in a rectangle (20 × 5 mm) at one end of the head. The rectangle was positioned so that the flame was about 90 mm from the monochromator entrance slit.

With this burner, and a flame based on an air flow of 7·0 l minute^{-1}, it was found that the lowest burner position on the A3000 spectrophotometer still allowed light from the primary cones to enter the monochromator. This is undesirable as the background emission from this part of the flame is one to two orders more intense than that from the interconal region, and the high background light level seriously impairs the signal-to-noise ratio. A mask was therefore fitted to the end of the burner so that light from the primary cones could not pass through the entrance slit.

The arrangement of the burner, cavity and monochromator is shown in Fig. 1. The 210L cavity was used for all work for reasons already described; a quartz plate was interposed between the cavity aperture and the flame as it was found that when a strong current of cooling air was required by the tube the resultant turbulence around the cavity aperture disturbed the flame and gave rise to unstable readings.

Flame conditions—

Flame conditions for the atomic fluorescence of the eight elements for which tubes had been made were investigated, by using an air - acetylene flame. The flame was set in all instances with an air flow of 7 l minute^{-1} as this was the optimum for the nebuliser being used, and the flame composition was varied by alteration of the acetylene flow. It was found that for all elements, except arsenic, the flame characteristics and burner height were relatively unimportant; the burner was therefore set to the bottom of its travel and the flame adjusted to be slightly fuel rich, *i.e.*, with deep blue "soft" cones, as these conditions gave minimum background. For arsenic, the flame composition markedly affected the fluorescence signal, and a richer flame with the cones just tipped with yellow was found to be optimum.

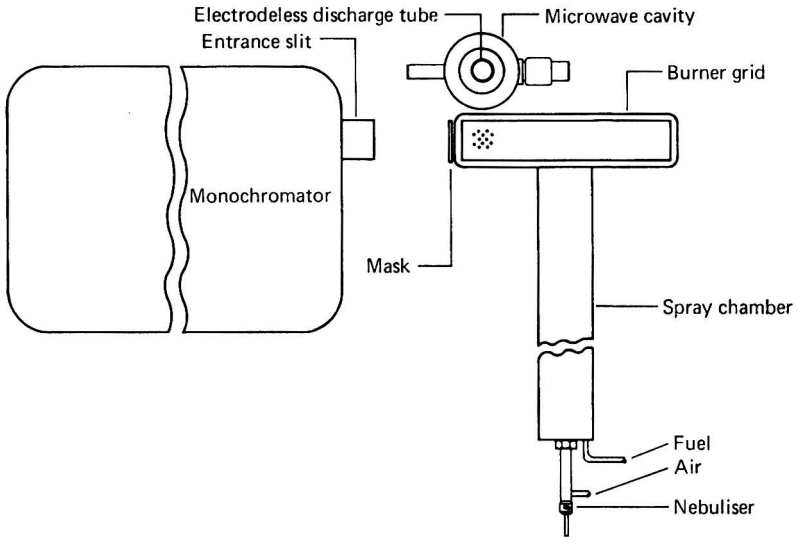

Fig. 1. Schematic lay-out of fluorescence equipment

SOURCE OPERATION—

The microwave tubes were operated under the optimum conditions determined and described in our earlier work [see Table I, reference 1 (p. 564)].

TABLE I

MAIN FLUORESCENCE LINES OF THE EIGHT ELEMENTS

Element	Wavelength, nm	Relative intensity
Cadmium	228·8	100
Zinc	213·9	100
Selenium	196·1	100
Selenium	204·0	87
Tellurium	214·3	100
Tellurium	238·3/238·6	77
Arsenic	189·0	97
Arsenic	193·7	100
Arsenic	197·2	90
Arsenic	235·0	64
Antimony	231·1	100
Antimony	217·6	90
Antimony	206·8	78
Bismuth	302·5	100
Bismuth	206·2	62
Mercury	253·7	100

SELECTION OF FLUORESCENCE LINES—

The wavelengths and apparent intensities of the main fluorescence lines for the elements studied are given in Table I; the line with the highest reading on our detector system is given the value 100, and the subsidiary lines are referred to it. These ratios apply to the different lines for single elements only, and no attempt has been made to correlate the relative intensities of different elements. The absolute values will probably differ slightly from those given, as no correction has been made for photomultiplier spectral response curves, etc.

For most elements, the optimum fluorescence line for highest sensitivity is the most intense line, but special considerations apply to the selection of a line for bismuth. The most intense bismuth line emitted by a bismuth tube is that at 306·8 nm, but its use for fluorescence measurements in an air - acetylene flame is rendered difficult as it is overlapped by the intense hydroxyl-band system present in this part of the spectrum. The 302·5 nm

line, with excitation by the 206·2 nm line, has been used successfully with the low background nitrogen - hydrogen flame,[4] but this also is overlapped by the hydroxyl bands in air - acetylene. The effect of the hydroxyl-band background is to impair the signal-to-background ratio and thus increase the limit of detection. Over-all, the 206·2 nm line is best in terms of fluorescence intensity and low flame background, but is only weakly emitted by a bismuth tube; the line used for excitation was therefore the very intense 206·2 nm iodine line, which overlaps the bismuth line sufficiently to give good fluorescence intensity. As reported in the earlier work, iodine tubes were not stable when modulated, and the iodine lines from arsenic and antimony tubes were used. The arsenic tube gave about three times greater sensitivity, possibly because of the lower stability of arsenic tri-iodide (AsI_3), and was therefore used for all work with bismuth.

The intensity of the 206·2 nm iodine line is very high, and this gave rise to scattered radiation from the various parts of the flame compartment of sufficiently high intensity to be detected. It was the intensity of this scattered light that defined the detection limit for bismuth, as it constituted a high background level upon which the small fluorescence signal was superimposed. The same effect was found with mercury, the other very intense tube studied, which emits an extremely intense line at 253·7 nm.

Sensitivity and linearity—

Calibration graphs were plotted for the optimum lines for all eight elements, and the concentration ranges over which they were found to be linear are shown in Table II. Linearity in this context is defined as calibration points falling within ±5 per cent. of the best straight line drawn through the points. The upper concentration range in which linearity is maintained can be raised by moving the source so that only the edge of the flame near to the entrance slit is irradiated, thus reducing self-absorption of the fluorescent radiation in the flame.

TABLE II

LINEARITY RANGES AND SENSITIVITY FOR THE EIGHT ELEMENTS

Element	Wavelength, nm	Linearity range, μg ml⁻¹	Detection limit, μg ml⁻¹		Band width, nm
			Modulated	Unmodulated	
Zinc	213·9	0·001 to 2	0·0005	0·002	3
Cadmium	228·8	0·001 to 2	0·0003	0·001	3
Mercury	253·7	0·5 to 80	0·2	2·2	1·2
Selenium	196·1	0·5 to 50	0·1	0·5	6
Selenium	204·0	1 to 100	0·15	0·5	6
Tellurium	214·3	0·2 to 100	0·05	0·25	3
Arsenic	193·7*	0·5 to 100	0·2	1·2	6
Antimony	231·1	0·2 to 100	0·04	0·5	3
Bismuth	206·2	2 to 150	0·4	2·0	6
Bismuth	302·5	5 to 500	1·0	Not determined†	1·2

* Not completely resolved from 189·0 and 197·2 nm at slit width used.
† Not determined because of very high flame background.

Table II also shows the detection limits attained for the elements studied (S:N = 2), by using the tubes in both the modulated and unmodulated modes but keeping all other measuring conditions constant. It will be seen that in all instances the detection limit is lowered by modulating and, as all other parameters remained the same, this improvement must be caused by the decrease in the effect of flame background.

Interferences

Possible interferences were studied with three sets of metals, which were chosen to represent different types of behaviour in the flame. Anions were not studied because it can be predicted that their effect in atomic fluorescence will closely parallel that found in atomic absorption and emission. The metals chosen were aluminium, calcium and magnesium, which form refractory oxides in the flame; sodium, potassium and calcium, which emit strongly; and copper, lead, zinc and iron, which are easily atomised. The results obtained were complex, sometimes being in accordance with fairly simple theory, and at other times differing widely from the expected behaviour. They can be summarised as follows.

CADMIUM AND ZINC—

One microgram per ml solutions were aspirated, with 100 μg ml^{-1} solutions of each "interfering" element. No interferences were observed, and it was possible to spray aluminium solutions containing 2000 μg ml^{-1} without effect. (Aluminium was selected as it was considered to be the most likely to cause source scattering interference.) The criterion of no interference was that the fluorescence-intensity reading for the sample solution alone should remain constant to within 5 per cent., *i.e.*, about three times the standard deviation; this criterion was applied throughout the interference work.

BISMUTH—

A 10 μg ml^{-1} solution was aspirated, with 500 μg ml^{-1} solutions of the interferants. No interference was observed at the 302·5 nm line, but aluminium caused some enhancement when the 206·2 nm line was used. A 500 μg ml^{-1} solution of aluminium raised the apparent intensity from 60 to 88 units; the aluminium solution alone gave a reading of 30 units, so that the rise would appear to be caused by source scatter.

ARSENIC—

An 8 μg ml^{-1} solution of arsenic and 400 μg ml^{-1} solutions of the postulated interfering elements were aspirated; the only interference noted was from aluminium, which raised the intensity reading from 51 to 53 units. An 800 μg ml^{-1} aluminium solution raised it further to 55 units, which again would seem to indicate source scattering.

MERCURY—

Mercury was more prone to source scattering interference than the other elements studied, presumably because of the very high intensity of the incident light and the relatively low fluorescence sensitivity of mercury. A 5 μg ml^{-1} solution of mercury gave an intensity reading of 37 units, which remained unaltered by 500 μg ml^{-1} solutions of potassium, copper, lead, zinc and iron, but was increased by aluminium, calcium, magnesium and sodium; 500 μg ml^{-1} solutions of the last three elements increased the reading to 42, 44 and 43, respectively, but a 500 μg ml^{-1} solution of aluminium increased it to over 100. A 100 μg ml^{-1} solution of aluminium raised the reading to 60 units, the same aluminium solution alone giving a reading of 20, so that source scatter appeared to be the cause.

ANTIMONY—

Antimony was found to be subject to two forms of interference, one from aluminium along now familiar lines, and a new form that suppressed the fluorescence intensity; 5 μg ml^{-1} of antimony and 500 μg ml^{-1} of the interfering elements were aspirated, and interference was noted from aluminium, calcium and sodium. Aluminium raised the signal from 45 to 51 units, and 1000 μg ml^{-1} of aluminium alone gave a scatter reading of 10 units; sodium reduced the reading to 40 units, and calcium behaved in a similar way.

SELENIUM AND TELLURIUM—

Source scattering interference was not noted with these elements, but sodium and calcium again caused suppression. All of the other interfering elements were without effect; 5 μg ml^{-1} solutions of selenium and tellurium and 500 μg ml^{-1} of the other metals were aspirated. Selenium intensity was reduced from 40 to 25 and 26 units, and tellurium from 35 to 27 and 28 units, by sodium and calcium, respectively.

DISCUSSION

It is fairly easy to explain the enhancement of fluorescence readings by elements such as aluminium, which may be expected to produce reflective particles of refractory oxides in the flame, but the origins of the suppression effects noted above are more obscure. These effects are not caused by a true decrease in the atom population in the flame, as they are not found in the absorption mode; some experiments showed that they were in many instances dependent upon the spectral band width used, and appeared to become less important as the band width was increased. The figures given above for selenium and tellurium, for example, were obtained at a spectral band width of 3 nm; at a band width of 6 nm no suppression was found, but it is possible that this merely represents a mutual cancellation of errors.

It is apparent from the figures in Table II and those published for the performance of single-beam atomic-absorption spectrophotometers that the sensitivity attained in this work is higher than would be expected from the same instrument in the absorption mode. Some direct comparisons have been made by using hollow-cathode lamps for absorption work, and it has been shown that zinc gives 1 per cent. absorption for 0·01 μg ml^{-1} concentration and a detection limit of about 0·002 μg ml^{-1}. Corresponding figures for arsenic, selenium and cadmium were 2·9 and 6·8 μg ml^{-1}, 1·7 and 0·6 μg ml^{-1} and 0·016 and 0·003 μg ml^{-1}, respectively, all with an air - acetylene flame. The high noise level arising from flame absorption at 193·7 nm explains the poor absorption-to-detection limit ratio for arsenic.

Atomic fluorescence is much more prone to interference, particularly source scattering interference and this hitherto unreported suppression effect, and this may prove to be the limiting factor in its use in many applications. To decide on further lines of development, it is necessary to ascertain which of the various forms of interference are inherent in the technique and which can be decreased or eliminated by improvement of the apparatus. The origin of interferences has been studied in the course of this work and will form the subject of a separate paper.

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Studies on the Analytical Chemistry of Hafnium and Zirconium

Part II.* Fluorimetric Determination of Hafnium in the Presence of Zirconium by Using Quercetin †

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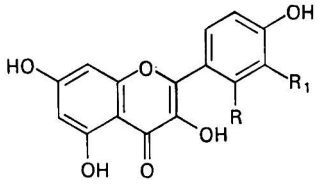
Hafnium (greater than 0·3 per cent.) in hafnium - zirconium mixtures can be determined by measuring the fluorescence of the hafnium complexes with quercetin or morin in 57·5 per cent. perchloric acid. The zirconium complex with quercetin does not fluoresce, and that with morin fluoresces only weakly at this acidity. Iron, titanium, vanadium, uranium and molybdenum interfere.

A mechanism for the selective fluorescence reaction is suggested.

QUERCETIN is well known as a reagent for the spectrophotometric[1] and fluorimetric[2] determination of zirconium. Hafnium behaves in an identical manner to zirconium in the spectrophotometric procedure, but hydrogen peroxide causes a much greater reduction in the absorbance of the zirconium complex than in that of the hafnium complex.[3] This effect was used to determine down to 0·1 mole per cent. of hafnium in zirconium. A similar differentiation has been made by using the effect of hydrogen peroxide on the xylenol orange complexes of the metals.[4]

The behaviour of hafnium in the fluorimetric procedure has not been reported. The method for zirconium is carried out with 2·5 M hydrochloric acid and approximately 80 per cent. ethanol. Hercules[2] found that a decrease in ethanol concentration caused a decrease in fluorescence intensity. Extrapolation of Hercules' results indicates that there is very little fluorescence with ethanol concentrations of less than 40 per cent. Preliminary experiments[5] confirmed that at low ethanol concentrations and high acidities zirconium gave little or no fluorescence, whereas microgram amounts of hafnium fluoresced strongly under the same conditions. This paper describes the development of methods for the determination of from 0·3 to 100 per cent. of hafnium in hafnium - zirconium mixtures, based on this observation.



I

ESTABLISHMENT OF OPTIMAL CONDITIONS—

The effect of perchloric acid concentration on the fluorescence intensity of quercetin (**I**, R = H, R_1 = OH) and its hafnium and zirconium complexes is shown in Fig. 1. The fluorescence maxima for quercetin in 57·5 per cent. perchloric acid are 437 nm (excitation) and 507 nm (emission), and for the hafnium and zirconium complexes are 421 nm (excitation) and 486 nm (emission). As the fluorescence intensity of quercetin is only slightly less at

* For details of Part I of this series, see reference list, p. 785.

† Paper presented at the Anglo-Dutch Symposium on Accurate Methods of Analysis for Major Constituents, London, 1970.

486 nm than at 507 nm, Fig. 1 shows that in about 60 per cent. perchloric acid, zirconium does not change the weak fluorescence of quercetin, whereas hafnium considerably increases the intensity of fluorescence. However, zirconium forms a non-fluorescent complex with quercetin under these conditions (as discussed later), so that zirconium competes with hafnium

TABLE I

EFFECT OF ZIRCONIUM ON FLUORESCENCE INTENSITY OF HAFNIUM - QUERCETIN COMPLEX IN 57·5 PER CENT. PERCHLORIC ACID

Solutions were 1×10^{-6} M in quercetin, and 4·3 per cent. in ethanol

Hafnium concentration, M $\times 10^{-7}$		9·0	17·8	26·5	35·0	43·5
Intensity	zirconium concentration, $2·2 \times 10^{-5}$ M ..	61	78	97	107	123
	zirconium concentration, $4·4 \times 10^{-5}$ M ..	62	71	79	90	100

for the quercetin if the concentration of quercetin is not appreciably greater than that of the zirconium, with a consequent reduction in fluorescence intensity from the hafnium (Table I). It is essential, therefore, that an excess of quercetin is present to form a complex with all of the zirconium. Suitable conditions in which zirconium (0·5 μmole) does not interfere in the determination of hafnium (0 to 0·2 μmole) are a solution 57·5 per cent. in perchloric acid, 8 per cent. in ethanol and $1·54 \times 10^{-4}$ M in quercetin (Table II). Under

TABLE II

EFFECT OF ZIRCONIUM ON THE FLUORESCENCE INTENSITY OF THE HAFNIUM - QUERCETIN COMPLEX UNDER THE RECOMMENDED CONDITIONS

Hafnium concentration, M $\times 10^{-7}$	0·0	7·7	15·4	23·1	30·7
Intensity	zirconium concentration, 0·0	35	41	46	53	59
	zirconium concentration, $3·85 \times 10^{-5}$ M ..	35	41	47	53	59

these conditions, identical linear calibration graphs are obtained for the determination of hafnium in the presence or absence of zirconium, which are applicable to the determination of from 1 to 20 per cent. of hafnium in admixture with zirconium. Larger amounts can be

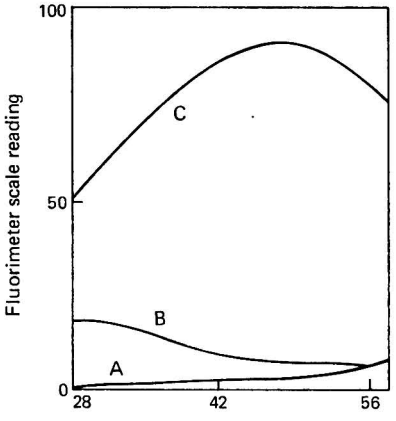


Fig. 1. Fluorescence intensity of A, 5×10^{-5} M quercetin; B, 5×10^{-5} M quercetin *plus* 5×10^{-6} M zirconium; and C, 5×10^{-5} M quercetin *plus* 5×10^{-6} M hafnium in perchloric acid, 5 per cent. in ethanol. λ (excitation), 421 nm; λ (emission), 486 nm

readily determined with smaller samples. The relative standard deviation for the determination of 1·785 μg of hafnium in pure solution is 0·5 per cent. (ten determinations); a series of results for the determination of hafnium in pure solution is given in Table III. A similar series of results for the determination of hafnium in the presence of zirconium is given in Table IV.

TABLE III

DETERMINATION OF HAFNIUM IN PURE SOLUTION

Hafnium, μg								
taken	0·0	1·79	3·57	5·4	7·1	8·9
found	0·13	1·83	3·57	5·4	7·2	8·9

TABLE IV

DETERMINATION OF HAFNIUM IN THE PRESENCE OF ZIRCONIUM

Hafnium*, per cent. w/w						
taken	0·00	3·76	7·25	11·05	13·55	16·3
found	−0·20	3·66	7·25	10·85	13·45	16·3

* Relative to zirconium (45 μg)

INTERFERENCES—

Under the conditions recommended for the determination of hafnium in the presence of zirconium, 0·5 mg of the following ions did not interfere: Cl^-, SO_4^{2-}, NO_3^-, acetate, Al^{3+}, Be^{2+}, Cr^{3+}, Co^{2+}, Mn^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Sn^{2+}, Sn^{4+}, Nb^{5+}, Ta^{5+}, Pb^{2+}, Th^{4+}, La^{3+} and Fe^{2+}. Sulphate decreased the fluorescence intensity of the hafnium complex when present in larger amounts, as did 0·5 mg of F^-, PO_4^{3-}, VO_2^+ and As^{3+}; Ti^{4+} and Fe^{3+} strongly suppressed the fluorescence of the hafnium complex and that of quercetin. Borate and UO_2^{2+} fluoresced, even in the absence of quercetin; and molybdate was precipitated. As the procedure adopted for the dissolution of the metal samples avoids the introduction of anions other than perchlorate, the only serious interferences are Ti^{4+}, Fe^{3+}, V^{5+} and U^{6+}. However, zirconium and hafnium are readily separated from these metals by precipitation with mandelic acid,[6] or by extraction with thenoyltrifluoroacetone, as carried out in the fluorimetric method for zirconium with quercetin.[2]

BEHAVIOUR OF MORIN—

Morin (**I**, R = OH, R_1 = H), as expected from its similar structure to quercetin, also discriminates between hafnium and zirconium at high acidities. It has also been used previously for the spectrophotometric[7] and fluorimetric[8] determination of zirconium, but no difference in the behaviour of zirconium and hafnium with the reagent was recorded. Experiments showed that morin can be used instead of quercetin for the fluorimetric determination of hafnium in the presence of zirconium but, at the recommended acidity, the zirconium - morin complex fluoresces slightly. An increase in acidity increases the fluorescence of morin. Thus, morin can only be used when the zirconium concentration is essentially constant, *e.g.*, for zirconium samples containing less than about 4 per cent. of hafnium. Fortunately, morin is three times more sensitive to hafnium than quercetin under these conditions, so that the working range is from 0·3 to 4 per cent. of hafnium. The calibration graph is again linear.

With both reagents the fluorescence can be measured immediately; the intensity does not decrease for at least 3 hours.

EXPERIMENTAL

Fluorescence measurements were made with an EIL 27A direct-reading fluorimeter. Spectrofluorimetric measurements were made with a Farrand spectrofluorimeter.

