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Summaries of Papers in this Issue

A Technique for Determining Matrix Correction Factors and Their Application to the X-ray Fluorescence Analysis of Low-grade Iron Ores over a Wide Range of Compositions

A new approach is described in which synthetic standards are used to determine factors that are required to correct accurately for matrix effects that occur in the X-ray fluorescence analysis of oxide materials when analysed over a wide range of compositions. This technique enables wider ranges to be analysed than hitherto possible using single calibration graphs for each constituent. The application of the correction factors determined by this technique to the X-ray analysis of a wide range of low-grade iron ore field survey samples is described.

H. HUGHES

British Steel Corporation, Research and Development Department, Swinden Laboratories, Moorgate, Rotherham.

Analyst, 1972, **97**, 161–170.

The Use of Elastic Particle Scattering for Preliminary Survey Examinations in Positive-ion Microprobe Analysis

The intensity of particles scattered elastically from a sample during bombardment with positive ions provides a useful complement to existing positive-ion microprobe techniques. This new approach can be used to give a rapid preliminary indication of gross elemental distributions, which is particularly valuable for identifying areas that require more detailed examination.

T. B. PIERCE, P. F. PECK and D. R. A. CUFF

Analytical Sciences Division, Atomic Energy Research Establishment, Harwell, Didcot, Berkshire.

Analyst, 1972, **97**, 171–173.

The Spectrofluorimetric Determination of Orthophosphate as Quinine Molybdophosphate

Between 0·02 and 1·2 μg of phosphorus is determined as orthophosphate via the formation of a complex between a molybdophosphate and the alkaloid quinine. Quinine molybdophosphate is precipitated in 0·5 M sulphuric acid, the excess of quinine reagent is removed by washing the precipitate with 0·5 M sulphuric acid, and the complex is dissolved in the solvent mixture acetone - 0·5 M sulphuric acid (9 + 1 v/v). The fluorescence intensity of this solution is measured at 445 nm with excitation at 352 nm. Optimum conditions for the determination have been established, and the effects of 100-fold weight excesses of each of thirty-eight foreign ions have been examined. The determination is selective; large amounts of silicate do not interfere, and arsenic(III) and tungsten(VI) are tolerated at 20 and 50-fold weight excesses, respectively. The nature of the quinine molybdophosphate complex has been examined.

G. F. KIRKBRIGHT, R. NARAYANASWAMY and T. S. WEST

Chemistry Department, Imperial College, London, S.W.7.

Analyst, 1972, **97**, 174–181.

The Direct Determination of Calcium in Zirconium and Zircaloy-2 by Flame-emission Spectrophotometry with the Nitrous Oxide - Acetylene Flame

A method is proposed for the direct determination of calcium impurities in nuclear-grade zirconium and Zircaloy-2 by flame-emission spectrophotometry with the nitrous oxide - acetylene flame. Because chemical manipulations are limited to the dissolution of the sample, the method is very simple and rapid, and it gives a precision that compares favourably with that of other available methods. By the method described, 6 to 20 p.p.m. of calcium in zirconium and Zircaloy-2 have been determined with standard deviations ranging from 0·8 to 1·8 p.p.m.

The spectral interference from zirconium, the choice of instrumental conditions and the procedures for zirconium background correction are discussed.

G. GHERSINI

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N. OMENETTO and P. BENETTI

Istituto di Chimica Generale ed Inorganica, Università di Pavia, Pavia, Italy.

Analyst, 1972, **97**, 182–188.

The Use of a Mixed-solvent System for the Determination of Calcium and Zinc in Petroleum Products by Atomic-absorption Spectroscopy

A mixed-solvent system that permits the use of inorganic compounds as standards has been applied to the determination of calcium and zinc in unused lubricating oils and automatic transmission fluids by atomic-absorption spectroscopy. By the incorporation of hydrochloric acid into the solvent system, it has been found possible to eliminate the systematic errors that may occur when an air - acetylene flame is used in the determination of calcium.

Results have been obtained for a wide range of unused lubricating oils and automatic transmission fluids and there is good agreement with those obtained by X-ray fluorescence and established Institute of Petroleum chemical procedures (I.P. 111/49T Method B and I.P. 117/66T Method B).