REAGENTS—

Quercetin and morin—(Available from Koch-Light Laboratories, Colnbrook, Bucks.). These were Soxhlet extracted with ethanol, recrystallised twice from ethanol, and dissolved in spectroscopically pure ethanol to give 10^{-3} M solutions.

Unless otherwise stated, all other reagents were of analytical-reagent grade.

Standard zirconium or hafnium solutions—Fuse 1 g of zirconium (⩾99·9 per cent. pure) or hafnium metal (see Note 1) with ammonium sulphate and concentrated sulphuric acid in a porcelain crucible. Dissolve the mixture in water, and precipitate the metal hydroxide completely by adding 0·88 ammonia solution. Dissolve the precipitate by boiling it with concentrated hydrochloric acid, and repeat the precipitation and dissolution processes until the hydroxide is free from sulphate. Dissolve the precipitate in 75 ml of grade A 60 per cent. perchloric acid (B.D.H. Chemicals, Poole, Dorset) and boil the solution. After cooling, make the solution up to 250 ml with 60 per cent. perchloric acid (9 N). Heat 10 ml of this solution nearly to boiling and dilute it with 90 ml of distilled water. Heat the the solution again nearly to boiling and titrate immediately with 0·05 M EDTA solution with xylenol orange as indicator.[9]

NOTE 1—

The hafnium may contain several per cent. of zirconium (we used hafnium containing about 2·4 per cent. of zirconium), and this must be taken into account when establishing the hafnium content of the standard hafnium solution.

DETERMINATION OF HAFNIUM IN ZIRCONIUM—

Take an accurately measured aliquot of the solution containing about 5×10^{-7} moles of hafnium *plus* zirconium, make it up to 10 ml with 60 per cent. perchloric acid and add exactly 1 ml of 10^{-3} M quercetin or morin solution and exactly 2 ml of 72 per cent. perchloric acid. Measure the fluorescence intensity, after calibration of the fluorimeter to give a reading of 100 on scale 3 with 10^{-6} M quinine sulphate in M sulphuric acid. Read the hafnium concentration from a calibration graph of fluorescence intensity *versus* hafnium concentration, prepared from the results of standards taken through this procedure.

DISCUSSION

The method described is accurate and precise and, unlike other "wet methods" for this determination,[10] does not require accurate knowledge of the total concentration of zirconium *plus* hafnium. The sensitivity of the method is limited by the appreciable fluorescence intensity of quercetin or morin in the absence of hafnium (Table II). Attempts to expand the fluorimeter scale, by using the reagent blank to give zero scale reading, however, gave a decreased precision, because of instrumental instability. It also showed a slight decrease in fluorescence with time for both reagents, which would seriously restrict any attempt to increase the sensitivity of the determination in this way. Alternatively, a systematic study of reagents similar to quercetin might reveal a less fluorescent reagent. The only other reagent studied in the present investigation was rutin. This is the 3-rutinoside of quercetin, and it is only weakly fluorescent. Unfortunately it slowly hydrolyses to form quercetin under the acidic conditions and is, therefore, useless as a fluorimetric reagent.

Perchloric acid must be used, because other acids, including hydrochloric acid, do not give the required high acidity without masking hafnium.

NATURE OF THE FLUORESCENCE—

Quercetin, QH, with several hydroxy groups, is involved in several acid - base equilibria, but spectrophotometry shows that at the acidity used in this investigation, an appreciable proportion of quercetin exists as a singly protonated species, QH_2^+. However, the fluorescence of quercetin becomes appreciable only at the highest acidities investigated, indicating that the fluorescence is not related solely to ground-state equilibria. It is suggested, therefore, that as greater acidity is required for fluorescence, an excited quercetin molecule is less basic than the ground-state molecule. More acid is required to stabilise the protonated excited state, which would be responsible for the fluorescence. Another, less likely, explanation is that the fluorescent species is doubly protonated quercetin, which exists in the excited state, but not in the ground state, in highly acidic solutions.

Complexes formed with zirconium are a little stronger than the analogous complexes with hafnium,[11] so the former persist at higher acidities than the latter, a property that has often been used for the spectrophotometric determination of zirconium in the presence of hafnium.[12 to 17] Spectrophotometric studies show that quercetin is not an exception. There is general agreement[1,2] that zirconium forms a 1 : 1 complex with quercetin. Job and mole-ratio plots for the hafnium - quercetin system, measured in 0·1 N perchloric acid at 430 nm,

give a clear inflection corresponding to the existence of a 1:1 complex. Previous workers, who used similar spectrophotometric measurements, detected another complex at higher quercetin concentrations, but its formula was not established unequivocally. In the present study a poorly defined inflection in the mole-ratio plot for hafnium at high quercetin concentrations was found, corresponding to a complex in which the hafnium-to-quercetin ratio is between 1:2 and 1:3. Under the present conditions, therefore, it can be assumed that the only important complexes are ZrQ^{3+} and HfQ^{3+}.

The acidity range over which the fluorescence of the hafnium complex increases with increasing acidity is similar to that over which the degree of dissociation of the complex also increases. Zirconium, on the other hand, which forms a more stable complex, does not give rise to any fluorescence. As with quercetin, therefore, the fluorescence of the complexes is not simply related to ground-state equilibria. Moreover, protonation of the ground-state complexes cannot be invoked, because the hafnium complex dissociates rather than protonates.

It can be postulated that HfQ^{3+}, like QH_2^+, changes its basicity and stability in the excited state. If the excited complex is more stable than in the ground state, it would exist at higher acidities than the ground-state complex. But this would not account for the decreased fluorescence at lower acidities. Thus it is necessary to assume that the excited complex is more basic than the ground-state species, and can be protonated without causing dissociation:

$$HfQ^{3+*} \xrightarrow{H^+} HfQH^{4+*} \longrightarrow \text{fluorescence.}$$

The acidity necessary for protonation would be appreciable, and a further increase in acidity would cause the excited complex to dissociate, causing the observed reduction in fluorescence. As hafnium is a slightly weaker base than zirconium, there will be a greater electron density on certain oxygen atoms when quercetin is bonded to hafnium than to zirconium. Thus protonation of the hafnium complex should be more favourable than that of the zirconium complex, so the formation of the fluorescent species will occur at lower acidities for hafnium than for zirconium.

Accurate titration of zirconium or hafnium with EDTA requires complete de-polymerisation of the metal-ion species, which is usually achieved by boiling it with moderately concentrated acid; 3 N nitric acid has been recommended for this, although Přibil and Vesely[18] had previously found 0·3 to 0·6 N nitric acid to be satisfactory, and similar concentrations of perchloric and hydrochloric acids to give low titres. In the present instance, however, the metal ions are dissolved in 60 per cent. perchloric acid, so that after boiling in this medium polymerisation is negligible. Dilution of this solution to N perchloric acid and subsequent boiling gave sharp end-points, without the fading characteristic of slow de-polymerisation.

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The Spectrofluorimetric Determination of Sulphides

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The spectrofluorimetric determination of trace amounts of sulphide with mercury(II) - 2,2′-pyridylbenzimidazole is described. The sulphide can be determined in solutions with concentrations in the range 300 pg to 300 ng ml^{-1}, with a standard deviation of 0·75 per cent. for a concentration of 60 ng ml^{-1}. Interferences can be avoided by the use of standard distillation procedures. The main advantages, apart from the extremely high sensitivity of the method, are the high stability of the reagent and of the reacted sulphide system. The over-all time of the method after liberation of the sulphide can be as little as 2 to 5 minutes, and the time lapse between the reaction and measurement of solution is not critical as up to 48 hours' delay has no significant effect.

The extreme toxicity of hydrogen sulphide is caused by the great ability of the sulphide ion to co-ordinate with many metals in the human metabolism. This leads to a requirement for sensitive analytical methods for the determination of sulphide ion.

Many methods have been proposed for the determination of trace amounts of sulphide in atmospheres and effluents; these methods usually involve a colorimetric determination. However, for further progress in extending the limits of determination it is considered necessary to use spectrofluorimetric analysis. The advantages of the latter when compared with absorptiometric methods of analysis have been reviewed by several workers, including Hercules.[1]

Only one author has previously reported[2] the increase in the fluorescent intensity of a system by the reaction between sulphide ions and a metal complex; a mercury(II) acetate - fluorescein complex was allowed to react with sulphide ions, the liberated fluorescein causing fluorescence.

We have previously reported the use of 2,2′-pyridylbenzimidazole as a highly sensitive fluorimetric reagent for the determination of zinc,[3] and noted that the presence of trace amounts of mercury(II) ions caused noticeable interference both by decreasing the fluorescence of the system when zinc was being determined and also quenching the fluorescence of the free reagent.

If sulphide ion is now allowed to enter the system containing the mercury(II) complex, mercury(II) sulphide is formed and an equivalent amount of the fluorescent organic ligand is released. Thus the fluorescence intensity of the system should be increased.

The complex formed by the reaction between the mercury(II) ions and 2,2′-pyridylbenzimidazole has been investigated as a reagent for the determination of trace amounts of sulphide ion by using the above reaction.

Experimental

Preparation of reagent solution—

Aliquots of the reagent solution were prepared by adding 2,2′-pyridylbenzimidazole (1 ml of 10^{-3} M solution made by dissolving 0·0195 g in 100 ml of re-distilled 95 per cent. ethanol) and a 5-ml aliquot of a buffer (pH range 6·2 to 7·3) to mercury(II) solution (0·8 ml of 10^{-3} M aqueous solution prepared by dissolving 0·0272 g in 100 ml of fluorimetrically pure water) contained in a 100-ml conical flask. The solution was diluted to about 60 ml with fluorimetrically pure water. The solution, which is then ready to be treated with sulphide solution, has a storage life in excess of 3 months.

Preparation of standard sulphide solutions—

Sodium sulphide (0·2402 g of $Na_2S.9H_2O$) was dissolved in fluorimetrically pure water. To ensure that the solution was alkaline, 1 ml of 4 M sodium hydroxide solution was added to the stock solution before making it up to 100 ml to give a 10^{-2} M solution. Subsequent dilution with water gave the required concentrations of sulphide ion. All solutions were prepared immediately before use because of the instability of the sulphide-ion solutions towards aerial and hydrolytic oxidation.

Spectrum—

The emission and excitation spectra were recorded by using an Aminco Bowman spectrofluorimeter. The maximum fluorescence emission for the mercury(II) - 2,2′-pyridylbenzimidazole complex occurs at the same wavelength as the maximum for 2,2′-pyridylbenzimidazole, *i.e.*, at 381 nm; the presence of mercury(II) and sulphide ions does not alter the wavelength of maximum fluorescence. Excitation occurs at 311 nm.

Effect of pH variation on the intensity of fluorescence—

The emission spectrum of the uncomplexed organic ligand is known to vary with the acidity of the medium.[3] The variation of the spectrum of the metal complex caused by varying the pH was thus investigated. It was essential to use sodium acetate - acetic acid buffers, because the large excesses of ammonium ions in ammonium acetate - acetic acid buffers reduced the amount of mercury(II) - 2,2′-pyridylbenzimidazole chelate formed. Other organic buffers generally gave precipitates or colloidal suspensions. The effect of variations in the pH of the system on the over-all fluorescence of the system (caused by the small excess of ligand present over that required by stoicheiometry, and by any ligand in the solution from the dissociation of the metal complex) was investigated. A series of sodium acetate - acetic acid buffers was used. A plateau of maximum quenching, *i.e.*, maximum stability of metal - ligand complex, exists at pH 6·2 to 7·3 (see Fig. 1). (Over this pH range the variation in the intensity of a sample of the ligand is insignificant.[3])

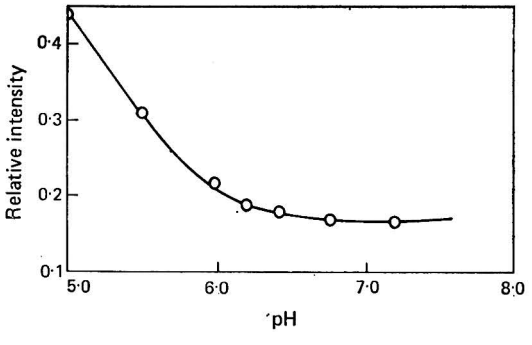


Fig. 1. Effect of pH on over-all fluorescence of system

PREPARATION OF THE CALIBRATION GRAPH—

Sulphide reacts with mercury(II) at any pH from 1 to 14 to give a precipitate (the solubility of mercury(II) sulphide is $4{\cdot}6 \times 10^{-25}$ g l^{-1}).[4]

For practical convenience in the preparation of the calibration graph, a buffer of pH 6·40 was chosen as this maintained the pH of the solution on dilution; 1 to 10-ml volumes of 10^{-5} M sulphide solution were added to the prepared buffered reagent solutions and each total volume was made up to 100 ml with water. The fluorescence intensity was measured at wavelength 311 nm for excitation and 381 nm for emission. A linear plot was obtained, which passed through the origin. Thus a Beer - Lambert relationship is obtained for the system.

EFFECT OF ETHANOL—

It is necessary to use an ethanolic solution of the 2,2′-pyridylbenzimidazole to ensure a suitable concentration of the ligand for formation of the mercury complex.

However, as alteration in the characteristics of the solvent often gives rise to variation in intensity of fluorescence, the effect of altering the amount of ethanol was investigated. Ethanol, if in excess of the 1 ml added with the 2,2′-pyridylbenzimidazole solution, caused a higher fluorescence intensity. Thus the amount of ethanol must be kept constant for all calibrations and determinations.

EFFECT OF TEMPERATURE AND TIME—

All solutions were maintained at 25° ± 0·1° C. At this temperature the mercury complex was stable for up to 3 months. Addition of sulphide ions also yielded a solution that was stable for at least 24 hours.

EFFECT OF FOREIGN IONS—

The sulphide ion in solution is fairly readily oxidised to the sulphate ion. Many anions can facilitate this reaction; those tested were chlorate, bromate and iodate. Therefore, if these oxidants are present in an effluent, it is unlikely that any sulphide will be present, and examination of these incompatible ions as interferences in this method becomes unnecessary. It is only essential to investigate those ions which can co-exist with sulphide in solution, and their reactions with the reagent solution.

Metal ions that cause a change in the fluorescence of the reagent solution can be removed from the sample solution by distilling the sulphide from acidic media as hydrogen sulphide. Some other anions are also likely to be carried over by this procedure and ions of this type must be examined as possible interferences. The anions tested were F^-, Br^-, I^-, Cl^-, $C_2O_4^{2-}$, HPO_3^{2-}, HSO_4^-, CH_3COO^-, CN^- and SCN^-. The last two strongly interfere in the method but the others can be tolerated in concentrations of at least 100 times that of the sulphide ion.

STATISTICAL DEVIATION—

A series of twenty standard solutions containing 64 ng ml^{-1} of sulphide ion was prepared and each solution was tested separately. The standard deviation was found to be 0·75 per cent.

PROPOSED METHOD

If the presence of interfering substances is suspected or if the analysis has to be delayed the sulphide is fixed by allowing it to react with zinc acetate, by following the recommended method.[5,6] The whole of the treated sample is then taken. If no interfering substances are present and the analysis is to be made immediately, then 1 l of sample solution is used directly without addition of zinc acetate solution.

The 1-l sample (with the sulphide fixed or in its original condition) is transferred to a flask fitted with a dropping funnel and a gas inlet so that nitrogen can be bubbled through the solution. Two flasks fitted with sintered-glass gas scrubbers are connected in series with the flask containing the sample. The first flask contains 40 ml and the second flask 20 ml of the mercury(II) - 2,2′-pyridylbenzimidazole reagent solution. Glacial acetic acid (20 ml) is added slowly, from the dropping funnel, to the sample solution and a steady stream of nitrogen is maintained for 1 hour. The two reagent solutions are then transferred to a 100-ml graduated flask and made up to volume with water. The fluorescence intensity of the solution is measured at 381 nm, and the sulphide concentration calculated from the calibration graph.

ANALYSIS OF THE COMPLEX—

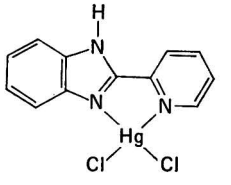
A sample of the mercury(II) - 2,2′-pyridylbenzimidazole complex was prepared as follows. 2,2′-Pyridylbenzimidazole (80 mg) was dissolved in an aqueous ethanolic solution of potassium chloride (30 ml of a 1 + 1 mixture of ethanol and water containing 3 g of potassium chloride); pH 6·4 buffer solution (2 ml) was added, followed by mercury(II) nitrate (100 mg dissolved in 10 ml of water). The mixture was warmed at 60° C for 30 minutes and the product separated by filtration under suction. It was then washed with water followed by ethanol, and dried at 105° C.

Elemental analysis for carbon, hydrogen, nitrogen and chlorine is given below.

	C	H	N	Cl
Experimental results	30·90	1·99	9·07	15·18
Required for $C_{12}H_9N_3Cl_2Hg$ ..	30·90	1·93	9·01	15·23

DISCUSSION

The probable structure of the complex is given below.



In a 10^{-5} M solution of reagent in a large excess of acetate buffer some of the chloride may be replaced by acetate and the suggested reactions for release of the ligand are

$$HgLCl_2 + H_2S = L + HgS + 2HCl$$
$$HgLAc_2 + H_2S = L + HgS + 2HAc$$

where L represents ligand and Ac represents CH_3COO^-.

Most of the other methods reported for the determination of sulphide have one or more practical disadvantages. The American standard method[5] for determining sulphide involved the formation of a methylene-blue complex, and in this method all of the air must be flushed from the apparatus before the reaction commences; the rate of colour formation is highly dependent both on the temperature and on the total volume, *i.e.*, concentration of sulphide. The limit of detection is about 0·01 p.p.m. The British standard method[6] involves the use of a reaction liberating iodine, which is then determined titrimetrically; the limit of detection is 5 p.p.m.

Other methods used include that based on the action of sulphide on silver thiocyanate.[7] The liberated thiocyanate can then be determined colorimetrically by its reaction with iron(III). In this indirect determination, halides, especially bromide and iodide, strongly interfere and the limit for the determination of sulphide is 0·05 p.p.m. The method in which the reaction between Bindschindler's green[8] and hydrogen sulphide in acidic media is used has the severe disadvantage that the reagent is destroyed in acidic conditions, and hence a strict time control must be observed. The limit of determination in this method is 4 p.p.m. The sensitivity previously reported[2] for the determination of sulphide by a reaction analogous to that described here is 0·005 μg ml^{-1} of S^{2-}.

Catalysis reactions provide sensitive methods of determining sulphide but their main disadvantage is that time is a critical factor. The use of the sulphide-catalysed iodine-azide reaction[9] has been reported. The limit of concentration of sulphide for use in enzyme-catalysed reactions[10] has been reported as 10^{-7} M.

The procedure given in this paper provides a highly sensitive method for determining sulphide by using simple and easily prepared systems that are stable for long periods of time. The limit of determination with this method is 300 pg ml^{-1}, *i.e.*, 3×10^{-10} g ml^{-1} of the original solution. The interferences from substances such as metals, which react with the mercury complex, or anions, which liberate the ligand, are readily overcome by the normal distillation procedures advocated in either of the standard methods mentioned above.

The over-all time of determination is about 2 to 3 minutes from the addition of the sulphide solution to the reagent solution. The time is not critical; the lapse between mixing the solutions and measuring the fluorescence can be extended to 1 to 2 days without any significant alteration in the final result.

The shelf-life of the reagent is acceptable, being at least 3 to 4 months without deterioration. The pH range (6·2 to 7·3) over which the method is viable is readily obtained. The standard deviation of the method is 0·75 per cent.

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Kinetic and Analytical Investigations of the Chlorate and Iodate Oxidations of Vanadium (IV) in a Perchloric Acid Medium

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Kinetic studies of the chlorate and iodate oxidations of vanadium(IV) in perchloric acid have shown that the rates of the reactions are described by two different equations. Possible mechanisms for the two reactions are described and values for the rate constants and equilibrium constants involved in the reactions are reported, together with ionic strength and temperature dependence data for the two rate constants. The feasibility of using either the chlorate or the iodate oxidation of vanadium(IV) as a titrimetric reaction is discussed in relation to both the kinetic results obtained, and to similar data reported previously for the bromate oxidation of vanadium(IV).

Comparative kinetic results for reactions between the halates (chlorate, bromate and iodate) as oxidising agents and common reducing agents are at present restricted to their reactions with halide ions.[1] Many other direct reactions, for example those with arsenic(III) and antimony(III),[2] are extremely slow and difficult to elucidate because of the occurrence of subsequent, more rapid, competing reactions. A kinetic investigation of the oxidation of vanadium(IV) by potassium bromate in acidic medium and the application of the reaction as an analytically useful system has recently been described,[3] and the present paper reports a study of the chlorate and iodate oxidations of vanadium(IV) under similar conditions. The kinetic results obtained and the analytical feasibility of these reactions are compared with those previously reported for the bromate oxidation.

Little previous information has been reported on either the chlorate or iodate oxidations of vanadium(IV). That the reaction with chlorate takes place can be deduced from the investigation of its reactions with other, lower oxidation states of vanadium,[4,5,6] and in addition Riolo and Soldi[7] have reported that the reaction was too slow to be of analytical use in alkaline solutions. The similar chemical behaviour of chlorate, bromate and iodate is well known and their oxidation - reduction properties also show similarities; for example, the redox potentials for the ClO_3^- - Cl_2, BrO_3^- - Br_2 and IO_3^- - I_2 couples are 1·47, 1·52 and 1·20 V, respectively.[8] In addition, many of the analytical reactions of chlorate and bromate[9] bear strong resemblances although differences in the rates of the reactions are often encountered as, for example, in their reactions with arsenic(III) in hydrochloric acid medium.[9]

It might be expected, therefore, that the reactions of the halates with vanadium(IV) would show similar mechanisms but possibly differing rates, and this has been confirmed.

Experimental

Reagents—

Vanadium(IV) solution, 0·153 M—Vanadyl sulphate was dissolved in 0·0596 M perchloric acid and the solution standardised against potassium bromate by photometric titration.[3]

Sodium chlorate solution, 0·5007 M—Sodium chlorate was dried at 120° C for 2 hours, then the required weight was dissolved in water.

Sodium iodate solution, 0·3856 M—Sodium iodate was dried at 120° C for 2 hours and the required weight was then dissolved in water.

Perchloric acid, 2·465 M—Perchloric acid, 60 per cent. w/w, was diluted and standardised against sodium carbonate with methyl orange - xylene cyanol FF as indicator.

Sodium perchlorate solution, 2·075 M—Analytical-reagent grade sodium perchlorate was dissolved in water and standardised by evaporating a known volume of the solution to dryness and drying to constant weight.

TITRATION PROCEDURES—

Photometric titrations were carried out by a similar procedure to that described previously for the bromate titrations of vanadium(IV).[3] Potentiometric titrations were performed with a platinum-wire indicator electrode and a saturated calomel reference electrode with an Electronic Instruments Ltd. 23A pH meter.

KINETIC STUDIES—

Kinetic investigations were carried out spectrophotometrically by measuring the decrease in absorbance of the solution at 770 nm, an absorption maximum for vanadium(IV), with a Hitachi - Perkin-Elmer 139 spectrophotometer to which was attached a Honeywell Electronik 15 strip-chart recorder. A two-limbed reaction vessel was used to contain the reactant solutions during the 30-minute thermostatting period, prior to the start of the reaction. The acidified vanadium(IV) solution was placed in one limb of the vessel while the sodium chlorate or sodium iodate solution was placed in the other limb. After mixing of the solutions a sample was transferred to a 10-mm spectrophotometer cell that had previously been housed in the thermostatically controlled cell compartment of the spectrophotometer.

Concentrations of vanadium(IV) in the range 0·007 to 0·015 M, sodium chlorate in the range 0·05 to 0·20 M and perchloric acid in the range 0·05 to 0·25 M were studied at 25·0° $\pm$ 0·2° C and 0·500 M ionic strength in a total reaction volume of 100 ml for the chlorate oxidation of vanadium(IV). The effects of ionic strength variations from 0·26 to 0·80 M and variations of the temperature from 15° to 29° C were also investigated. Sodium perchlorate was used to maintain or vary the ionic strength at the levels indicated.

For the kinetic studies of the iodate oxidation of vanadium(IV) the effects of vanadium(IV) in the concentration range of 0·007 to 0·015 M, sodium iodate in the range 0·07 to 0·23 M and perchloric acid in the range 0·05 to 0·37 M were studied at 50·0° $\pm$ 0·5° C and an ionic strength of 0·500 M in a total reaction volume of 100 ml. The effect of ionic strength variations in the range 0·29 to 0·81 M and temperature variations from 42° to 57° C were also studied.

In all experiments, the chlorate or iodate was kept in large excess (minimum ratio 6:1) over the vanadium(IV) and, as the products are chlorine or iodine and vanadium(V), the stoicheiometry of both reactions requires a ratio of 5:1. The maximum consumption of chlorate or iodate for the complete reaction of vanadium(IV) in any experiment is thus only 3 per cent. and their concentration therefore remains virtually constant throughout all the reactions. Under these conditions, pseudo first-order kinetics are maintained and are defined by equation (1)

$$-\frac{d\,[\mathrm{V(IV)}]}{dt} = k\,[\mathrm{V(IV)}] \quad .. \quad .. \quad .. \quad .. \quad (1).$$

RESULTS AND DISCUSSION

KINETICS—

The half-life, $t_{\frac{1}{2}}$, for the chlorate oxidation of vanadium(IV) was found to be independent of the initial concentration of vanadium(IV) used (Table I), confirming a first-order dependence on the vanadium(IV) concentration [equation (1)]. The chlorate and hydrogen-ion dependences for the reaction are also given in Table I and an over-all experimental rate equation can be written for the reaction in the form given by equation (2)

$$-\frac{d\,[\mathrm{V(IV)}]}{dt} = \frac{k_1\,[\mathrm{ClO_3^-}]\,[\mathrm{V(IV)}]}{1 + k_2\,[\mathrm{ClO_3^-}]} \quad .. \quad .. \quad .. \quad (2).$$

By comparison with equation (1), it can be seen that

$$k = \frac{k_1\,[\mathrm{ClO_3^-}]}{1 + k_2\,[\mathrm{ClO_3^-}]} \quad .. \quad .. \quad .. \quad .. \quad (3).$$

$$\text{or } \frac{1}{k} = \frac{1}{k_1[\mathrm{ClO_3^-}]} + \frac{k_2}{k_1} \quad .. \quad .. \quad .. \quad .. \quad (4).$$

A plot of $1/k$ *versus* $1/[\mathrm{ClO_3^-}]$ was found to be a straight line with a positive intercept on the $1/k$ axis. The slope and intercept yielded values for k_1 and k_2 of 3·33 l mole^{-1} minute^{-1}

and 0·748 l mole^{-1}, respectively, at 25·0° C and 0·500 M ionic strength at a hydrogen-ion concentration of 0·1263 M. This rate equation (2) is identical with the one obtained for the bromate oxidation of vanadium(IV)[3] and suggests that a similar reaction path is being followed in this case, *i.e.*,

$$ClO_3^- + VO^{2+} \overset{K_1}{\rightleftharpoons} ClO_3^- VO^{2+} \quad \text{fast} \quad .. \quad .. \quad .. \quad (5)$$

$$ClO_3^- VO^{2+} \xrightarrow{k_0} \text{products} \quad \text{slow} \quad .. \quad .. \quad .. \quad (6).$$

The rate-determining step is then the decomposition of the $ClO_3^- VO^{2+}$ species. Equations (5) and (6) give rise to the rate equation[3]

$$\text{Rate} = \frac{k_0 K_1 [ClO_3^-] [VO^{2+}]}{1 + K_1 [ClO_3^-]} \quad .. \quad .. \quad .. \quad .. \quad (7).$$

A comparison of equations (2) and (7) leads to values for k_0 and K_1 of 4·45 minute^{-1} and 0·75 l mole^{-1}, respectively, for the conditions outlined above.

TABLE I

EFFECT OF VANADIUM(IV), HYDROGEN-ION AND CHLORATE CONCENTRATIONS ON THE PSEUDO FIRST-ORDER RATE CONSTANT OF THE VANADIUM(IV) - CHLORATE REACTION AT 25·0° C AND 0·500 M IONIC STRENGTH

Initial [V(IV)]	[$NaClO_3$]	[$HClO_4$]	$t_{\frac{1}{2}}$, minutes	k, minute^{-1}
0·0153	0·1262	0·1293	1·83	0·378
0·0107	0·1262	0·1275	1·88	0·369
0·0077	0·1262	0·1263	1·80	0·394
0·0061	0·1262	0·1257	1·84	0·385
0·0077	0·1262	0·0523	1·82	0·381
0·0077	0·1262	0·2495	1·87	0·371
0·0077	0·0501	0·1263	4·33	0·160
0·0077	0·1001	0·1263	2·19	0·317
0·0077	0·1502	0·1263	1·53	0·453
0·0077	0·2003	0·1263	1·19	0·582

As with the bromate oxidation of vanadium(IV), only slight variations of the pseudo first-order rate constant, k, are observed for variations of hydrogen-ion concentration (Table I) and similarly the ionic strength has little effect on the rate constant k_0 (Table II). These effects also probably arise from an effect on the equilibrium described by equation (5), as occurred for the comparable equilibrium in the bromate - vanadium(IV) reaction.[3]

The variation of the first-order rate constant, k_0, with temperature at 0·500 M ionic strength over the range 15° to 29° C is shown in Table II. Application of the Arrhenius equation for the variation of the rate constant with temperature gives a value of 19 kcal. mole^{-1} for the activation energy.

TABLE II

EFFECT OF IONIC STRENGTH AND TEMPERATURE ON THE RATE OF THE VANADIUM(IV) - CHLORATE REACTION

Initial [V(IV)] = 0·0077 M [$NaClO_3$] = 0·1262 M [$HClO_4$] = 0·1263 M

Ionic strength, M	T,°C	$t_{\frac{1}{2}}$, minutes	k_0, minute^{-1}
0·2602	25·0	1·70	4·74
0·3835	25·0	1·78	4·52
0·5000	25·0	1·80	4·47
0·6460	25·0	2·03	3·96
0·8000	25·0	2·05	3·92
0·5000	14·8	5·77	1·39
0·5000	18·3	3·63	2·22
0·5000	21·7	2·63	3·06
0·5000	26·8	1·53	5·26
0·5000	28·6	1·20	6·70

Possible structures for the intermediate $ClO_3^- VO^{2+}$ species can be assigned which are identical with those postulated for the comparable $BrO_3^- VO^{2+}$ species.[3] The most probable structure, however, involves bonding between the vanadium and one oxygen atom on the chlorine, with the breakage of this chlorine - oxygen bond occurring in the rate-determining

step. The final product from chlorate is chlorine and it is assumed that the rate of reaction of all the intermediates with vanadium(IV) is fast.

The kinetics of the iodate oxidation of vanadium(IV) proved to be slightly more complex but they also gave a clearer picture of the possible mechanisms occurring in all three halate oxidations.

TABLE III

EFFECT OF VANADIUM(IV), HYDROGEN-ION AND IODATE CONCENTRATIONS ON THE RATE OF THE VANADIUM(IV) - IODATE REACTION AT 50° C AND 0·500 M IONIC STRENGTH

All concentrations given in mole l^{-1}

Initial [V(IV)]	$[NaIO_3]$	$[HClO_4]$	$t_{\frac{1}{2}}$, minutes	k, $minute^{-1}$
0·0153	0·1540	0·1293	12·6	0·0550
0·0107	0·1540	0·1275	12·0	0·0578
0·0077	0·1540	0·1263	12·2	0·0568
0·0077	0·0771	0·1263	41·4	0·0167
0·0077	0·1156	0·1263	20·4	0·0340
0·0077	0·1927	0·1263	7·6	0·0912
0·0077	0·2312	0·1263	5·8	0·1194
0·0077	0·1032	0·0523	22·7	0·0306
0·0077	0·1032	0·1016	24·7	0·0281
0·0077	0·1032	0·2003	28·7	0·0241
0·0077	0·1032	0·2495	33·3	0·0208
0·0077	0·1032	0·3730	38·6	0·0180

TABLE IV

EFFECT OF IONIC STRENGTH AND TEMPERATURE ON THE RATE OF THE VANADIUM(IV) - IODATE REACTION

Initial $[V(IV)] = 0·0077$ M $[HClO_4] = 0·1263$ M

Ionic strength, M	T, °C	$[NaIO_3]$, M	$t_{\frac{1}{2}}$, minutes	k_o', $minute^{-1}$
0·288	50·0	0·1540	10·3	5·04
0·402	50·0	0·1540	11·0	4·72
0·500	50·0	0·1540	12·2	4·25
0·641	50·0	0·1540	12·7	4·08
0·807	50·0	0·1540	13·3	3·90
0·500	42·5	0·1032	52·6	2·03
0·500	45·8	0·1032	41·3	2·59
0·500	54·3	0·1032	16·7	6·41
0·500	57·3	0·1032	12·3	8·70

The reaction was found, as expected, to be first order with respect to vanadium(IV) concentration and to exhibit only small dependences on hydrogen-ion concentration (Table III) and ionic strength (Table IV). The iodate dependence, however, was shown to be almost second order (Table III), with a graph of the reciprocal of the pseudo first-order rate constant against the reciprocal of the square of the iodate concentration giving a small, but definite, positive intercept on the ordinate axis, indicating that there is an equilibrium occurring during the reaction giving rise to a back-reaction. The probability of two iodate molecules forming a complex with one vanadium(IV) species in a single equilibrium step is unlikely, so the possible two-step equilibrium process (equations (8) and (9)) is much more likely, with the rate-determining step for the oxidation then arising from the decomposition of the $(IO_3^-)_2VO^{2+}$ species [equation (10)].

$$IO_3^- + VO^{2+} \overset{K_1}{\rightleftharpoons} IO_3^- VO^{2+} \quad \text{fast} \quad \ldots\ldots \quad (8)$$

$$IO_3^- VO^{2+} + IO_3^- \overset{K_2}{\rightleftharpoons} (IO_3^-)_2VO^{2+} \quad \text{fast} \quad \ldots\ldots \quad (9)$$

$$(IO_3^-)_2VO^{2+} \overset{k'_o}{\rightarrow} \text{products} \quad \text{slow} \quad \ldots\ldots \quad (10).$$

This type of reaction sequence would give rise to an iodate dependence of the type shown in equation (11) and would also account for the observed small effects due to hydrogen-ion and ionic-strength variations.

$$k = \frac{k_0' K_1 K_2 [IO_3^-]^2}{1 + K_1[IO_3^-] + K_1 K_2 [IO_3^-]^2} \quad .. \quad .. \quad .. \quad (11)$$

The over-all rate equation is then given by equation (12)

$$-\frac{d\,[V(IV)]}{dt} = \frac{k_0' K_1 K_2 [IO_3^-]^2 [VO^{2+}]}{1 + K_1[IO_3^-] + K_1 K_2 [IO_3^-]^2} \quad .. \quad .. \quad .. \quad (12).$$

Solving equation (11) for several iodate concentrations gives values for K_1 and K_2 of 1·67 l mole^{-1} and 0·43 l mole^{-1}, respectively, while plotting a graph of the reciprocal of the pseudo first-order rate constant against the function $\left(\frac{1}{[IO_3^-]^2} + \frac{K_1}{[IO_3^-]} + K_1K_2\right)$ gives a straight line passing through the origin, with better agreement between the experimental and theoretical values than that obtained from an equation based only on an inverse iodate squared dependence. The slope of this line allows a value for the first-order rate constant k_0' of 4·42 minute^{-1} to be obtained at 50° C.

The temperature dependence of the rate constant k_0' (Table IV) gives a value for the activation energy of 20 kcal. mole^{-1}. This value for the activation energy, as with the values obtained for bromate and chlorate, is only approximate because it has been assumed that that there are negligible variations in the values of the equilibrium constants with variations in temperature. Over the temperature ranges used this approximation seems to be acceptable because good linearity is obtained by using the Arrhenius equation in the determination of the activation energies.

By using the value for the activation energy of 20 kcal. mole^{-1} a value of 0·35 minute^{-1} was obtained for k_0' at 25° C and 0·500 M ionic strength.

A comparison of the rate equations and mechanisms proposed for the three halate oxidations shows strong similarities in all cases with a slight variation with the iodate oxidation. It therefore seems likely that an identical mechanism occurs in all three reactions, *i.e.*,

$$XO_3^- + VO^{2+} \underset{\text{fast}}{\overset{K_1}{\rightleftharpoons}} XO_3^- VO^{2+} \underset{\text{slow}}{\overset{k_0}{\rightarrow}} \text{products} \quad .. \quad .. \quad .. \quad (13)$$

$$XO_3^- VO^{2+} + XO_3^- \underset{\text{fast}}{\overset{K_2}{\rightleftharpoons}} (XO_3^-)_2 VO^{2+} \underset{\text{slow}}{\overset{k_0'}{\rightarrow}} \text{products} \quad .. \quad .. \quad (14)$$

with the values of the formation constants and the rate constants (collected together in Table V) associated with the two types of halate (XO_3^-) - vanadium(IV) species determining which decomposition mechanism predominates. For bromate and chlorate, the values favour the decomposition of the 1 : 1 intermediate species, while for iodate they favour the 2 : 1 species, with decomposition via the alternative routes almost certainly occurring but only to a very minor degree.

TABLE V

COMPARISON OF THE FIRST-ORDER RATE CONSTANTS AND EQUILIBRIUM CONSTANTS FOR THE FORMATION OF THE INTERMEDIATE COMPLEXES OBSERVED IN THE CHLORATE, BROMATE AND IODATE OXIDATIONS OF VANADIUM(IV) AT 25° C AND 0·5000 M IONIC STRENGTH

	k_0, minute^{-1}	k', minute^{-1}	K_1, l mole^{-1}	K_2, l^2 mole^{-2}
ClO_3^-	4·45	—	0·75	—
BrO_3^-	11·0	—	88·8	—
IO_3^-	—	0·35	1·67	0·43

Comparison of the values given in Table V shows that the bromate reaction is by far the fastest of the three. Although the rate constant is not much more favourable than that for chlorate, the equilibrium constant is more than two orders higher. Bromate would therefore be expected to be a more suitable titrant for vanadium(IV) because of its ability to form a more stable intermediate during the reaction. The iodate oxidation is much slower because of both rate and equilibrium factors and will be useless as an analytical reaction.

TITRATIONS—

Mainly because of the unfavourable value of K_1, the over-all rate of oxidation of vanadium(IV) by chlorate is three to four hundred times slower than the oxidation by bromate.

Vanadium(IV) could be conveniently titrated photometrically with bromate by allowing a 3 to 4-minute time interval between titrant additions.[3] A comparison between the rates of the two reactions shows that in the case of the chlorate oxidation of vanadium(IV) a time interval between titrant additions of about 15 to 20 hours would be required to allow photometric titrations to be feasible. A potentiometric titration would necessitate even longer time intervals at the end-point to ensure that complete reaction occurred. The effect of variations in the time interval between titrant additions in the photometric titration of vanadium(IV) by chlorate is illustrated in Fig. 1. The apparent induction effect, observable particularly for small time intervals, is caused by the accumulation of potassium chlorate at the beginning of the titration, which then causes a gradual increase in the rate of reaction. The experimental titration curves observed agree closely with those predicted from the kinetic results, as even with a time interval of 12 hours the theoretical titration end-point has not been achieved, although it is closely approached.

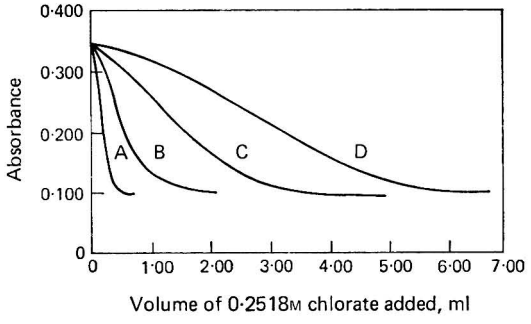

Fig. 1 Photometric titrations of 2 ml of 0·1530 M vanadium(IV) with 0·2518 M potassium chlorate in a 0·40 M sulphuric acid medium. The titration volume was about 50 ml. Time intervals between 0·2-ml increment additions were: A, 12 hours; B, 1 hour; C, 15 minutes; and D, 5 minutes

Erdey and Mazor,[4] during the potentiometric titration of vanadium(II) with potassium chlorate, reported a small potential increase which only became apparent when the titration was performed at 80° C and which they ascribed to the oxidation of vanadium(IV) to vanadium(V). Extrapolation of the temperature dependence data for the rate constant (Table II) to 80° C shows that the oxidation could be followed quantitatively by a photometric technique within a practical time limit at this temperature. To obtain quantitative results by a potentiometric technique, however, would necessitate titrations at 90° to 100° C. From potentiometric titrations at 95° C chlorate appears to act as a 6-electron oxidant, at least at these high temperatures. At lower temperatures the attainment of stable potential is very slow and the titration becomes most unsatisfactory.

The controlling factor in the over-all rate of reaction, and therefore the controlling factor in the analytical application of the reaction, is thus seen to be the small equilibrium constant observed for the formation of the intermediate species. Because of even smaller over-all equilibrium ($K_1 \times K_2$) and rate constants observed in the case of the iodate oxidation, it is apparent that this reaction is of even less use analytically.

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The Determination of Traces of Beryllium in Human and Rat Urine Samples by Gas Chromatography*

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The toxic nature of beryllium necessitates the use of a sensitive method for its detection. Present methods are not entirely satisfactory and the use of gas chromatography for the detection and determination of beryllium has been advocated. In the procedure described, beryllium is isolated by solvent extraction in the form of a volatile, thermally stable chelate with trifluoroacetylacetone, and is then determined by gas chromatography with an electron-capture detector. Results are presented for the determination of aqueous beryllium solutions and for beryllium contained in urine. Satisfactory results were obtained with both direct solvent extraction and solvent extraction after wet oxidation of the sample. The rate of excretion of beryllium by rats was measured by this method. The procedure is rapid, reliable and sensitive; the limit of detection is 1 ng ml^{-1}.

Beryllium has a number of important industrial uses, but in view of the extremely toxic nature of both the metal and its compounds, strict control of the use of beryllium is necessary.[1,2,3,4] As a result it is essential to have available methods for determining beryllium concentrations in the atmosphere, in body tissues and in urine. A method can only be regarded as satisfactory if it can be used to determine reliably amounts of beryllium significantly below the toxic levels.

Several procedures for the determination of microgram amounts of beryllium are available, and are based on absorption spectroscopy,[5,6] arc spectroscopy[7,8,9] and fluorimetry.[10,11,12] None of these techniques is ideal and all suffer from limitations induced by, for example, interference phenomena, impurities, the necessity for substantial clean up and the need for large volumes of sample.

In recent years several papers have been published describing the use of gas chromatography for the separation and determination of metals in the form of volatile derivatives,[13 to 19] and a procedure for the determination of beryllium has been given.[20] Some of the problems associated with the determination of beryllium by gas chromatography have been studied by the present authors, and the results of this study are reported.