S. T. HOLDING and P. H. D. MATTHEWS

Shell Research Ltd., Thornton Research Centre, P.O. Box No. 1, Chester, CH1 3SH.

Analyst, 1972, **97**, 189–194.

Silver Contamination from an Electric Furnace

Silver contamination encountered in a synthetic base, which is used in the preparation of standards for the spectrographic analysis of rocks and soils, was successfully traced to the silver thermal fuse of an electric furnace used during the sintering process. The degree of contamination is related to temperature.

P. J. PARLE and G. A. FLEMING

An Foras Talúntais (The Agricultural Institute), Johnstown Castle Research Centre, Wexford, Republic of Ireland.

Analyst, 1972, **97**, 195–197.

MARCH, 1972 Vol. 97, No. 1152

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A Technique for Determining Matrix Correction Factors and Their Application to the X-ray Fluorescence Analysis of Low-grade Iron Ores over a Wide Range of Compositions

BY H. HUGHES*

(British Steel Corporation, Research and Development Department, Swinden Laboratories, Moorgate, Rotherham)

A new approach is described in which synthetic standards are used to determine factors that are required to correct accurately for matrix effects that occur in the X-ray fluorescence analysis of oxide materials when analysed over a wide range of compositions. This technique enables wider ranges to be analysed than hitherto possible using single calibration graphs for each constituent. The application of the correction factors determined by this technique to the X-ray analysis of a wide range of low-grade iron ore field survey samples is described.

IT is well established that inter-element effects occur in X-ray fluorescence analysis as in most forms of physical methods of analysis and, when correction factors are not known, analyses have to be limited to "narrow-range" calibration graphs derived from samples that are similar to the material to be analysed or a dilution technique has to be used with consequent loss of sensitivity.

Considerable efforts have therefore been directed towards the determination of factors to correct by both empirical and theoretical methods for inter-element effects, especially for metal alloys. Claisse[1] developed a fundamental equation relating measured X-ray intensity to mass concentration in a binary AB mixture, and Criss and Birks[2] used this type of equation to obtain correction factors. They applied regression analyses to X-ray results obtained from standard samples. Lachance[3] and Traill and Lachance[4,5] have developed Claisse's original equation and determined correction factors both from alloy standards and mass-absorption values. With oxide materials, Holland[6] has used a mass-absorption correction procedure for silicate analysis, again by using a mathematical model and an iteration procedure. Johnson[7,8] has also reported work on correction procedures for the analysis of steel-making slags.

In order to exploit more fully the potential of X-ray fluorescence when applied to a variety of oxide materials involved in the production of iron and steel, this paper describes a practical method that was developed to determine correction factors by using synthetic standards. These factors have in turn been successfully used to establish a correction procedure for the analysis of field survey samples obtained from Lincolnshire and Northamptonshire low-grade iron ore fields that vary over a wide range of compositions.

DETERMINATION OF CORRECTION FACTORS

METHOD—

The basic equation used was a modified form of Lachance and Traill's relationship[3–5] between the X-ray fluorescence intensity for a constituent (an oxide in the present work, although the characteristic X-ray line of the metal was measured), the concentration of that constituent in the sample and the concentration of other constituents present in the sample. The modification was necessary in the present work as the reference sample used was not the pure oxide.

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The modified relationship states that

$$R_A = \frac{\beta_A C_A}{1 + \alpha_{AB}C_B + \alpha_{AC}C_C + \ldots} \quad .. \quad .. \quad .. \quad (1)$$

where R_A is the ratio of the X-ray intensity for oxide A in the sample to the intensity for the same oxide in a reference sample. The ratio is corrected for line overlap, if present, counter dead-time losses and background intensity; β_A is a constant depending on the reference sample used; C_A is the concentration of the oxide A being determined (expressed as a proportion of 1); C_B and C_C . . . etc. are the concentrations of other oxides present in the sample (again expressed as proportions and $C_A + C_B + C_C \ldots = 1$); and α_{AB}, α_{AC} . . . etc. are the interference factors of oxides B, C . . . etc. on the measured X-ray intensity from oxide A.