Discussion

The use of gas chromatography for the detection of a specific metal does not rely on the prior separation of interfering materials including metals (provided that sufficient complexing agent is present), as under suitable conditions they can be separated by chromatography. For gas chromatography it is essential that the metal derivative be thermally stable and at the same time sufficiently volatile. If the metal derivative used is a complex that permits removal of the metal from aqueous solution by extraction with the complexing agent in a suitable organic solvent, such an extraction may permit the isolation of the metal in a chemical form suitable for direct application of the gas-chromatographic step; the extraction can also concentrate the metal if required. The thermal characteristics of a number of β-diketonates have been studied,[21,22,23] and some appear to be sufficiently stable and volatile to undergo gas-chromatographic analysis. In the determination of beryllium, extraction efficiency from the aqueous sample into a solution of the complexing agent in an organic

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solvent should preferably be complete, or at least known and reproducible. Extraction efficiency is dependent on pH and the pH at which maximum efficiency occurs varies with the metal for any given complexing agent.[24] Preliminary separation from some other metals may thus be achieved by choice of suitable pH. With beryllium it is a simple matter to retain most other metals in the aqueous layer during the solvent-extraction step by complexing them with EDTA.

Although the gas-chromatographic procedure does not rely on the prior separation of extraneous metals, it is convenient to prevent most of these from forming β-diketonates. The presence of other metal β-diketonates would increase the gas-chromatographic analysis time as many have retention times significantly longer than the beryllium complex. The use of a fluorinated β-diketone has the advantage that an electron-capture detector can be used, enabling the lower limit of detection to be significantly improved over that attainable with the flame-ionisation detector.

The analysis of biological samples for beryllium can either be carried out by direct solvent extraction of the beryllium, or by extraction after wet oxidation. Direct extraction has the advantage of minimising the analysis time and the risk of beryllium losses during processing, but interference from organic matter is possible. On the other hand, although wet oxidation removes all organic matter, it is time consuming, losses of beryllium may occur, and traces of inorganic impurities may be introduced from the mineral acids used in the process. Urine containing beryllium has been analysed by both techniques.

Experimental

Reagents—

The complexing agent, 1,1,1-trifluoropentan-2,4-dione(trifluoroacetylacetone), is commercially available and was used for all the beryllium determinations. It can be used without purification for the determination of relatively large concentrations of beryllium (greater than 1 $\mu g\ ml^{-1}$) but for trace analysis it is necessary to distil fractionally the commercial product. Simple distillation is not satisfactory as this effects the concentration of an impurity, which itself is electron capturing.

Bis-(trifluoroacetylacetonato) - beryllium(II), $[Be(tfa)_2]$, was prepared by dissolving beryllium carbonate in perchloric acid, diluting with water and neutralising. The solution was buffered with sodium acetate and a solution of trifluoroacetylacetone in benzene added. After shaking the mixture, the bulk of the benzene was removed by distillation, followed by evaporation with a stream of dry air. The residue was dissolved in hexane, recrystallised, sublimed and finally dried under vacuum. The thermal stability was studied with a differential scanning calorimeter and the compound was found to be stable up to 200° C (Fig. 1).

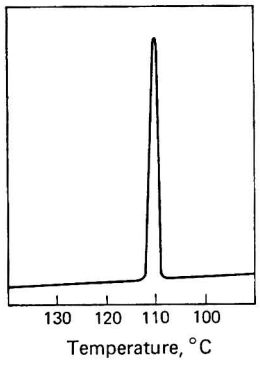

Fig. 1. Thermogram of $Be(tfa)_2$

For trace analysis it is advisable to use Aristar grade mineral acids if wet oxidation precedes the extraction, and to use thiophen-free benzene for the extraction step.

APPARATUS—

All determinations were carried out with a Pye 104 chromatograph fitted with an electron-capture detector or with a flame-ionisation detector. Operating conditions were chosen so that the beryllium complex was eluted in a reasonable time (several minutes) without decomposition, and was resolved from the bulk of the solvent and from solvent and complexing-agent impurities. Fig. 2 compares the response of the flame-ionisation and electron-capture detectors to solutions of the complex.

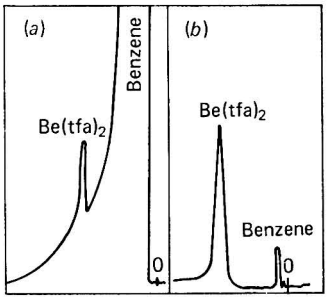

Fig. 2. Chromatograms of $Be(tfa)_2$ solutions in benzene: (*a*), response of flame-ionisation detector to 0·2 μl of sample containing 50 μg ml^{-1} of beryllium (1×10^{-8} g of beryllium); (*b*), response of electron-capture detector to 0·2 μl of sample containing 1 μg ml^{-1} of beryllium (2×10^{-10} g of beryllium)

Considerable difficulty was encountered in finding columns suitable for the satisfactory elution of trace amounts of the beryllium complex. Adsorption by the column and support resulted in significant losses of beryllium. The use of metal columns was avoided to prevent the possibility of exchange reactions on the metal surface. Several different columns were prepared and the most suitable stationary phase was found to be the methyl phenyl silicone gum SE52. Table I lists these columns, which are arranged in order of increasing suitability. Clearly glass is of no use and the difference between the two diatomaceous supports Chromosorb W and Gas-Chrom Z is striking. Different batches of Chromosorb W gave different results and this may also be true of Gas-Chrom Z.

TABLE I

COMPARISON OF COLUMNS FOR BERYLLIUM COMPLEX ANALYSIS

Sample: 1 μl of 0·5 μg ml^{-1} $Be(tfa)_2$ in benzene

Column material	Support	Stationary phase	Recovery,* per cent.
Glass	Chromosorb W, DMCS	5% SE52	Zero
PTFE	Voltalef	5% SE52	2·6
PTFE	Chromosorb 101	—	5·2
PTFE	Chromosorb W, DMCS	5% SE52	18·2
PTFE	PTFE	5% SE52	25·0
PTFE	PTFE	1% Cetrimide + 5% SE52	45·0
PTFE	Gas-Chrom Z, DMCS	5% SE52	100·0 (standard)

* Relative figures, assuming complete recovery with the most satisfactory column.

Adsorption effects can be further minimised by using column plugs of PTFE yarn rather than glass yarn. Total adsorption occurred on the porous polymer bead column. The treatment of a PTFE support with cetrimide gave a significant improvement over a similar, untreated support. The most satisfactory columns were those of PTFE containing SE52

on Gas-Chrom Z and these were used for all subsequent work. The use of PTFE columns in conjunction with an electron-capture detector does not cause contamination of the detector, provided that, both prior to and after packing, the column is conditioned for several days at 200° C. The detector standing current was checked regularly and no current decrease was observed over several months' operation (see Fig. 3).

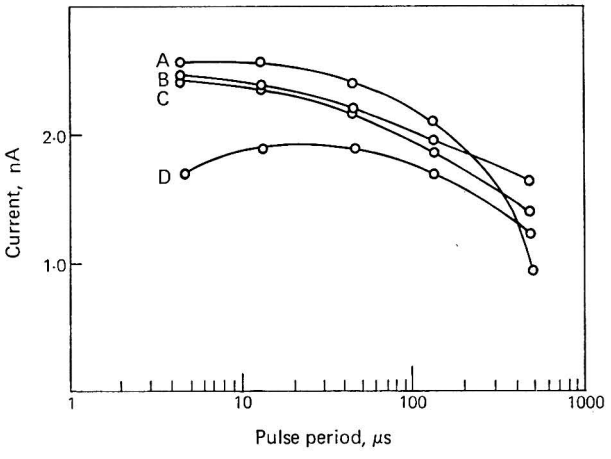

Fig. 3. Electron-capture detector standing currents: A, after 2 months' operation; B, new detector system; C, after 1 week; and D, makers' "typical" values

CALIBRATION—

Calibration of the electron-capture detector was carried out with standard solutions of bis-(trifluoroacetylacetonato) - beryllium(II) in benzene by successive dilution of a 100 μg ml^{-1} primary standard. The detector is readily overloaded and this can give rise to some curious peak shapes making quantitative analysis difficult. Fig. 4 shows the effect of overloading on peak geometry, and Fig. 5 a response curve in which overloading occurs at 0·5 μg ml^{-1}. The amounts and concentrations of beryllium in the injected sample are shown in the figures.

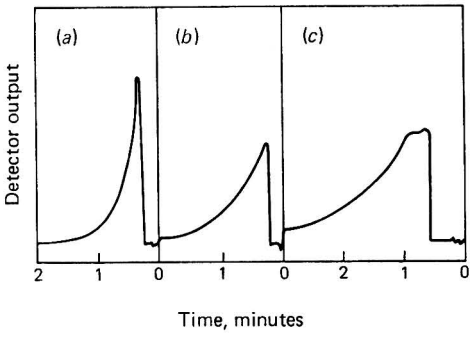

Fig. 4. Effect of electron-capture detector overloading on peak geometry: (*a*), 0·1 μl of a 25 μg ml^{-1} beryllium solution with purge gas (2·5 × 10^{-9} g of beryllium); (*b*), 0·1 μl of a 25 μg ml^{-1} beryllium solution without purge gas (2·5 × 10^{-9} g of beryllium); and (*c*), 0·5 μl of a 50 μg ml^{-1} beryllium solution (2·5 × 10^{-8} g of beryllium)

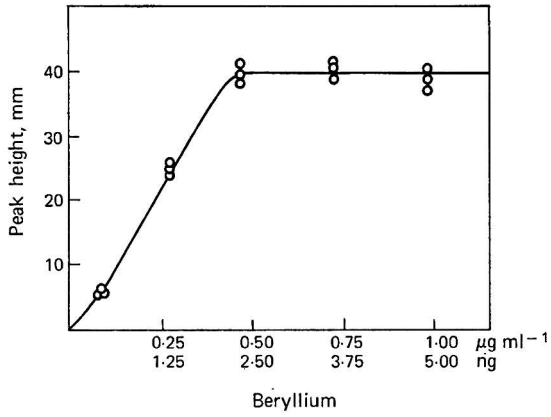

Fig. 5. Electron-capture detector response curve. Concentration (upper scale) and amount (lower scale) of beryllium

The upper limit of detection can be increased by changing the operating conditions, and concentrations up to 10 μg ml^{-1} can be accommodated. All standards were stored in borosilicate glassware to minimise adsorption effects. Comparison of the response of the detector to fresh standards and old standards on the same day showed that no deterioration of the standards had occurred over a period of several months. This is illustrated in Fig. 6.

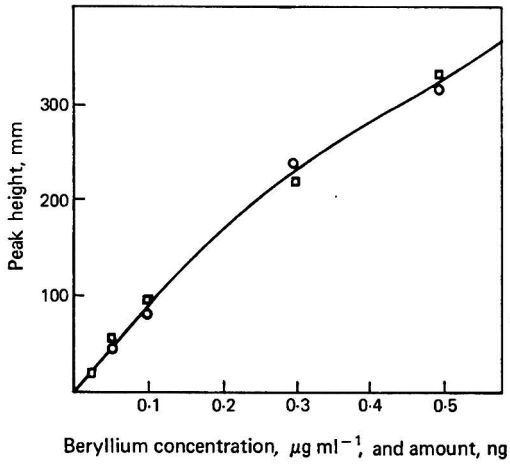

Fig. 6. Ageing of beryllium complex standard solutions: ○, standard; □, standard 6 months' old

With standard solutions of the beryllium complex the smallest concentration of beryllium detected is 0·5 mg ml^{-1}. The value is limited by solvent impurities rather than the noise level of the detector (Fig. 7).

PROCEDURE—

EDTA is added to a known volume of the aqueous sample which is then adjusted to pH 6 with ammonia solution and buffered with M sodium acetate. The same volume of 0·05 M trifluoroacetylacetone in benzene is added, and extraction carried out for 30 minutes. In a typical analysis 100 mg of EDTA disodium salt, 5 ml of sample and of complexing solution

and 1 ml of sodium acetate solution are used. The excess of complexing agent in the organic extract is destroyed by shaking it with 1 ml of 0·1 M sodium hydroxide for several seconds. Microlitre aliquots of the extract can then be analysed. Failure to destroy the excess of trifluoroacetylacetone results in gross interference in the gas-chromatographic analysis and the electron-capture detector may remain unusable for many minutes.

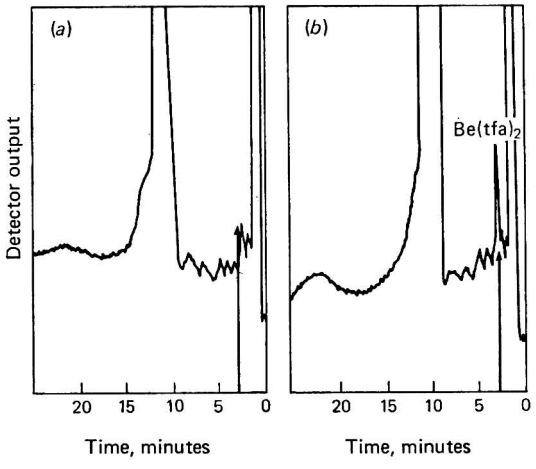

Fig. 7. Chromatograms of (*a*), 20 μl of benzene; and (*b*), 20 μl of a solution of $Be(tfa)_2$ in benzene (0·5 mg ml^{-1} of beryllium, 10^{-11} g)

Results

Aqueous standard solutions of beryllium sulphate, covering the range 0·05 to 0·5 μg ml^{-1}, have been analysed with the above method. Nearly complete recovery of the beryllium was observed in all cases. The effects of storage on these samples was studied, and little difference was observed between fresh standards and those stored for 6 and 12 months in borosilicate glassware.

The analysis of samples containing beryllium in the presence of large amounts of other metals is open to error when these metals also form β-diketonates. It is necessary to ensure that sufficient β-diketone is added to complex all the metals or, if convenient, the interfering metals can be complexed by the addition of EDTA prior to adding the β-diketone. To demonstrate this the analysis of an aqueous solution containing 0·1 μg ml^{-1} of beryllium and 50 μg ml^{-1} of iron was carried out. The results are summarised in Table II. In the absence of EDTA, most of the β-diketone was used in complexing the excess of iron, and little beryllium was detected. In the presence of excess of EDTA, however, a satisfactory beryllium result was obtained. A similar effect was observed on analysis of a lung sample, and these results also appear in Table II.

Table II

Influence of EDTA on beryllium recoveries

Sample	EDTA concentration, mg ml^{-1}	Beryllium detected, μg ml^{-1}
Aqueous solution of— 50 μg ml^{-1} of Fe, 0·10 μg ml^{-1} of Be, 1·8 mg ml^{-1} of H_3PO_4	Nil	0·02
	10	0·10
Wet-oxidised human lung containing 0·20 μg ml^{-1} of beryllium	Nil	Nil
	40	0·05
	Saturated	0·16

Human urine, to which known amounts of beryllium sulphate solution had been added, was analysed under the following operating conditions: the injection, column and detector temperatures were 100°, 100° and 125° C, respectively; column and purge gas flow-rates were 67 ml minute^{-1} (nitrogen gas); the column was of PTFE, 4 feet long by $\frac{1}{8}$ inch o.d., containing 5 per cent. SE52 on 72 to 85-mesh Gas-Chrom Z; and the electron-capture detector had a pulse period of 150 μs, width 0·75 μs, amplitude 47 to 60 V and a source of 10-mCi

TABLE III

SPIKED URINE ANALYSIS

Extraction after wet combustion			Direct extraction		
	Beryllium concentration, μg ml^{-1}			Beryllium concentration, μg ml^{-1}	
Sample number	Found	Added	Sample number	Found	Added
1	2·7	3·4	10	0·82	1·0
2	1·6	1·3	11	0·80	1·0
3	1·3	1·3	12	0·11	0·10
4	1·0	1·0	13	0·11	0·10
5	0·40	0·37	14	0·050	0·050
6	0·25	0·37	15	0·050	0·050
7	0·10	0·10	16	0·009	0·010
8	0·036	0·050	17	0·005	0·005
9	Nil	Nil	18	0·001	0·001
			19	Nil	Nil

nickel-63. Beryllium was recovered both by wet oxidation and by direct extraction by shaking buffered urine with the complexing agent in benzene, in the manner described above. Background interference can be minimised by successively shaking the organic extract with water. The results are summarised in Table III, from which it can be seen that both procedures yield satisfactory results, which are the mean values of triplicate analyses. The minimum amount of beryllium detected in urine is 1 ng ml^{-1}. Chromatograms of urine extracts are shown in Fig. 8.

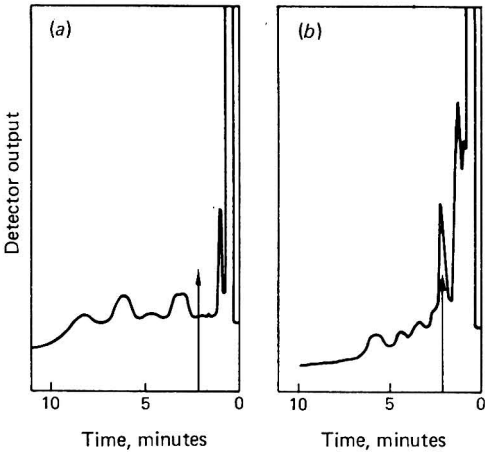

Fig. 8. Chromatograms of urine extracts: (*a*), 1 μl of human urine extract; and (*b*), 1 μl of human urine extract containing 10 ng ml^{-1} of beryllium (10^{-11} g of beryllium)

A series of experiments to measure the rate of excretion of beryllium from rats was undertaken. Two rats were injected with a solution of beryllium sulphate and two others acted as controls. The results are presented in Table IV. Only about 10 per cent. of the total

amount injected was detected in the urine, which was virtually free from beryllium 2 days after injection. No beryllium or interfering material was found in the control rats.

TABLE IV

ANALYSIS OF RAT URINE

Animals injected with 25·4 μg of beryllium

Time after injection, hours	Beryllium detected, μg	
	Rat 1	Rat 2
6·5	1·56	1·81
24	0·79	1·34
30·5	0·18	0·54
48	0·15	0·08
54·5	Nil	Nil
71	Nil	Nil
Total	2·68	3·77

CONCLUSIONS

The determination of beryllium as the trifluoroacetylacetonate can conveniently be carried out with a gas chromatograph fitted with an electron-capture detector. The analysis of urine samples for beryllium, by direct extraction, is satisfactory and a total sample volume as small as 1 ml can readily be handled. The method is rapid and reproducible, and concentrations as low as 1 ng ml^{-1} in urine have been determined.

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Flame-photometric Determination of Bromine in Urine

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A flame-spectrophotometric method is given for the determination of bromide and other bromine-containing compounds in human urine, involving the application of the indium bromide flame technique. This method can be used, for example, to analyse excretions of bromine-containing narcotics, sleeping pills and other bromine-containing compounds, such as halothane, in a simple, quick and specific manner.

A method for determining organochlorine with the indium chloride band spectrum has been published,[1] and this has subsequently been improved and further applications described.[2] Another technique involving the use of indium salts has been developed and can be used not only for chlorine but also for bromine and iodine.[3] Our first mentioned chlorine method has been adapted for the determination of bromine,[4] and this paper reports the use of this method for determining the bromine from sleeping pills in urine. This method is also suitable for the detection of other bromine residues in urine, and of bromine in urine after halothane narcosis. Its advantage over the X-ray fluorescence method is its relative cheapness.[5]

Physiological and Chemical Principles

Because chlorine and bromine are chemical analogues, nine tenths of the bromine in bromine-containing compounds absorbed into the body is replaced by chlorine, and the bromine is normally eliminated as sodium bromide.[6] This elimination as sodium bromide is almost independent of the type of chemical bonding of the bromine in the compound ingested.[7] The analytical method had to take into account that sodium bromide would be present with large concentrations of sodium chloride, and also that, in pathological cases, unsubstituted or only partially substituted organically bound bromine might be found in small concentrations (maximum of 10 per cent.) in urine.[6,7] Further, the speed of bromine elimination is relatively slow. Examination of urine after a large dose of bromine-containing sleeping pills had been taken showed that maximum elimination occurs between 3 hours and 3 days,[7,8] resulting in the bromine being distributed among a large number of urine samples. Bromine concentrations are therefore low, so that the detection method not only has to be very sensitive, but enable small bromine concentrations to be detected in very large chlorine concentrations (ratio of chlorine to bromine, 9 : 1) and, in addition, enable the bromine that is not converted into sodium bromide to be determined accurately.

Experimental

Large amounts of some sodium and potassium salts are present in urine and cause considerable background interference in flame emission resulting from luminescence (recombination of ions and electrons).[9] Consequently there is a strong aspecific continuous background, which cannot be compensated for. This background is dependent on the concentrations of the other salts in urine and varies from sample to sample, and cannot be eliminated by improving the spectral resolution. Weak band intensities therefore have to be measured on a strong continuum background, and we found it advisable to introduce into the sample standard cations, which do not cause a continuum background, with a cation - ion exchange connected in series. Ammonium ions were used which, as well as the suppressing of luminescence of recombining ions and electrons, have the additional advantage that the evaporation of the samples from the evaporation coil is more uniform than when the alkali metal salts are added, as the ammonium salts have low boiling-points.

CALIBRATION AND BLANK SOLUTIONS—

An aqueous solution containing 710 mg of chloride and 40 mg of bromide per 100 ml, each added as the ammonium salt, was used as the calibration solution. Dissolve 10·70 g of ammonium chloride and 4·88 g of ammonium bromide in distilled water and make up to 1 litre. The blank solution contained 10·70 g l^{-1} of ammonium chloride.

BURNER WITH EVAPORATION EQUIPMENT—

The special burner for hydrogen - compressed air with a separated flame has been described and illustrated.[10] (The primary and secondary combustion zones are separate, the secondary zone being above the primary.) The indium surface is increased by introducing two sheets of indium-coated copper - beryllium metal, joined as a cross, and not, as before,[10] by introducing wire coils coated with indium. The indium-coated surface could thus be increased by about 450 mm^2.

Just above the primary air supply to the burner, where the exit of the gas chromatograph is usually connected, is a ground bead of quartz, on which is a ground-brass tube with a holding device for the platinum wire with one platinum coil attached to the front. To apply the analytical sample, pull out the ground-brass tube with the platinum coil. With a Hamilton syringe, pipette the sample on to the platinum coil and introduce it immediately into the burner. The time taken for each introduction should be the same. Full details have been published.[10] So that the platinum coil is always introduced into the burner at the same height and the evaporation conditions are always the same, arrange a V-prism, with the opening on top, at the same height as the ground bead from the burner. Place the ground-brass tube with the platinum coil on this prism so that it can be pushed forward directly into the flame. The optimum position is marked by a stop on the V-groove.

Operating conditions for the burner are: primary air supply, 1·3 l $minute^{-1}$; secondary air supply, 3·0 l $minute^{-1}$; and hydrogen supply, 1·1 l $minute^{-1}$.

OPTICAL - ELECTRICAL MEASURING ARRANGEMENT—

The following measuring arrangement is used for the spectrophotometric determination: Bausch & Lomb 0·5-m grating monochromator; blaze-angle, optimised for 300 nm; entrance slit and exit slit 0·4 mm; spectral band width 0·67 nm; wavelength 375·8 nm, RCA 1P28 radiation receiver; Keithley type 610A d.c. amplifier; and recording on the band of maximum emission with the Kipp micrograph BD 2.

SAMPLE PREPARATION—

Inorganic bromine—Insert a plug of glass-wool, cleaned with chromic acid - sulphuric acid and dried, into a glass column of 40 mm i.d. Fill the column with 200 g of cation exchanger Dowex 50W-X8, 100 to 200-mesh, H^+ form, which should fill it to a height of 160 mm. The lower end of the glass column ends in a tube of 5 mm diameter, and is closed by a one-way tap. Before use, load the cation exchanger by allowing 500 ml of N ammonia solution to percolate through. Then wash it with the same volume of distilled water, ensuring that the exchanger column does not run dry. When the water level has reached the resin surface, add 0·2 ml of a 0·02 per cent. ethanolic solution of methyl red to mark the level of the water - sample solution interface. Add 10 ml of the urine sample to the column. When the exchanger has absorbed the sample, add 500 ml of distilled water. When the yellow solution of methyl red has passed through, coloured by the less alkaline region, collect 5 ml of the pre-treated urine sample in a test-tube. It is recommended that after each urine sample has been run through, the ion exchanger is regenerated with ammonium chloride solution and washed with distilled water. Introduce 1 μl of this solution to the platinum coil and use the peaks recorded as a standard for the amount of bromine introduced after calibration.

Organobromine—Extract the urine sample with one tenth of its volume of light petroleum in a separating funnel. The organic components are extracted. The aqueous phase can be analysed as above by introducing 1 μl on to the evaporation coil and measuring it against a light petroleum calibration solution. An ion-exchange operation is not necessary in this case.

ANALYSIS—

For the calibration graph, prepare solutions with a chlorine content of 710 mg per 100 ml, equivalent to the average chlorine content of urine, and bromine contents of 0 to 100 mg of bromine per 100 ml. The main calibration solution was that containing 40 mg of bromine per 100 ml. The ratio is—

$$\frac{\text{Intensity}_{\text{calibration or sample solution}}}{\text{Intensity}_{\text{main calibration solution}}}.$$

Apply this ratio to the resulting bromine solution. After measuring the ratio, the concentration, in milligrams of bromine per 100 ml of sample, can be found directly from the straight-line calibration graph.

CONTROL OF THE METHOD AND RESULTS

REPRODUCIBILITY—

The reproducibility was found by using the ratio method to make twenty measurements on 1 μl of a solution containing 0·10 μg μl^{-1} of bromine. The relative error is $\pm$2·19 per cent.

FLAME-PHOTOMETRIC DETECTION LIMIT—

The detection limit was found by making twenty measurements, each with 1 μl of a blank solution and with 1 μl of an ammonium bromide solution containing 0·10 μg μl^{-1} of bromine.

The detection-limit definition used was that proposed by Kaiser and Menzies[11]—

$$\bar{x} - x_B = 3\sqrt{2} \cdot \sigma_B$$

where $\bar{x}$ is the mean of the measurements of the blank value and the bromine peak; x_B is the mean of the blank value variation only; and σ_B is the standard variation of the fluctuations of these blank values. The detection limit was 0·0062 μg of bromine, as ammonium bromide, in 1 μl of delivered sample. This detection limit does not take into account fluctuations caused by sample preparation.

RECOVERY OF ADDED BROMINE IN URINE—

Addition of sodium bromide—Between 10 and 100 mg per 100 ml of bromine as sodium bromide are added to each 10 ml of urine and the samples analysed by the above method. The results are given in Table I.

TABLE I

RECOVERY EXPERIMENTS

Theoretical value, μg μl^{-1}	Experimental value, μg μl^{-1}	Recovery, per cent.
0·10	0·03	30·0
0·20	0·11	55·0
0·40	0·30	75·0
0·60	0·49	81·7
0·80	0·55	68·7
1·00	0·935	93·5

If the recovery rates are low, they can be increased by changing the method of sample preparation. As mentioned above, the ion-exchange column is loaded with 10 ml of urine containing 0·2 μg μl^{-1} of bromine and is then washed with 300 ml of distilled water. The urine and water were collected and evaporated to 10 ml. On analysing 1 μl, instead of the expected 0·2 μg μl^{-1} of bromine 0·21 μg μl^{-1} of bromine was found, *i.e.*, 105 per cent. recovery. If this procedure is used for the sample preparation, it is possible to determine the bromine without waste.

Addition of sleeping pills containing bromine—It is possible in pathological cases that residues of sleeping pills or decomposition products are not converted into sodium bromide in the body but appear unchanged, or in a similar organic form, in the urine. It was therefore interesting to see if the method for inorganic bromine (under Sample preparation) could also be used to determine the organobromine. For this 72·5 mg of a Bromural pill (bromoisovalerylurea) were heated with 20 ml of water, allowed to cool and filtered. This solution

was used for the relative value. In a second sample, 72·5 mg of Bromural were dissolved in urine in the same way. These solutions were used as calibration and blank solutions, respectively. A 1-μl aliquot of the filtrate of the aqueous solution was analysed; the recovered bromine content was 0·03 $\mu g\ \mu l^{-1}$ of bromine. A 10-ml portion of the filtered urine sample was put on the exchanger, run through with 300 ml of distilled water, and the volume reduced to 10 ml, of which a 1-μl aliquot was analysed. The recovered bromine content was 0·015 $\mu g\ \mu l^{-1}$ of bromine. Compared with the aqueous solution only 50 per cent. of the dissolved bromoisovalerylurea was found.

Discussion

The proposed flame-photometric method for the determination of bromine in urine is simple and specific, and can be used to detect small bromine concentrations in large concentrations of chlorine. The costs of sample preparation are relatively low. Recoveries are between 50 and 100 per cent. The unchanged bromine compounds in urine (a maximum of 10 per cent.) that did not form sodium bromide in the human body could be separated and analysed by prior extraction with light petroleum, which does not affect the sodium bromide. In practice, this light petroleum extraction is unnecessary because the concentrations are usually below 10 per cent. of the total bromine, and the recoveries for these small organic residues are 50 per cent. with the sodium bromide technique. The total bromine content in urine is not noticeably affected by this. In a separate determination of inorganic bromine and organobromine the recovery rates were 100 per cent. for each by the appropriate procedures.

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The Rapid Determination of Fat in Cocoa Products by Using a Differential Density Technique

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The rapid determination of fat for process control purposes in cocoa powders, extrusion cakes and chocolate liquors has been accomplished by rapid extraction of the fat with tetrachloroethylene, followed by indirect determination of the density of the resulting solution by using a differential density technique. The difference in apparent weight loss, which is indicated by an aperiodic balance, between a plummet immersed in the fat solution and an identical plummet immersed in pure tetrachloroethylene is correlated with the fat content of the sample. The correction factor associated with batch-to-batch variations of solvent density required by previous density methods is eliminated. The method is simple to operate and produces results at 10-minute intervals, although 20 minutes are required to complete each determination.

For cocoa powders, 95 per cent. of the results obtained for the fat content by the differential density technique were within $\pm 0{\cdot}46$ per cent. w/w of those obtained by a Soxhlet extraction method, but for extrusion cakes and chocolate liquors the confidence limits were slightly wider.

THE fat, *i.e.*, cocoa butter, content is an important factor to consider in cocoa production because of its influence on product quality and because it is subject to statutory specifications. Consequently, the fat content must be controlled with reasonable precision. Soxhlet extraction with light petroleum is a reliable method of fat determination, but it is too time consuming for process control purposes and a more rapid method is necessary. A considerable number of rapid methods based on a variety of techniques has been developed for food products, many of which are described in an extensive review recently published.[1] The conventional rapid procedures applicable to cocoa products depend on extraction of the fat with a suitable solvent and measurement of the refractive index or density of the resulting fat solution. Both techniques have been examined by the authors' company, but the density procedure was preferred because it was easier to carry out the manipulations.

About 1930, Harris[2] developed a density method for chocolate products in which 1,2-dichlorobenzene was used as the solvent, and the density was determined with a hydrometer. Leithe[3] used carbon tetrachloride and determined the density with a pycnometer. In both methods the fat was extracted at room temperature, but in the method[4] developed by this company boiling tetrachloroethylene was used. Each solvent satisfied the criterion that its density should differ greatly from that of cocoa butter, but the last solvent was preferred because of its relatively low toxicity compared with other high density chlorinated solvents. The pycnometric density determination was used initially, but the method was later simplified, although with a slight loss in precision, by using a hydrometer. The minimum time required to complete the determination was 20 minutes. To make the procedure capable of operation by semi-skilled personnel, a further modification was to determine the density indirectly by immersing in the solution a plummet, which was permanently attached to one arm of an aperiodic balance, and measuring the plummet's apparent weight loss. This apparatus was known as the single plummet apparatus (S.P.A.). The fat was extracted from $25{\cdot}0 \pm 0{\cdot}025$ g of sample by refluxing for 5 minutes with a fixed weight of tetrachloroethylene, which was

obtained by taking a volume equal to 100 ml at 20·0° C. (For brevity, this amount will hereafter be referred to as "100 ml at 20° C," although the volume actually measured depends on the temperature of the solvent.) After cooling the extraction flask for 1 minute in a water-bath, its contents were siphoned on to a filter-paper and filtered into a cylinder containing the plummet, the cylinder being immersed in a cold water bath. The fat solution was equilibrated in the water-bath for 5 minutes and the balance reading noted. The apparent weight loss of the immersed plummet was correlated to the fat content of the sample. The method was applied to a variety of cocoa powders and ground extrusion cakes, but was insufficiently precise for application to chocolate liquor. It was successfully used for process control for several years, but improvements that further simplify the procedure have recently been applied.

Hitherto, the method required the use of correction factors to take into account slight temperature variations of the fat solution and batch-to-batch variations in the density of the pure solvent. Because of the latter variations it was essential to determine accurately, with a pycnometer, the density of every batch of solvent before use. This inconvenience has now been eliminated by carrying out the measurement on a differential basis. In the new procedure use is made of the difference in apparent weight loss between a plummet suspended from one arm of the aperiodic balance and immersed in the fat solution, and an identical plummet suspended from the other balance arm and immersed in the pure solvent. The apparatus, known as the differential plummet apparatus (D.P.A.), also incorporates a constant-temperature water-bath, which eliminates the need for temperature corrections. An additional advantage of this apparatus has proved to be a slight improvement in precision, possibly caused by the elimination of the correction factors, which has allowed its use for chocolate liquors as well as cocoa powders and extrusion cakes. It is now in routine use, and its reliability and rapidity lead us to believe that it might be applied in other fields.

Experimental

Relationship between balance reading and fat content of the solution—

As the volume of the fat solution varies with the amount of fat it contains, the relationship between the balance reading and the weight of fat, expressed in grams, is curvilinear. However, over small ranges of fat content, the deviation from linearity is slight, and the relationship can be taken to be linear. The fat contents of extrusion cakes and cocoa powders lie within the ranges 0 to 20 per cent. w/w and 20 to 32 per cent. w/w, respectively, and, for sample weights of 25 g, produce fat solutions containing between 0 and 5 g, and 5 and 8 g of cocoa butter, respectively. For practical purposes, the balance reading to weight of fat relationship over each of these ranges is assumed to be linear.

Effect of variation in the size of the plummets—

The optimum plummet size with regard to precision of analysis and convenience of use is 20 ml. However, it is difficult to make plummets of identical volume and it was necessary to determine the maximum permissible difference in volume between the two plummets of the D.P.A. Similar limits had previously been calculated when the S.P.A. was introduced because of the possibility of breakage of the standard 20-ml plummet in use at that time. It was subsequently shown that a slight deviation from 20 ml is permissible, providing certain precautions are taken.

For purposes of clarity, the effect of a variation in the size of the single plummet of the S.P.A. is initially discussed. The samples required to be analysed varied in fat content between 0 and 30 per cent. w/w and, by using the specified extraction conditions, produced solutions containing between 0 and 7·5 g of fat. When using tetrachloroethylene of density 1·6232 g ml^{-1}, the densities of the solutions varied between 1·6232 and 1·5682 g ml^{-1}, respectively. If a standard 20-ml plummet were immersed in turn in the two solutions containing 0 and 7·5 g of fat, the apparent weight losses would be 32·464 and 31·364 g, respectively, the difference being 1·100 g. However, if the plummet volume were 20·1 ml, the apparent weight losses and their difference would then be 32·626, 31·521 and 1·105 g, respectively. Thus, the effects of using a plummet differing in volume from the standard 20 ml would be 2-fold, an offsetting of the calibration line from that obtained when using the standard plummet, and a slight change in slope.

If necessary, the offsetting of the calibration line could be corrected by using an appropriate balance counterweight so that a non-standard plummet gave the same balance reading as a standard plummet when immersed in the same fat solution. It was not possible to correct fully for the change in slope, but a partial adjustment could be achieved by repeating the above standardisation with a second fat solution. Thus two standard cocoa butter solutions were prepared with fat contents in the middle of the cocoa powder and extrusion cake ranges. With the standard plummet immersed in each of the standard solutions, suitable counterweights were made so that balance readings were obtained at about the middle of the scale. The two balance readings became the standardisation points for all further work. Thus, in the event of a non-standard plummet being used, new counterweights could be made so that the same balance readings were obtained with the plummet immersed in the two standard solutions. Provided that the apparatus was standardised at these two points, it was calculated that an acceptable tolerance for the volume of a non-standard plummet was $\pm 0{\cdot}05$ ml, corresponding to about $\pm 0{\cdot}05$ per cent. of fat.

The same considerations apply to the two plummets of the D.P.A. technique, but further calculations show that the permissible difference in volume between the plummets is more critical. Consider two standard 20-ml plummets, one immersed in fat-free tetrachloroethylene of density 1·6232 g ml^{-1}, and the other in the fat solution of density 1·5682 g ml^{-1}. The difference in the apparent weight loss of the two plummets would be 1·100 g. However, if the fat solution plummet had a volume of 20·1 ml, the difference would then be 0·943 g. Thus, for this fat solution a volume difference of 0·1 ml between the D.P.A. plummets introduces an error of about 157 mg, which is equivalent to an error of about 4·5 per cent. of fat according to S.P.A. calibration results. For a maximum error of 0·05 per cent. of fat, the volume tolerance between the plummets is 0·001 ml.

Standardisation of the apparatus—

The D.P.A. standardisation points for cocoa powders and extrusion cakes are identical with those arbitrarily selected for the S.P.A. but, in addition, the D.P.A. is provided with a third standard for use with chocolate liquor. The cocoa powder standard solution consists of 6·25 g of filtered, pressed cocoa butter dissolved in "100 ml at 20° C" of tetrachloroethylene, and the extrusion cake and chocolate liquor standard solutions of 3·00 g and 11·00 g of cocoa butter, respectively, dissolved in a similar amount of tetrachloroethylene. The D.P.A. is standardised with regard to the counterweights when these solutions give balance readings of 210, 277 and 284 mg, respectively, provided the reference plummet cylinder contains tetrachloroethylene identical with that used for the standard solutions.

Conditions of fat extraction—

The optimum conditions for fat extraction from cocoa powders and extrusion cakes were established during the development of the S.P.A. method. By using a sample weight of 25 g and "100 ml at 20° C" of tetrachloroethylene, it was shown that most of the fat was extracted after refluxing for 1 minute and that refluxing times longer than 5 minutes did not increase further the efficiency of fat extraction. However, before applying the D.P.A. procedure to chocolate liquor, the extraction conditions were re-examined for this product because of its higher fat content. Satisfactory extraction was again obtained with a refluxing time of 5 minutes, but the sample weight was reduced to 20 g. A smaller weight would decrease precision and a larger weight possibly lead to fat deposition within the apparatus. Because of the higher fat content, chocolate liquor samples should be weighed with greater accuracy than other cocoa products. Because of the molten condition of the chocolate liquors, it was more convenient to transfer 20 ± 1 g of sample to the extraction flask, weigh it accurately, and then adjust the results for the deviation from the standard 20·0-g sample weight.

Determination of optimum equilibration time—

Hot tetrachloroethylene was discharged into the apparatus and, with identical tetrachloroethylene equilibrated to 20° C in the right plummet cylinder, the balance reading was noted at 15-s intervals. A zero balance reading indicative of temperature equilibrium was obtained after 5 minutes, and thus the S.P.A. 5-minute equilibration period was also applicable to the D.P.A.

EFFECT OF TEMPERATURE VARIATIONS—

The balance reading was determined by using the standard 6·25-g fat solution with the bath temperature adjusted to 15°, 20° and 25° C. The 5° C change from the standard temperature caused a mean change in balance reading of 8 mg, as compared with a change of about 150 mg, which would be expected with the S.P.A. for the same temperature change. Clearly, each plummet of the D.P.A. is subject to changes in apparent weight loss resulting from temperature variations, but the differential nature of the apparatus largely prevents a change in the balance reading, and thus the temperature dependence of the apparatus is considerably lower than that of the S.P.A. Complete prevention of a temperature effect is not possible because of the slight difference in the coefficient of expansion of the fat-free solvent and that of the fat solution. It was considered that the optimum practical range of water-bath temperature would be 20·0° ± 0·5° C.

EFFECT OF PARTICLE SIZE—

The cocoa powder samples received in the laboratory are finely ground, but the extrusion cake samples are in the form of particles varying in size between fine dust and about 3 mm diameter. Hence, following the development of the S.P.A., a series of experiments was undertaken to determine if the efficiency of fat extraction is affected by particle size. A dry-sieving analysis was carried out on an extrusion cake sample, and Soxhlet and S.P.A. fat determinations were carried out on the sieved fractions. The fat content of the sieved fractions was also determined by using the Werner-Schmidt method,[5] in which an acid-hydrolysis procedure is used to break down the material. Thus, the last series of results was obtained by using conditions of maximum extraction and would be expected to be independent of particle size.

It was shown that as the particle size increased, the actual fat content (Werner-Schmidt method) decreased, and that the Soxhlet method was capable of total fat extraction over the complete particle-size range. The S.P.A. procedure gave low results for the coarse material, clearly indicating inefficient extraction, but a further series of experiments in which the coarse samples received a preliminary grinding in a small domestic coffee mill before fat extraction produced S.P.A. results consistent with the Soxhlet determinations.

APPARATUS

The differential plummet apparatus shown in Fig. 1 is comprised of three sections: the fat extraction unit; the siphon; and the measurement assembly.

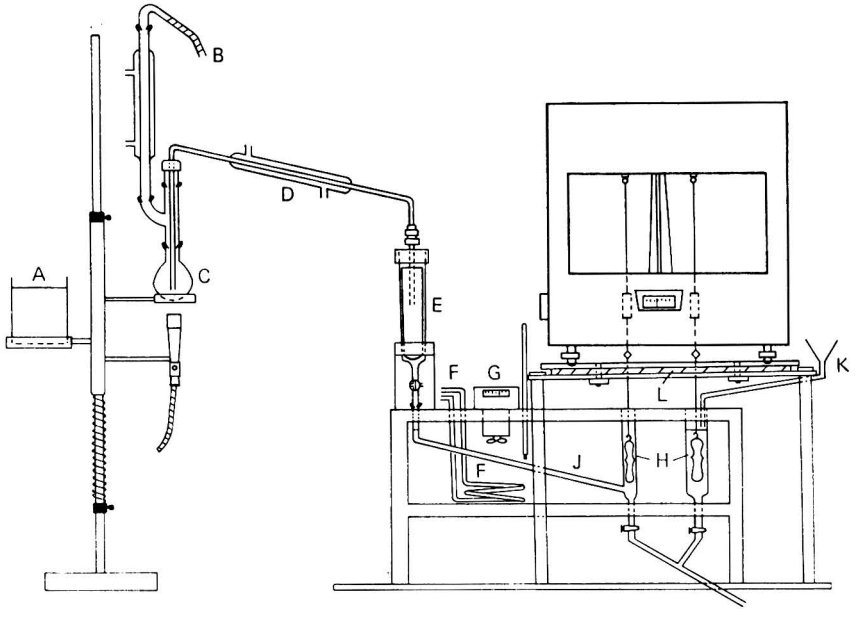
The fat extraction unit, C, consists of a 250-ml flask connected through one arm of a two-necked parallel adaptor to a condenser. The outlet of the condenser is connected to a small piece of copper piping, with a hole in its side, which is in turn connected to a small hand-bellows.

The heating and cooling arrangements comprise a spring-loaded tube on a main upright support carrying an asbestos-bound ring for the flask, with a burner fixed directly underneath. At an angle of 90° is fixed a circular platform supporting a vessel of water, A, used for cooling purposes. The tube and its attachments can easily be rotated and depressed to allow the rapid installation and removal of the flask.

The siphon, D, is made of 6 mm i.d. copper piping, the centre portion of which is water-cooled. One end of the pipe extends from the bottom of the flask and passes through a cone - screw adaptor, which makes an air-tight joint with the extraction unit. The other end is fitted with a threaded union for easy detachment from the measuring assembly, and a short extension from the union passes through a polythene cap fitted over the filter tube, E. This cap, which reduces evaporation during filtration, has two small holes to prevent the build-up of back-pressure, which would stop the filtration.

The measurement assembly consists of an aperiodic balance supported by a large steel plate, a water-bath containing a stirrer - heater unit, two copper cooling coils and two glass plummet cylinders, H. The legs of the balance fit into holes in a rectangular steel sheet bolted to the steel plate, with a rubber pad between the sheet and plate to reduce vibration. The holes for the bolts are sufficiently large to allow the plate to be moved relative to the water-bath to obtain the optimum position of the plummets within the plummet cylinders.

The balance should be a manual aperiodic two-pan model, with a 0 to 500-mg scale. We use an Oertling B.O.6, modified to allow unhindered suspension of the plummets. Both the pans and pan supports are removed, and from the upper pan hooks are suspended two thin brass rods, each passing through the pan-support holes and each ending in a brass ring below the balance floor. The spindle of the beam-release mechanism passes through the brass rings. A further length of rod, with a small loop at its lower end, is bolted to the lowest portion of each brass ring. Large diamond-shaped brass slip-rings connect each loop with a short length of platinum wire (40 s.w.g.) from which the plummets are suspended.



A = Cooling bath
B = Hand-bellows connection
C = Fat extraction unit
D = Siphon
E = Filtration tube
F = Water-bath cooling coil
G = Stirrer - heater unit
H = Plummet tubes
J = Fat solution cooling coil
K = Reference solvent delivery tube
L = Anti-vibration pad

Fig. 1. Differential plummet apparatus

The two glass plummets, each with a flange round the middle to minimise surface-tension effects, are weighted with lead shot to increase their weight in air to between 35 and 50 g. Although it is only necessary to ensure that their weights are of the same order, ideally their volumes should be identical. When totally immersed in distilled water at 20·0° C, the weight of each plummet should be reduced by 19·944 g (equivalent to a volume of 20·000 ml). A latitude of $\pm$0·05 ml is permissible in the volume of both plummets, provided that the difference between the two is no greater than 0·001 ml.

To minimise the volume of fat solution required for complete immersion of the plummet, the diameter of the fat solution cylinder is only slightly larger than that of its plummet. The diameter of the right-hand plummet cylinder is considerably larger to aid the optimum positioning of the plummets.

The water-bath, which is supported within an angle-iron framework, is constructed from 12-mm thick Perspex sheet and is fitted with a Perspex lid. The plummet cylinders are positioned in the bath by rubber bungs at the bottom, and by accurately aligned holes in the lid into which the cylinders protrude. The bottom of each cylinder is connected to a stopcock outside the water-bath to allow drainage.

The filtration tube terminates in a T-bore stopcock connecting the tube, the fat solution cooling coil, J, and the atmosphere. The exit to atmosphere is used for the relief of any air locks that may occur. The filtration tube, of about 150-ml capacity, contains a 320-mm Whatman No. 111 filter-paper folded in the form of a thimble. This shape is obtained by the use of a wooden former, but care must be taken to ensure that the paper is not split during shaping.

The fat solution cooling coil (shown, for convenience, as a straight tube in Fig. 1) is made of about 0·6 m of 6 mm i.d. copper piping, with bends to increase the path length of the solution. The lower end is attached to a side-arm at the base of the fat solution plummet cylinder; the upper end is attached to a glass tube seated on the Perspex water-bath lid and ending in a cone into which the filtration tube fits. The cooling coil is assembled so that complete drainage is possible.

A funnel and delivery tube are clamped to the right side of the steel plate to facilitate filling of the solvent reference cylinder.

METHOD

PREPARATION OF TETRACHLOROETHYLENE—

Fresh supplies of tetrachloroethylene should, if necessary, be dried over calcium chloride and fractionally distilled, the fraction within 1° C of the boiling-point (121° C) being collected. A clear bright distillate should be obtained. If it is not clear and bright, allow it to stand overnight and filter through an oven-dried filter-paper. Store the prepared solvent in a dark bottle or in the dark.

MEASUREMENT OF SOLVENT—

The tetrachloroethylene is measured from a pipette-shaped vessel with a tap, the volume delivered, 100·0 ml at 20° C, being given between two marks. The apparatus is calibrated for temperature over the range 15° to 25° C, the correction being 0·1 ml $°C^{-1}$.

PREPARATION OF SAMPLE—

Cocoa powder samples are tested as received, but extrusion cake samples are initially broken down by passage through fluted rolls, and then 30 g of this material are finely ground in a domestic coffee mill for 15 s, in three bursts of 5 s. After this treatment, the sample should be sufficiently fine for more than 90 per cent. to pass through a B.S. 22-mesh sieve.

PREPARATION OF APPARATUS—

Before testing the first sample, the standardisation of the apparatus is checked. If a zero balance reading is obtained when the plummets are suspended in air, but not when suspended in tetrachloroethylene, the solvent in one of the plummet cylinders has become contaminated since being removed from the bulk supply, and should be discarded.

DETERMINATION OF FAT CONTENT—

The appropriate weight of prepared sample is transferred to the 250-ml flask, "100 ml at 20° C" of tetrachloroethylene are added and the suspension refluxed for 5 minutes. The flask is immersed in the cooling water for 1 minute, and then the contents are siphoned into the filtration tube containing a folded filter-paper by placing a finger over the small hole in the copper tube and gently squeezing the bellows.

The first few millilitres of filtrate are used to flush the fat solution cooling system and are discarded, but the remainder is allowed to immerse fully the plummet in the cylinder. After a minimum equilibration period of 5 minutes, the balance beam release is actuated and the balance reading noted, ensuring that the appropriate counterweight is in use for the material being tested.

When a series of determinations is to be carried out, the results can be obtained more rapidly by timing the various operations so that a sample is being refluxed while the clear fat solution of the previous sample is being equilibrated. When a series of samples of similar fat content is being tested it is not necessary to flush the apparatus between each determination. However, if there is a marked difference in fat content between one sample and the next, it is advisable to flush the measuring system with a cocoa butter - tetrachloroethylene solution between determinations, the concentration of the solution being similar to that anticipated for the sample fat solution to be tested.

CALIBRATION—

Carry out the procedure as described above, and then determine the fat content of a further portion of the sample (extrusion cake and cocoa powder, 4 g; chocolate liquor, 2 g) by Soxhlet extraction with low boiling light petroleum for a minimum of 16 hours. Select samples so that the entire range of results expected to be met in practice is covered by the calibration.

RESULTS AND DISCUSSION

COCOA POWDERS AND EXTRUSION CAKES—

Several samples of five cocoa powders of differing specification, and two extrusion cakes produced by different processes, were tested in duplicate by the D.P.A. procedure and by the Soxhlet method. The results for each product were statistically analysed by the method of least squares. An analysis of covariance of the cocoa powder regression equations showed that two of the equations were significantly different, but that the remaining three equations were from the same population and could be combined. An analysis of covariance of the extrusion cake equations showed that these were also significantly different from each other. Table I summarises the ranges of fat content, regression equations and their 95 per cent. confidence limits for the various products. By using the calibration results for standard cocoa butter solutions, it was calculated that, for some products, total fat extraction with these experimental conditions was not fully achieved. The difference between the regression equations was probably caused by a slight variation between products in the efficiency of fat extraction.

The standard deviations of replicate D.P.A. readings for the cocoa powders and extrusion cakes were 3 and 7 mg, respectively. The poorer precision for the latter materials was consistent with the greater standard deviations from regression of the equations, and was probably related to the greater sample heterogeneity that would be expected for this type of material.

TABLE I

RELATIONSHIP BETWEEN D.P.A. READING AND FAT CONTENT FOR THE VARIOUS PRODUCTS

Product	Number of samples	Range of fat content, per cent. w/w			Regression equation*	95 per cent. confidence limits, per cent. w/w
		Minimum	Mean	Maximum		
Extrusion cake—						
A	9	7·9	12·1	18·8	F = 0·026R + 5·0	±1·10
B	10	8·5	9·9	13·2	F = 0·025R + 4·7	±0·60
Cocoa powder—						
A	9	21·8	23·2	23·6	F = 0·024R + 19·5	±0·32
B	8	21·1	23·3	28·1	F = 0·034R + 17·9	±0·36
C	9	22·0	23·9	26·3	F = 0·027R + 19·3	±0·46
D	18	23·5	24·5	26·0		
E	10	18·8	22·4	25·7		

* F = Fat, per cent. w/w; R = Reading of balance, mg.

Effect of solvent density variations—During the calibration tests, several batches of redistilled tetrachloroethylene were used, but the range of density was considerably smaller than that normally met in routine practice and did not adequately evaluate the differential technique. Consequently, a specially prepared solvent consisting of a 2·5 per cent. v/v solution of trichloroethylene in tetrachloroethylene was used in the D.P.A. to determine the fat content of a series of samples that had previously been tested by using pure tetrachloroethylene. The density of this solvent, 1·6190 g ml^{-1}, was considerably lower than that of pure tetrachloroethylene.

The results are summarised in Table II. The differences between the Soxhlet and D.P.A. mixed solvent results were well within the 95 per cent. confidence limits of the method. The difference between corresponding D.P.A. results was attributed to random error.

TABLE II

COMPARISON OF SOXHLET AND D.P.A. RESULTS OBTAINED BY USING TWO SOLVENTS OF DIFFERENT DENSITY

	Fat, per cent. w/w, by		
		D.P.A. method	
Product	Soxhlet method	Tetrachloroethylene (density 1·622 to 1·623 g ml^{-1})	Mixed solvent (density 1·619 g ml^{-1})
Extrusion cake—			
A	8·5	9·0	8·4
A	9·3	9·5	9·6
B	9·8	9·5	10·1
Cocoa powder—			
A	23·5	23·6	23·7
A	23·6	23·6	23·9
A	23·5	23·4	23·4

CHOCOLATE LIQUOR—

Seventeen samples of four chocolate liquors of differing specification were tested in duplicate by the Soxhlet and D.P.A. methods. Insufficient results were available to allow separate statistical analyses, but a regression analysis of the complete set of results produced the equation

$$F = 0{\cdot}0376R + 44{\cdot}6.$$

Graphical inspection indicated that the equation was independent of the chocolate liquor specification. The 95 per cent. confidence limits on the regression equation were $\pm 0{\cdot}64$ per cent. of fat, and the replicate standard deviation of the D.P.A. balance reading was 3 mg, corresponding to 0·10 per cent. of fat. As would be expected, this was slightly poorer than that by the Soxhlet method (0·06 per cent. of fat).

CONCLUSIONS

The differential plummet apparatus enables the fat content of certain cocoa powders, extrusion cakes and chocolate liquors to be determined within the accuracy and precision required for process control purposes. The operational simplicity of the method permits its use by semi-skilled personnel, and results can be obtained at 10-minute intervals. Batch-to-batch solvent density variations have no effect on the accuracy of the method.

The authors are indebted to the late Dr. H. C. Lockwood for applying the plummet technique to cocoa products, and to Mr. A. Turner for his guidance during the modification and improvement of the S.P.A. procedure. We also thank Miss S. Scadding for the statistical interpretation of the results, Miss D. M. Palmer for her help with the practical work, and the Confectionery Group Board of Cadbury Schweppes Limited for permission to publish this paper.

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A Rapid Procedure for Preparing Gas Samples for Nitrogen-15 Determination

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A method, which is faster and more convenient than previous techniques, is described for converting ammonium sulphate into nitrogen gas for isotopic analysis on a mass spectrometer. The dry ammonium sulphate sample is contained in a small screw-topped vial, in which it can be stored pending analysis. This vial can be coupled with a neoprene O-ring to a special assembly, evacuated, and the sample oxidised with air-free hypobromite solution from a reservoir in the assembly. By using this procedure the usual inconvenience of de-greasing ground-glass joints on sample tubes is avoided, the difficulty of storing samples prior to analysis is overcome and the de-gassing of solutions prior to the conversion of each sample into nitrogen gas is eliminated. The procedure is particularly suitable when many hundreds of samples are to be analysed.

Although the use of the isotope nitrogen-15 in biology and agriculture has increased markedly in recent years, analytical techniques for determining it have not changed greatly since the pioneering work of Rittenberg.[1] The techniques have recently been reviewed in detail by Bremner,[2] who described a procedure that avoids many of the difficulties previously encountered. Martin and Ross[3] have further discussed errors encountered in nitrogen-15 determinations.

While the procedure described by Bremner is much more rapid than those previously published, certain improvements become desirable when large numbers of samples are to be analysed. In this laboratory the nitrogen programme gives rise to many hundreds of samples per year on which nitrogen-15 determinations are required. Under these conditions, with existing procedures a disproportionate amount of time has to be spent in transferring samples to and from storage vessels prior to analysis, in de-greasing glassware and in de-gassing hypobromite and ammonium sulphate solutions before the conversion into nitrogen gas. The procedure reported here does not suffer from these defects.

Experimental

Sample preparation—

In the procedure to be described it is assumed that the nitrogen in the sample has been converted into a solution of ammonium sulphate, possibly containing boric acid and a nitrogen-free indicator but with no other contaminants. (Methods of achieving this and the associated difficulties have been discussed previously.[1,2,3,4])

Acidify up to 50 ml of solution, containing not more than 1 mg of nitrogen, to between pH 3 and 4 with sulphuric acid, and evaporate it to dryness on a water-bath in an ammonia-free atmosphere. [It is important to avoid excess of acid as the hypobromite (to be discussed later) is less alkaline than that recommended by previous workers.] Dissolve the residue in 1 ml of distilled water and transfer the solution to a glass screw-topped tube, 58 mm tall and 17 mm in diameter (Trident 2-dram vial, tall, manufactured by Johnsen and Jorgensen, London). Evaporate it to dryness in an oven at 105° C.

The ammonium sulphate will be left as a solid deposit around the base of the tube and possibly on the walls if boric acid is present. When cool the tube can be capped and the contents stored indefinitely.

APPARATUS—

The glass assembly used for converting the ammonium sulphate into nitrogen gas is shown in Fig. 1 (*a*). The 100-ml reservoir, Q, contains air-free hypobromite solution under an atmosphere of helium. To the upper portion of the assembly is attached a fitting, S, from a commercially available greaseless stopcock (G. Springham and Co. Ltd., Harlow, Essex), which contains a Viton A seat, R. Normally S is bolted to the retaining ring, P, but these components are separated in Fig. 1 (*a*) for clarity. The vial, T, containing the dry ammonium sulphate is coupled to the lightly greased 19/26 socket, U, by using a neoprene O-ring (11 mm i.d., 16 mm o.d.). With the stopcock S closed and on evacuation of the assembly through the 10/19 cone, V, the vial is forced into the socket by atmospheric pressure to give a completely vacuum-tight seal. Hypobromite solution can then be admitted into the vial by opening stopcock S.

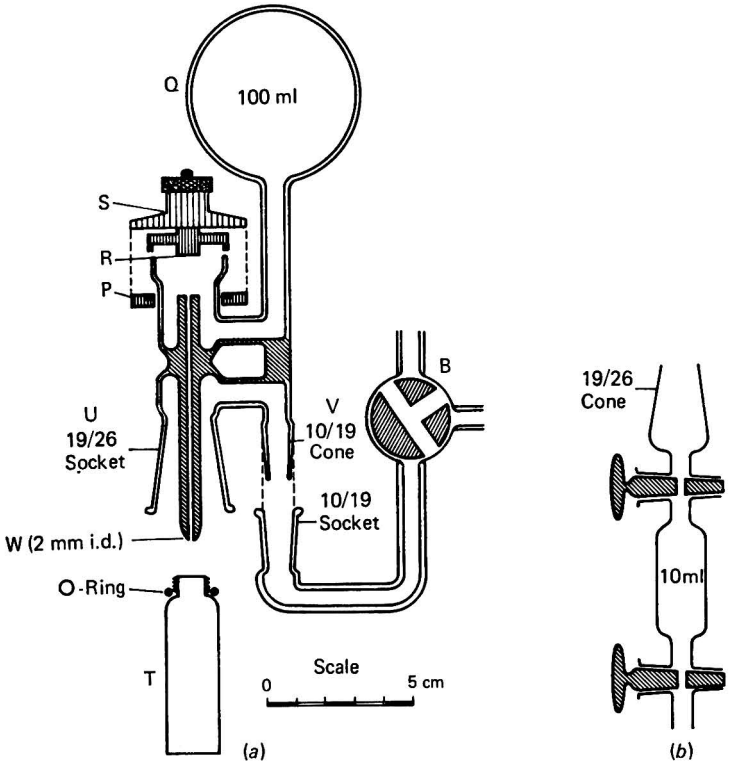

Fig. 1. (*a*) Conversion assembly for preparing nitrogen gas from ammonium sulphate. B, 3-way glass vacuum stopcock; P, R and S, Springham valve assembly; Q, hypobromite reservoir; T, vial; U, 19/26 socket (Quickfit or Clearfit); V, 10/19 cone; and W, capillary delivery tube. (*b*) Helium vessel

The complete preparative unit for converting the ammonium sulphate into nitrogen gas and admitting this into the mass spectrometer is shown diagrammatically in Fig. 2 (*a*). A is the conversion assembly shown in detail in Fig. 1 (*a*). B is a 3-way glass stopcock used to control the vacuum manipulations. C is a 100-ml cold trap immersed in a dry ice - acetone freezing mixture; its purpose is to prevent contamination of the vacuum system with water during evacuation of the assembly. D is a cold trap consisting of a 300-mm stainless-steel tube (1 mm i.d., 1·5 mm o.d.) wound into a helix, which is immersed in liquid nitrogen just before the nitrogen-gas sample is admitted, so that impurities such as nitrous oxide are condensed. It is attached through a length of 2 mm i.d. stainless-steel tubing, E, and the

3-mm polythene diaphragm valve 2 (G.E.C. - A.E.I., Manchester) to the 500-ml mass spectrometer expansion vessel, F, whence the sample can be admitted to the ion source by opening valve 3. Epoxy resin (Araldite AW 106 adhesive with HV 953 U hardener, Ciba Ltd., Basle) is satisfactory for constructing the glass-to-metal seals required.

REAGENTS—

Alkaline lithium hypobromite solution—To 60 ml of cold (0° to 5° C) 10 per cent. w/v solution of analytical-reagent grade lithium hydroxide, $LiOH.H_2O$, add 2 ml of analytical-reagent grade bromine and shake the mixture until the bromine dissolves.

FILLING THE RESERVOIR—

To remove spent hypobromite from the reservoir of the conversion assembly, shown in Fig. 1 (*a*), admit air by opening stopcock S and suck the hypobromite solution out through the central capillary tube W with a water-pump. Roughly evacuate the reservoir with the pump, close S, and dip the end of the capillary in distilled water contained in an evaporating basin. Open S so that water is forced into the reservoir for rinsing. Remove this rinse water and suck up the freshly prepared hypobromite solution by using the same technique. As the hypobromite is forced up into the evacuated space by atmospheric pressure it is effectively de-gassed. Close stopcock S before all of the solution is removed from the evaporating basin, so that no air enters the reservoir.

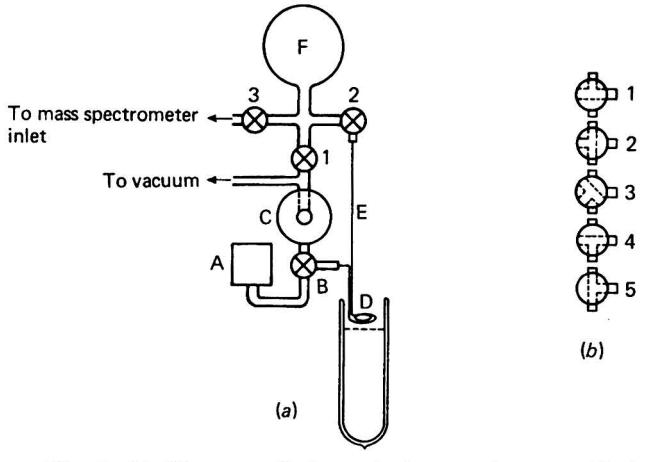

Fig. 2. (*a*) Diagrammatic lay-out of conversion assembly in relation to the mass-spectrometer inlet system. A, conversion assembly as in Fig 1 (*a*); B, 3-way glass vacuum stopcock as in Fig. 1 (*a*); C, dry ice - acetone cold trap; D, stainless-steel helical cold trap; E, stainless-steel tubing; F, expansion vessel; and 1, 2 and 3, inlet valves. (*b*) Positions of the 3-way stopcock B during vacuum manipulations (see text)

Pass helium from a cylinder through the glass assembly shown in Fig. 1 (*b*), and trap 10 ml by closing the stopcocks. Lightly grease the 19/26 cone and attach it to the conversion assembly at U. Invert the conversion assembly, making sure that all of the hypobromite solution runs out of the well of the greaseless stopcock. Evacuate the assembly in this position, for example through the cold trap C in Fig. 2 (*a*). Open the stopcock S and continue to evacuate for 2 minutes, or until the hypobromite solution begins to boil. Shut off the vacuum and open the stopcock on the helium fitting to admit the helium into the reservoir. Close S. Remove the conversion assembly and turn it the right way up, making sure that the well of S contains no trapped bubbles of helium. The assembly is now ready for use.

Whichever method is used to evacuate the assembly in the above procedure, the volume between the assembly and the stopcock to the vacuum system should not be more than 10 to 20 ml, or the final pressure of helium in the reservoir may be too low, in which event nitrogen gas produced in the sample vial may be forced into the reservoir, where it will contaminate the hypobromite.

CONVERSION PROCEDURE—

Between conversions, valve 1 is open while valves 2 and 3 are closed [Fig. 2 (*a*)]; stopcock B is in position 1 [Fig. 2 (*b*)]. The procedure is then as follows.

Attach a sample vial to the conversion assembly, close valve 1 and turn B anti-clockwise to position 2 to evacuate the sample; allow 1 minute for evacuation. During this time, focus the mass spectrometer on the peak at mass 28 so that it can be used as a vacuum gauge, and open valve 3 to check that the expansion vessel F is thoroughly evacuated. When the 1-minute period has elapsed, open valve 1 to check for leaks in the conversion assembly. If there is no appreciable rise in the 28 peak the vacuum is satisfactory. Immerse the sample vial to a depth of 5 to 10 mm in a dry ice - acetone freezing mixture, taking care that no acetone contaminates the grease on the 19/26 socket U. Turn B to position 3 and, by opening S, quickly admit into the vial sufficient hypobromite solution (0·5 to 1·0 ml) to oxidise all of the ammonium sulphate. Immerse the stainless-steel helix D in liquid nitrogen and wait until all of the hypobromite solution is frozen. Then close valve 1 and turn B to position 4. By using valve 2 as a throttle, allow the required amount of nitrogen to leak into F; this will register on the mass spectrometer. Turn B to position 1, remove the liquid nitrogen from D, and remove the dry ice - acetone from the sample vial.

Carry out isotope-ratio analysis of the nitrogen gas. When this is complete, close valve 3 and open valve 1. Remove the conversion assembly and place it upright in a clamp. Insert a 1 mm o.d. polythene tube attached to a water-pump into the capillary tube W as far as the Viton seat. Rinse the outside of the latter tube with distilled water from a plastic wash-bottle; this water will be sucked into the capillary, thus rinsing it. Occasionally, some of the solution will splash out of the vial during oxidation of the ammonium sulphate. If this occurs, rinse the upper part of the socket U also. Carefully withdraw the polythene tube so that no droplets of water remain to block the capillary during evacuation. Replace the conversion assembly and repeat the procedure with the next sample.

The entire procedure, including isotope-ratio analysis, takes about 5 minutes.

RESULTS

Twenty-five determinations on each of a labelled and an unlabelled standard ammonium sulphate salt were carried out over a 6-month period. The two standards were inserted as a pair into batches of samples, the unlabelled standard being analysed directly after the labelled to check for memory effects. The mean enrichments obtained were 0·8908 and −0·0002 atom per cent. excess of nitrogen-15, with standard deviations of 0·0048 and 0·0016 atom per cent. excess of nitrogen-15, respectively. The recommended procedure therefore appears to be satisfactory for routine work.

DISCUSSION

The foregoing procedures call for several comments.

The sample size is restricted to 1 mg of nitrogen or less, so that 0·5 ml of hypobromite solution is adequate for complete oxidation and that the pressure of the nitrogen gas produced does not exceed that in the reservoir. The sample is acidified to prevent possible losses of nitrogen on evaporation. If too much acid is added, however, carbon dioxide (from carbonate in the alkali) and free bromine may be formed during oxidation of the sample. Such contamination can be avoided. Acidification to between pH 3 and 4 appears to be a satisfactory procedure.

We have not had any difficulties with the apparatus described above. The hypobromite does not affect the Viton A adversely, even when in contact with it over long periods. The O-ring seal is completely vacuum-tight, but slight leakage occurs if this seal is not greased, although it is possible to prevent this by using a Clearfit socket that is not ground (Quickfit and Quartz). However, once the socket is greased it needs no further attention.

Procedures given in the literature[1,2] for the preparation of hypobromite solution are unnecessarily complicated; that recommended here takes only a few minutes, and therefore fresh hypobromite solution can be prepared as required. Sodium hydroxide solution (10 per cent. w/v) can be used in place of lithium hydroxide solution if desired, but 0·1 g of potassium iodide per 100 ml of reagent must then be added to suppress evolution of oxygen; with the more stable lithium hypobromite,[5] this precaution is unnecessary. The reagent tends to

deposit small amounts of lithium bromide, but this does not cause difficulty. One millilitre of this solution will oxidise about 5 mg of nitrogen.

The conversion procedure has been described for a preparative unit that forms part of a mass-spectrometer gas-handling system. However, it is not restricted to this arrangement. For example, if the isotope-ratio determinations were to be carried out by another laboratory that required the samples in the form of nitrogen gas, the expansion vessel, F in Fig. 2 (*a*), could be replaced by a Toepler pump and the mass-spectrometer inlet by a Pirani gauge. The procedure would then be almost identical, except that the sample would be pumped into a break-seal tube (see, for example, Beynon[6]) instead of being analysed immediately.

We have found that evacuation of the conversion assembly for 1 minute is adequate unless the sample is low in nitrogen (say less than 0·1 mg). In this instance B should be turned to position 5 for a further 2 to 4 minutes after the initial 1-minute evacuation period, then turned to position 3 as before. Although other vacuum systems may require different evacuation times, it seems likely that desorption of gases from the assembly is the critical factor. Contamination from air dissolved in the hypobromite solution appears to be negligible. However, if the conversion assembly is left for a considerable time, slight diffusion through the greaseless stopcock may occur; the assembly should then be inverted to ensure that fresh solution surrounds the outlet to the capillary. If the upper part of the socket U has been rinsed, the assembly should be evacuated until no water remains, otherwise this water may collect in the steel helix and block it. Small amounts of rinse water remaining in the capillary W are pumped away during the first few seconds of evacuation.

When alkaline hypobromite solution is added to an ammonium salt under vacuum, ammonia will be produced unless there is sufficient hypobromite present to oxidise it before it can escape from the solution. The persistence of ammonia on glassware is well known from memory effects encountered in the distillation of ammonia from samples labelled with nitrogen-15.[2,4] When the procedure described above is carried out without immersing the sample vial in dry ice - acetone, similar memory effects occur because of adsorption of ammonia on the conversion assembly. By freezing the vial, the vapour pressure of the ammonia is reduced to a very small value and no contamination of the conversion assembly occurs. An unlabelled sample analysed immediately following a sample containing 30 atom per cent. of nitrogen-15 shows no detectable (0·003 atom per cent. excess of nitrogen-15) enrichment. After sufficient hypobromite solution has been allowed to enter, the vial can be warmed in a small beaker of water without risk of evolution of ammonia. We have found, however, that about two thirds of the sample nitrogen is evolved when the vial is frozen, and thus unless the sample is low in nitrogen (less than 0·1 mg of nitrogen) it is unnecessary to waste time warming it. The sample vials are cheap enough to discard after use, but they can be washed with 2 per cent. aqueous hydrofluoric acid solution and then in water, to remove contamination, and used again.

When liquid nitrogen traps are used there is a risk of memory effects caused by the adsorption of nitrogen gas on particles of ice in the trap. For example, in one trial unlabelled samples were analysed after samples containing 50 atom per cent. of nitrogen-15; each sample was admitted into a 20-ml liquid nitrogen trap before it was allowed to enter the mass-spectrometer expansion vessel. Enrichments of −0·001 atom per cent. excess of nitrogen-15 were obtained for the unlabelled samples. The trap was then exposed to water vapour, a further 50 atom per cent. sample admitted and pumped away, and an unlabelled sample analysed. This sample showed an enrichment of 0·045 atom per cent. excess of nitrogen-15. A similar trial with a dry ice - acetone trap gave a value of −0·002.

Nitrous oxide is produced during the oxidation of ammonium salts with hypobromite and interferes in the measurement of the mass 30 peak. It is therefore necessary to remove this gas if highly enriched samples are to be analysed (for lower enrichments the 28 and 29 peaks only are used). We have found nitrous oxide difficult to remove from a gas sample unless a very efficient liquid nitrogen trap is used or a considerable time allowed for condensation (about 1 minute for a 10-ml vessel).

These difficulties with memory effects and trapping of nitrous oxide are overcome in the procedure described by using a highly efficient liquid nitrogen trap with a small heat capacity, which is evacuated at room temperature between analyses. The use of dry ice - acetone for the trap C ensures that no memory effects arise here from adsorption of labelled samples, while freezing the sample tube in dry ice - acetone, rather than in liquid nitrogen,

prevents possible losses of small samples (and possible isotopic fractionation) caused by adsorption effects.

In the design of the assembly described, the volume of that part in contact with the gas sample has been kept to a minimum, so that when the nitrogen gas is admitted into the expansion vessel little remains in the assembly. This is particularly important in the analysis of small samples, as a small expansion vessel is necessary to obtain optimum peak heights for analysis. We work with samples containing 0·1 to 1·0 mg of nitrogen when possible; this enables us to use a 500-ml expansion vessel. Evacuation of larger expansion vessels with our pumping system reduces the speed of analysis.

Conclusions

When the procedure described in this paper is used, the mass-spectrometric determination is no longer the most time consuming step in the analysis of chemical and biological samples for nitrogen-15. Preparation of samples as dry ammonium sulphate in small, cheap vials, which serve as the reaction vessels during conversion of the sample into nitrogen gas, enables large numbers of samples to be stored before analysis and eliminates transfer of samples to and from special storage vessels. De-greasing of ground-glass joints on vessels containing the sample and the hypobromite solution during the conversion into nitrogen gas is avoided by using an O-ring seal, and de-gassing of solutions (which occupies much of the time in previous techniques) is eliminated by using air-free hypobromite solution from a reservoir. Air contamination of a 0·5-mg sample is normally less than 0·1 per cent. Results show that the procedure is satisfactory for routine work.

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Rapid Determination of Small Amounts of Sulphate in Cellulose Nitrate by a Water-digestion Procedure

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Oxidative decomposition of cellulose nitrate into a completely water-soluble material is accomplished by an aqueous digestion method that involves boiling it with water under pressure. Sulphate is quantitatively liberated, and most of the nitrate groups are decomposed, thus minimising errors from co-precipitation of the nitrate ions. The digests, which are completely free from inorganic additions, allow rapid sulphate determination by direct titrimetric procedures.

In explosives laboratories it is frequently necessary to determine very small amounts of sulphate present in cellulose nitrate to check the stability of the ester on long storage. Several methods for this have been described, in most of which preliminary decomposition of cellulose nitrate is accomplished by wet digestion with different chemical reagents such as aqua regia,[1] hydrochloric acid,[2] alkali[3] and nitric acid to which small amounts of either sodium chlorate,[4] potassium chlorate,[5,6,7] potassium nitrate[8] or perchloric acid[9] are added. Macorkindale and Lamond[10] recommended the use of perchloric acid, either alone or with nitric acid. The method was described as rapid, and oxidation was completed in about 1 hour.

Combustion with oxygen was attempted earlier by Kullgren[2] on the residue obtained by wet digestion, whereas Touratier[11] has recently recommended the direct combustion in a bomb calorimeter, the sulphate being subsequently determined gravimetrically. Although the latter procedure yields a digest that is free from inorganic additions (almost a "pure" solution[12,13]), in samples of stabilised materials the analysis has to be carried out on combined digests of a number of successive combustions to avoid the risk in igniting large amounts in a single experiment.

The measures for the safe handling of cellulose nitrate favour the use of wet-digestion procedures. However, those cited above do not offer the advantage of working with an almost pure solution, especially at the low levels of sulphate existing in all finished forms of the industrial types of cellulose nitrates (stabilised products), and do not, therefore, allow freedom in the choice of methods of completing the determination.[12]

In this investigation, the oxidative decomposition of cellulose nitrate by boiling it with water under pressure has been attempted. Although this phenomenon was noticeable during a study on the stabilisation of cellulose nitrate,[14] its suitability for use in sulphate determination has not yet been reported. The water-digest treatment described in this paper involves the safe use of different samples (either large or small, wet or dry) and also provides a digest that is completely free from inorganic additions. The method as such seems sufficiently promising for it to be adapted for use with rapid, direct titrimetric procedures, which are successfully used for the determination of micro and sub-micro amounts of sulphate.[15,16,17] Of these, the barium perchlorate - thorin titration method is usually preferred,[12,17] and was therefore adopted.

Method

The oxidative decomposition of cellulose nitrate into a completely water-soluble material was brought about safely by digesting it with water under pressure at a temperature approaching its explosion point (or ignition temperature of about 186° C). Sulphate was quantitatively liberated in the large amount of water used in the digestion, and most of the nitrate groups were decomposed, thus minimising the errors from co-precipitation of the nitrate ions and simplifying the sulphate determination by either gravimetric or titrimetric procedures, or both.

APPARATUS—

A small stationary high pressure autoclave of 1·5-litres capacity, provided with a suitable pressure measuring device, a thermometer tube and a regulating valve, was used in these experiments. The apparatus was fitted with two strong heaters and thermostatic control was maintained by a contact thermometer and a relay. Before each digestion, 100 to 150 ml of distilled water were placed in the stainless-steel autoclave pot. To avoid excessive dilution, sample digestion was carried out in a 400-ml, tall-form beaker that had two small holes near its rim, and was placed in a wider shorter one containing about 10 ml of distilled water, which acted as a trap for any losses that might result from boiling. A loose cup-type cover was placed on the tall beaker, and this extended downwards over the two beakers to the middle of the wider one.

DIGESTION PROCEDURE—

An accurately weighed 4 or 8-g sample of well dried cellulose nitrate was put in the tall beaker with 80 ml of distilled water (the ratio of water to sample was always about 20 to 1). The covered beakers were placed in the autoclave, which was tightly closed and heated. The pressure was kept at between 50 and 60 lb inch^{-2} for about 20 to 30 minutes then rapidly raised to a maximum of 160 to 170 lb inch^{-2} for an additional period of 10 minutes. The time taken to heat it to the maximum pressure was about 10 minutes. The heating was then stopped and the pressure rapidly released. After cooling, the autoclave was opened and the digest solutions filtered (see Note).

Filtered digests of stabilised cellulose nitrates (see Discussion) were passed into an ion-exchange column, as described by Fritz and Yamamura,[15] containing settled Dowex resin (H^+ form) to eliminate cationic interferences in the subsequent titrimetric sulphate determination. After quantitative collection the solution was concentrated to about 90 ml and the digests were finally made up to exactly 100 ml or a multiple. A 25-ml portion of aqueous digestion solution was equivalent to 1 g of sample.

NOTE—

If the resulting colour is not pale yellow (see Discussion) it is advisable to heat either the filtered solution of unstabilised cellulose nitrate, or that resulting from ion-exchange treatment for stabilised material, or both, with 5 to 7 ml of 30 per cent. hydrogen peroxide (micro-analytical-reagent grade) at 70° to 80° C for 10 to 15 minutes, followed by boiling for 5 minutes. The solution is finally made up to volume.

GRAVIMETRIC DETERMINATION OF SULPHATE IN THE WATER-DIGEST SOLUTIONS

A 50-ml solution containing 2 g of sample (or a multiple of this for purified cellulose nitrate) was evaporated almost to dryness in a beaker with 0·3 to 0·5 ml of concentrated hydrochloric acid, and 25 ml of distilled water added. The sulphate was then determined gravimetrically.[18]

TITRIMETRIC DETERMINATION OF SULPHATE IN THE WATER-DIGEST SOLUTIONS

REAGENTS—

Barium perchlorate solution—Anhydrous barium perchlorate (2 g) was dissolved in 200 ml of water and 800 ml of ethanol. The apparent pH was adjusted to about 3·5 with perchloric acid and the solution was standardised against 5-ml portions of 0·005 M sulphuric acid according to the general procedure.

Ethanol—Pure absolute ethanol (or isopropyl alcohol) was used.

Thorin indicator—An aqueous 0·1 per cent. solution.

Methylene blue indicator—An aqueous 0·002 per cent. methylene blue screening solution.

Sulphate-free cellulose nitrate—A sample of sulphate-free cellulose nitrate was prepared by nitrating purified unbleached cotton linters with a mixture of pure nitric and glacial acetic acids (75 + 25 w/w). The ratio of cellulose to acids was 1 : 100 and the time taken for nitration was 15 minutes. The nitrated sample was stabilised by boiling with 2 litres of distilled water for about 10 hours, filtering and then washing it with ethanol. The sample was stored under water until required, when a wet sample was taken and the moisture content determined on an aliquot.

PROCEDURE—

The apparent pH of water-digestion solutions of different samples examined ranged from 2 to 3·5. Aliquots of 5 or 10 ml (and preferably 15 ml for purified cellulose nitrates) were transferred by pipette into 250-ml conical flasks. For every 5-ml portion 40 ml of absolute ethanol were added, and then 2 to 3 drops of thorin indicator and, if desired, 1 drop of methylene blue screen. The solution was titrated with 0·005 M barium perchlorate to the first pink colour, 0·01-ml increments being added near the end-point.

The blank value was found by taking a sample of sulphate-free cellulose nitrate through the whole procedure of digestion and titration. The normal blank value obtained during the work was almost zero. (The blank together with other samples was digested in an autoclave that took up to four samples.)

RESULTS AND DISCUSSION

The water-digestion method was examined over a wide range of temperatures above 150° C with their respective pressures. The oxidative decomposition of different types of cellulose nitrates (Table I) into a completely water-soluble material was instantaneously brought about by heating at a pressure of 250 lb inch^{-2} or higher. One disadvantage of this digestion technique, however, was that it gave brownish solutions. The colour of digests was greatly affected by heating conditions, *e.g.*, digestion at a maximum pressure of 175 lb inch^{-2} for a period of about 30 minutes gave dark yellow solutions. Under such conditions, an additional decolorisation treatment by heating the solution with a few millilitres of hydrogen peroxide was essential before it could be adapted for use in the titrimetric determination of sulphate.

TABLE I

SULPHATE CONTENT IN DIFFERENT TYPES OF CELLULOSE NITRATES

Sample	Nitrogen, per cent.	Sulphate content, per cent.		
		Water-digestion method		Method of Keirstead and Myers[6,7]
		Gravimetric	Titrimetric	
Crude nitrates, gun-cotton type[a1]				
Nitrated cotton	13·14	0·66	0·66	0·65
Nitrated wood pulp cellulose	13·10	0·76	0·76	—
Crude nitrates, low-nitrated type[a2]				
Nitrated cotton	12·13	0·86	0·86	—
Nitrated wood pulp cellulose	12·01	0·99	0·99	0·99
"Industrial"[b] nitrated cotton, gun-cotton type				
Crude nitrated cotton	13·23	0·38	0·38	0·37
			0·38[c]	
Stabilised nitrated cotton	—	0·038	0·037	0·04
			0·039[c]	
"Industrial"[b] nitrated cotton, low-nitrated type				
Crude nitrated cotton	12·22	1·2	1·21	1·20
			1·21[c]	
Stabilised nitrated cotton	—	0·042	0·042	0·045
			0·043[c]	

[a1,2] Pure samples prepared in the laboratory.
[b] Industrial type, nitrated cotton linters cellulose.
[c] Digests subjected to an ion-exchange treatment.

A pale yellow digest or a colourless solution (such as that with unstabilised products) was obtained by heating at a relative pressure of 50 to 60 lb inch^{-2} for 20 to 30 minutes and then at a maximum pressure of 160 to 170 lb inch^{-2} for 10 minutes; the longer the period at a lower temperature the lighter the colour. The use of this technique was favoured as, during the preliminary treatment at the comparatively lower temperature corresponding to 50 to

60 lb inch^{-2}, most of those sulphate esters[19,20,21] that might be present in unstabilised cellulose nitrate could easily be hydrolysed to sulphuric acid, and thus contribute to the accuracy of titrimetric procedures for sulphate determination—

$$RSO_4H + HOH = H_2SO_4 + ROH^{19}$$
$$R_2SO_4 + 2HOH = H_2SO_4 + 2ROH.$$

The considerable decolorisation of solutions noted above could be explained by the fact that, at comparatively low temperatures of digestion, the hydrolysis rather than oxidative de-nitration predominates (the oxidative de-nitration effect during water digestion is almost comparable with that of the merely thermal decomposition of cellulose nitrate[14]). The small amount of nitric acid produced in this way will, in turn, affect the autocatalytic decomposition of the molecules at such low temperatures. This is supported by experiment, whereby heating of a brownish digest with a few drops of concentrated nitric acid effected complete decolorisation; alternatively, adding a few drops of this reagent to water before digestion always gave a colourless solution. Similar observations were noted on the addition of a few millilitres of hydrogen peroxide to the mother liquor.

In the course of these experiments, the aqueous digestion method was carried out without any additions as it was realised that even the pale yellow solutions were suitable for titrimetric sulphate determinations.

Finally, tracing the presence of the nitrate ion in digests by the Schultze - Tiemann method[22] showed that nearly all of the nitrate groups were decomposed into gaseous products, most of which presumably escaped when the pressure was released; residual nitrogen ranged from 0·09 to 0·4 per cent. This minimised the co-precipitative interference of nitrate. Gravimetric sulphate determinations in different digests gave results that were in good agreement with those obtained by the method of Keirstead and Myers[7] (Table I), which is recommended for sulphate determination in cellulose nitrate.[6] The blank values determined on digests of sulphate-free cellulose nitrate were practically nil. On the other hand, the results of blank experiments made according to Keirstead and Myers' method were high because of the presence of sulphate impurities in the potassium chlorate available (pure reagent). To minimise inaccuracies, the mean of three blank values was determined, the corrected sulphate values being shown in Table I. This made the determination tedious.

Gravimetric methods are time consuming and unsuitable for the determination of low concentrations of sulphate such as those present in carefully stabilised cellulose nitrates, and so the barium perchlorate - thorin titration procedure was used. The use of this method on the milligram scale[15] for the determination of sulphate in variable types of unstabilised cellulose nitrates presented no difficulty. Reproducible results that agreed very closely with the gravimetric values were consistently obtained. On the other hand, pronounced

TABLE II

SULPHATE RECOVERY

Sulphate added*		Sulphate found		
mg	per cent.	mg	mean, mg	per cent.
2·4	0·06	2·47 2·43 2·43	2·44	0·06
4·8	0·12	4·86 4·78 4·86	4·83	0·12
9·60	0·24	9·53 9·59 9·65	9·59	0·24
14·40	0·36	14·41 14·36 14·36	14·38	0·36
50·40†	1·26	50·37 50·25 50·25	50·29	1·26

* Added to 4 g of sulphate-free cellulose nitrate.
† Added as 0·05 M H_2SO_4.

differences were noted for stabilised materials. The causes were investigated in view of modifications reported for the microgram adaptation,[12,17] *i.e.*, titration in an 87 to 90 per cent. ethanolic medium. The use of an almost 89 per cent. alcoholic concentration gave reliable results. Because of the presence of comparatively small amounts of sulphate in some types of unstabilised cellulose nitrates (gun-cotton type) and the use of small samples (0·2 to 0·4 g), the microgram method was recommended for all types of cellulose nitrates.

The very small amounts of sodium usually present in highly purified cellulose nitrates were found to be tolerated by the titrimetric procedure (Table I) and the ion-exchange treatment could therefore be omitted. Conversely, in most industrial types containing higher amounts of adulterants such as calcium carbonate, removal of cationic interferences is advisable. A rapid approximation could, however, be obtained by direct use of these digests.

In conclusion, checking the method on sulphate-free cellulose nitrate containing different amounts of sulphate (added as 0·005 M sulphuric acid) showed that the theoretical and the actual amounts agree closely within the limits of experimental error. Moreover, the sulphate content determined experimentally gave excellent agreement with the theoretical value, as shown in Table II.

The above results clearly indicate that this water-digest treatment, providing a digest completely free from inorganic additions, enables minute amounts of sulphate present in cellulose nitrate to be determined rapidly by direct titrimetric procedures. In addition, the method is safe and requires no special precautions.

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A Simple Sample Introduction Device for Use with High Vacuum Apparatus

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A simple sample introduction device is described which enables samples to be readily introduced into a high vacuum system. The device incorporates greaseless vacuum stopcocks, and is especially useful with certain types of vacuum-fusion and vacuum-extraction apparatus used for determining gases in metals.

For the determination of gases in metals by the method of vacuum extraction or vacuum fusion, it is usual with certain types of equipment to load a batch of twenty or more samples into the apparatus before the system is out-gassed. The analysis of these samples, including the preliminary out-gassing, often extends over 2 days and during this time it is not possible to meet requests for urgent analyses, or to repeat a determination (*e.g.*, when the weight originally taken of a sample of unknown gas content is inadequate).

A method of introducing single samples into the high vacuum system after the initial loading and out-gassing has, therefore, obvious advantages, and this has led the authors to develop the device illustrated in the diagram (Fig. 1).

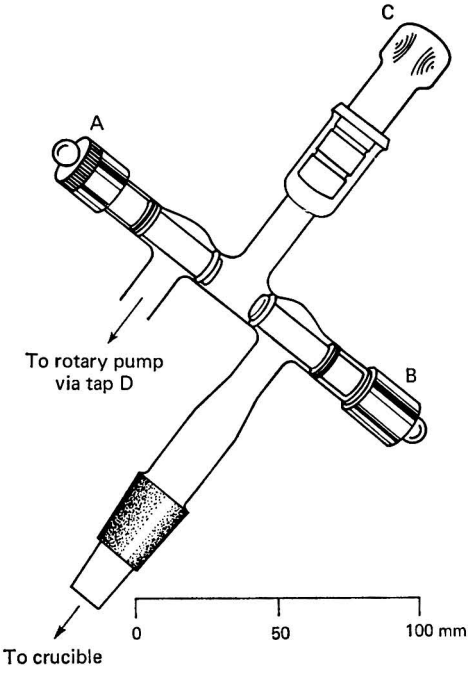

Fig. 1. Sample introduction device

CONSTRUCTION AND OPERATION—

The sample introduction device is constructed of Pyrex glass and incorporates greaseless vacuum stopcocks.* These are each fitted with a screw-cap (anodised light alloy or polypropylene), which enables the glass piston carrying Viton or PTFE O-rings to be moved along the glass barrel, thus closing or opening the stopcock. The plug, C, is fitted with two PTFE O-rings (see Note).

The side-arms on the stopcocks (Fig. 1) have a nominal 10-mm bore, but stopcocks with side-arms of up to 25-mm bore are obtainable*, thus permitting larger samples to be used.

The device is operated as follows.

Close B and open A, then open the two-way high vacuum tap, D, to the atmosphere (this action immediately closes the line to the rotary pump). Remove the plug, C, introduce a sample, then re-insert the plug. Turn tap D to the rotary pump and, after about 2 minutes, close A and partially open B (so that the sample still rests against the end of the piston). When a normal working vacuum has been reached (after about 2 minutes), open B fully to allow the sample to fall into the crucible of the apparatus, then close B.

PERFORMANCE—

This sample introduction device has been used intermittently over a period of about 12 months and has always proved reliable. Except for the need to change an O-ring occasionally, no replacements have been required.

NOTE—

A greaseless vacuum stopcock* can be used instead of the plug C, provided that the side-arm, and the PTFE O-ring at the base of the piston, are removed. To facilitate withdrawal of the stopcock for sample loading, it is preferable to use a relatively shorter piston from a 4-mm bore stopcock in the barrel of one of 10-mm bore.

Modified stopcocks of this type can also be fitted to the ends of the sample-loading arms normally used on vacuum-fusion or vacuum-extraction apparatus, and they are preferable to the usual greased or vacuum-waxed plugs.

* Available from J. Young (Scientific Glassware), 11 Colville Road, Acton, London, W.3.

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Book Reviews

ANALYTICAL METHODS FOR PESTICIDE RESIDUES IN FOODS. Compiled and Edited by H. A. McLEOD, P. J. WALES, R. A. GRAHAM, M. OSADCHUK and N. BLUMAN. Pp. ii + 247. Ottawa: The Queen's Printer. 1969. Price $5.00.

The stated purpose of the manual is "to provide a systematic compilation of methods for determining pesticide residues in foods for human consumption," and in this it is generally successful.

The publication has a loose-leaf format, so, as newer techniques are developed, additions and alterations can be made to maintain the usefulness of the manual. This is important in such a rapidly changing field as pesticide residue analysis, because even a manual such as this, presenting the most recently developed techniques, can rapidly become out of date.

Inevitably one compares this work with the U.S. Food and Drug Administration's "Pesticide Analytical Manual," and the similar U.S. Public Health Service's "Guide to the Analysis of Pesticide Residues." However, the greater part of these deals with the methodology used in the analysis of specific pesticides, whereas this work is orientated towards systematic multi-residue detection determinations, involving broad-screening procedures.

The book is divided into fifteen sections, each dealing with a particular aspect of the procedures involved, such as sampling, sample preparation, liquid - liquid partition systems, determinative procedures and confirmatory chemical reactions. Alternative procedures are given in all sections from which the analyst can choose the one most suitable for his particular substrate.

The procedures are described in such detail that one feels they could be followed by analysts who have little or no experience of residue analysis, although the introduction stresses the need for analytical skill. Great uniformity can be attained by analysts using the manual, because of the great detail with which each step is treated. However, it is felt that some of the material in the publication can be described as superfluous, particularly Fig. 15. 1 (*a*), which is a photograph of a plastic screw-capped bottle. Similarly the several simple chromatograms, Figs. 11.2 (*a*) to 11.2 (*l*), are given as full diagrams, and one feels the number and size could have been reduced with advantage. Each description of apparatus is preceded with the injunction "All glassware must be rinsed with acetone and then with hexane before use." Surely this general instruction need be made only once in the introduction.

The list of references is remarkably small (47) for a manual covering such a wide field as pesticide residue analysis in foodstuffs. There are a great number of published papers that surely would interest anybody involved in such work.

Apart from fulfilling its prime requirement to serve as a guide for pesticide analysts of the Canadian Food and Drugs Directorate, analysts everywhere interested in pesticide residue analysis of foodstuffs will find this manual most useful. Very few printer's errors have been noticed and these are not likely to lead to misunderstanding. J. H. RUZICKA

CHEMISCHE SPEKTRALANALYSE: EINE ANLEITUNG ZUR ERLERNUNG UND AUSFÜHRUNG VON EMISSIONS-SPEKTRALANALYSEN. Volume 1. Sixth Edition. By WALTER ROLLWAGEN. Pp. xii + 185. Berlin, Heidelberg and New York: Springer-Verlag. 1970. Price DM 48; $13.20.

This volume is the sixth edition of a monograph intended as an introduction to practical emission spectrometry. The first three chapters of the book are devoted to an account of instrumentation, its methods of operation and the evaluation of results. An adequate treatment is given to arc and spark techniques, but only a cursory treatment of flame sources is presented. Although a small section of the sixth edition is devoted to direct-reading spectrometers the monograph is principally concerned with photographic methods. Chapters 4 to 6 of the book are concerned with qualitative, semi-quantitative and quantitative analysis, and contain much interesting information of practical value including descriptions of the use of spectrographic methods for the analysis of various materials, *e.g.*, "pure" metals, alloys and pigments. The final chapter presents thumb-nail sketches of several other topics of more specialised nature. These include rapid analysis with the photographic plate or by visual means, low voltage spark techniques, the determination of gases in metals (especially oxygen) and quantitative analysis with the glow-discharge (hollow cathode) source.

The volume contains many excellent illustrations of commercial equipment and typical spectra encountered with the practical samples, the analysis of which is discussed. The book forms a useful introduction to the practical aspects of the subject; it has been deliberately restricted to this rôle by the author. No treatment of the theory of the physical processes involved is given, and it would be necessary for students to use this text as a complementary work to existing text-books. Some recent developments in the technique, *e.g.*, inductively and capacitatively coupled plasma sources, are not covered and little description of more sophisticated arc and spark sources is given. Although it might provide a source of interesting general reading for the reader with some experience in emission spectroscopy, he will gain little new information.

G. F. KIRKBRIGHT

AN INTRODUCTION TO THE CHEMISTRY OF THE ALKALOIDS. By ALEXANDER MCKILLOP, B.Sc., Ph.D. Pp. viii + 212. London: Butterworth & Co. (Publishers) Ltd. 1970. Price 34s.

The name alkaloids formerly immediately provoked the association "Poisons," which, of course, they are. The closer acquaintance of the last quarter of a century has led to a greater appreciation of their more benign and valuable pharmacological and therapeutic properties. Although this book is described as an introduction to the subject, 50 years ago it could not have been written, but the tools that have since been developed have made the work of analysis in the elucidation of constitution a very much more rapid and, as expounded in this volume, a deceptively simple procedure, which, however, demands a deep knowledge of modern techniques and interpretative skills. The approach to the subject is in fact based on these newer chemical and physical techniques as distinct from the classification based on a structural unit as, for example, in the pyridine, isoquinoline and indole alkaloids.

A brief introduction is followed by a chapter on the occurrence, isolation and systems of classification, then by one on methods of structure elucidation, classical and modern, in which general principles rather than practical details are discussed. This is followed by an interesting chapter giving six examples of such elucidation, three by classical degradation methods and three by modern physico-chemical techniques. Next are chapters on the re-arrangements that are prone to occur with or without fragmentation of the molecule during such methods of structure elucidation, and on the synthesis and biosynthesis. This last subject has become of increasing interest in recent years although it was studied as long ago as 1917 by Sir Robert Robinson. The chapters on structure, re-arrangements, synthesis and biosynthesis conclude with problems that tax the reader's knowledge and imagination, but a final section with answers to the problems provides either the pride of satisfaction with one's knowledge or the humbling realisation of one's lack thereof.

Of the approximately 2000 alkaloids of known constitution, the structures of about 150 are given in this Introduction, including 17 examples of chemical synthesis and 27 of biosynthesis.

The reviewer found the book fascinating to read and intellectually stimulating in spite of revealing the inadequacy of his chemical knowledge in this field. The book being designed for advanced undergraduates or for the general information of non-specialists, the text is not complicated with references apart from a few suggestions for further reading where a greater interest is aroused.

J. I. M. JONES

A MODERN LABORATORY COURSE. PHYSICAL CHEMISTRY. Edited by HUGH W. SALZBERG, JACK I. MORROW, STEPHEN R. COHEN and MICHAEL E. GREEN. Pp. xx + 528. New York and London: Academic Press. 1969. Price 89s.

This is an interesting book which is designed for students taking a modern laboratory course in undergraduate physical chemistry. The first half of the text is written to provide the student with the theoretical information necessary to perform the experiments described in the second half of the text. The experiments cover a range of subjects, such as viscometry, X-ray diffraction, activity coefficients, calorimetry, thermal analysis, kinetics (including the stopped-flow method and use of analog computers), mass spectrometry, infrared spectrometry, fluorimetry and polarography.

The theoretical section is presented most clearly and concisely. The experimental section contains generally little detail, although the editors say their instructions are intended to supplement, not supplant, the manufacturers' instruction booklets.

Much of this book is relevant to the training of an analytical chemist. Although there may be dispute both as to how best this training can be accomplished and, more importantly, as to

what is analytical chemistry, there should be no dispute as to the value of education in the philosophy of measurement, which is inherent in physical chemistry. V. J. JENNINGS

PROBLEMS AND SOLUTIONS. PHYSICAL CHEMISTRY. By LEONARD C. LABOWITZ and JOHN S. ARENTS. Pp. x + 524. New York and London: Academic Press. 1969. Price 70s.

The preface to this book states that its purpose is to provide a study aid and a supplementary source of problems for advanced undergraduate and beginning graduate students in physical chemistry.

There are sixteen chapters covering problems in expected subjects such as thermodynamics, electrochemistry, surface chemistry and colloids, kinetics, radiochemistry and quantum chemistry and spectroscopy. With the exception of this last chapter, the problems are divided into three categories of relative difficulty. Comprehensive solutions to these problems are presented clearly and concisely.

This book is valuable because of its relevance to the advanced study of physical chemistry. Some of the problems presented incorporate results from recent and earlier literature. It also presents a view of physical chemistry, not in the light of factual information on measurements but on how this information can be interpreted by the use of mathematical principles. It seems that chemistry is becoming increasingly an abstract as well as an observational science.

V. J. JENNINGS

ENZYMATIC METHODS OF ANALYSIS. International Series of Monographs in Analytical Chemistry. VOLUME 34. By GEORGE G. GUILBAULT. Pp. xvi + 347. Oxford, London, Edinburgh, New York, Toronto, Sydney, Paris and Braunschweig: Pergamon Press. 1970. Price 75s.

With increasing interest in molecular biology and the study of the chemical processes that occur in the cells it has been necessary to find adequate analytical procedure to study the processes involved. It is largely due to this search that enzymatic methods of analysis have been developed. Whereas initially enzyme analyses were mainly concerned with research they are now being used in a wide variety of technical spheres. In clinical biochemistry it is routine to use such procedures for the determination of both normal and abnormal body constituents and in practically every biological discipline these procedures are now being increasingly adopted. The author states that the purpose of this book is to acquaint chemists, biochemists and biologists with the possible use of enzymes as analytical reagents and, in following this idea, the scope of enzyme analysis is outlined. The first two chapters are mainly devoted to consideration of enzymes as analytical tools and the methods for following reactions. Then follow chapters that deal specifically with the assaying of particular enzymes and define procedures in which enzymes are used for the determination of substrates, activators and inhibitors. The book ends with the consideration of the processes involved in the immobilisation of enzymes and the final chapter attempts to show how enzyme analysis can be applied to automation. The chapter on methods of assay is particularly interesting in that it describes chemical and instrumental methods, including manometric, polarimetric and spectrophotometric procedures and also fluorescence methods. Each chapter contains full references, and in the appendix are given the sources of the enzymes that are described in the book and the manufacturing source from which they can be obtained.

R. F. MILTON

LUMINESCENCE OF BIOPOLYMERS AND CELLS. By GRIGORII M. BARENBOIM, ALEKSANDR N. DOMANSKII and KONSTANTIN K. TUROVEROV. Translation Editor: RAYMOND F. CHEN. Pp. viii + 229. New York and London: Plenum Press. 1969. Price $12.50.

This work emanates from three research scientists at the Institute of Psychology, Leningrad Academy of Sciences. It is mainly concerned with the use of luminescence to study biochemical substances, primarily in an attempt to follow the reactions that recur inside a cell. The changing luminescence that occurs with biological enzyme reaction is suggested as a tool that can be used to follow these reactions, and examples are given where the procedure has produced useful results. The final chapters are concerned with apparatus used for bioluminescence measurements, and a very full bibliography on the subject is given. For the chemist not working in this specialised field it is doubtful whether the book can have much appeal. R. F. MILTON

Erratum

FEBRUARY (1970) ISSUE, p. 160, 2nd line. *For* "the range 0·5 to 100 per cent." *read* "the range 0·5 to 150 per cent."

The Rapid Determination of Fat in Cocoa Products by Using a Differential Density Technique

The rapid determination of fat for process control purposes in cocoa powders, extrusion cakes and chocolate liquors has been accomplished by rapid extraction of the fat with tetrachloroethylene, followed by indirect determination of the density of the resulting solution by using a differential density technique. The difference in apparent weight loss, which is indicated by an aperiodic balance, between a plummet immersed in the fat solution and an identical plummet immersed in pure tetrachloroethylene is correlated with the fat content of the sample. The correction factor associated with batch-to-batch variations of solvent density required by previous density methods is eliminated. The method is simple to operate and produces results at 10-minute intervals, although 20 minutes are required to complete each determination.

For cocoa powders, 95 per cent. of the results obtained for the fat content by the differential density technique were within $\pm 0{\cdot}46$ per cent. w/w of those obtained by a Soxhlet extraction method, but for extrusion cakes and chocolate liquors the confidence limits were slightly wider.

A. M. J. PERL, A. D. INCE and P. H. WIGGALL

Research and Development Department, Confectionery Group, Cadbury Schweppes Limited, Bournville, Birmingham.

Analyst, 1970, **95**, 809–816.

A Rapid Procedure for Preparing Gas Samples for Nitrogen-15 Determination

A method, which is faster and more convenient than previous techniques, is described for converting ammonium sulphate into nitrogen gas for isotopic analysis on a mass spectrometer. The dry ammonium sulphate sample is contained in a small screw-topped vial, in which it can be stored pending analysis. This vial can be coupled with a neoprene O-ring to a special assembly, evacuated, and the sample oxidised with air-free hypobromite solution from a reservoir in the assembly. By using this procedure the usual inconvenience of de-greasing ground-glass joints on sample tubes is avoided, the difficulty of storing samples prior to analysis is overcome and the de-gassing of solutions prior to the conversion of each sample into nitrogen gas is eliminated. The procedure is particularly suitable when many hundreds of samples are to be analysed.

P. J. ROSS and A. E. MARTIN

Division of Soils, C.S.I.R.O., Cunningham Laboratory, St. Lucia, Queensland, Australia.

Analyst, 1970, **95**, 817–822.

Rapid Determination of Small Amounts of Sulphate in Cellulose Nitrate by a Water-digestion Procedure

Oxidative decomposition of cellulose nitrate into a completely water-soluble material is accomplished by an aqueous digestion method that involves boiling it with water under pressure. Sulphate is quantitatively liberated, and most of the nitrate groups are decomposed, thus minimising errors from co-precipitation of the nitrate ions. The digests, which are completely free from inorganic additions, allow rapid sulphate determination by direct titrimetric procedures.

ABDEL FATTAH DAWOUD and A. A. GADALLA

National Research Centre, Dokki, Cairo, U.A.R.

Analyst, 1970, **95**, 823–827.

A Simple Sample Introduction Device for Use with High Vacuum Apparatus

A simple sample introduction device is described which enables samples to be readily introduced into a high vacuum system. The device incorporates greaseless vacuum stopcocks, and is especially useful with certain types of vacuum-fusion and vacuum-extraction apparatus used for determining gases in metals.

D. F. WOOD and D. A. SWANN

Research & Development Department, Imperial Metal Industries Ltd., Kynoch Works, Witton, Birmingham 6.

Analyst, 1970, **95**, 828–829.

Notice to Authors

The Editor welcomes papers on all aspects of the theory and practice of analytical chemistry, fundamental and applied, inorganic and organic, including chemical, physical and biological methods. Papers are submitted to referees, who will advise on their suitability for publication.

Intending authors should consult the current Notice to Authors, last published in full in *The Analyst*, 1968, **93**, 269–272, reprints of which can be obtained on application to The Editor, *The Analyst*, 9/10 Savile Row, London, W1X 1AF. All papers submitted will be expected to conform to the recommendations there laid down, and any that do not may be returned for amendment.

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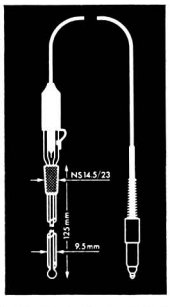

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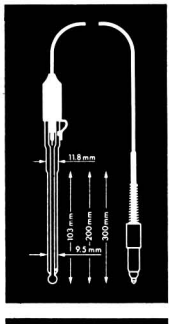

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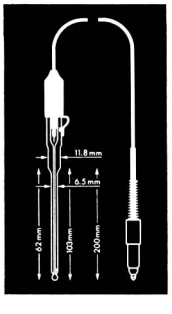

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