The basic equation neglects possible second-order terms, *i.e.*, it is assumed that the effect of constituent B on A is not modified by the introduction of a third constituent, C.

Johnson[9] has described a method for determining α-factors for interference effects in steels and the adaptation of the method for oxides by using a fusion procedure is described.

Equation (1) may be re-arranged to give

$$\frac{C_A}{R_A} = \frac{(1 + \alpha_{AB})}{\beta_A} - \frac{\alpha_{AB}}{\beta_A} \cdot C_A \quad .. \quad .. \quad .. \quad .. \quad (2)$$

for binary mixtures of A and B ($C_A + C_B = 1$) and

$$\frac{C_A}{R_A} = \frac{(1 + \alpha_{AB})}{\beta_A} - \frac{\alpha_{AB}}{\beta_A} \cdot C_A + \frac{(\alpha_{AC} - \alpha_{AB})}{\beta_A} \cdot C_C \quad .. \quad .. \quad (3)$$

for ternary mixtures of A, B and C ($C_A + C_B + C_C = 1$).

By determining R_A for a series of binary mixtures of A in B (with known values of C_A), the validity of the basic relationship can be tested in its form in equation (2). A graph of $\frac{C_A}{R_A}$ (ordinate) against C_A (abscissa) should give a straight line with slope (m) given by $-\frac{\alpha_{AB}}{\beta_A}$ and intercept (c) given by $\frac{(1 + \alpha_{AB})}{\beta_A}$.

In addition, the values of β_A and, more important, α_{AB} can be calculated if the basic equation applies.

Further, for a certain concentration, C_A, of A in a ternary mixture of A, B and C (containing C_C of C) the value of $\frac{C_A}{R_A}$ is given by equation (3) and may be compared with the value of $\frac{C_A}{R_A}$ for the same concentration, C_A, of A in a binary mixture of A and B given by equation (2). The difference is given by

$$\Delta\left[\frac{C_A}{R_A}\right] = \left[\frac{C_A}{R_A}\right]_T - \left[\frac{C_A}{R_A}\right]_B = \frac{(\alpha_{AC} - \alpha_{AB})}{\beta_A} \cdot C_C \quad .. \quad .. \quad .. \quad (4)$$

Hence the preparation of suitable ternary standards in addition to binary standards enables further tests of the basic equation to be made by plotting $\Delta\left[\frac{C_A}{R_A}\right]$ (ordinate) against C_C (abscissa). A straight line passing through the origin should be obtained and the slope, $\frac{(\alpha_{AC} - \alpha_{AB})}{\beta_A}$, facilitates the determination of α_{AC}, the interference factor of C on A.

With oxide materials, suitable binary and ternary standard samples can be prepared by a synthetic approach by fusing pure oxides in a borate matrix. Then A and C become the various oxides of interest and in the work described are re-designated F [iron(III) oxide], C (calcium oxide), S (silicon dioxide), P [phosphorus(V) oxide], etc., and B is always the fusion medium (lithium tetraborate in the present work and re-designated L).

Taking iron(III) oxide (F) as an example of the oxide of interest (A), if a series of samples with various concentrations of this oxide fused with lithium tetraborate (L) is prepared and the X-ray intensities (R_F) are measured, β_F and α_{FL}, the interference factor of lithium tetraborate on iron(III) oxide, can be determined.

The interference factors of other oxides, such as calcium oxide, silicon dioxide and phosphorus (V) oxide, on iron(III) oxide (α_{FC}, α_{FS}, α_{FP}, etc.) can then be determined by preparing ternary borate - iron(III) oxide - calcium oxide, borate - iron(III) oxide - silicon dioxide, borate - iron(III) oxide - phosphorus(V) oxide specimens, etc., and applying relationships of the form shown in equation (4), *e.g.*,

$$\Delta\left(\frac{C_F}{R_F}\right) = \frac{(\alpha_{FC} - \alpha_{FL})}{\beta_F} \cdot C_C$$

In the same way, the interference factors of lithium tetraborate and other oxides on calcium oxide (α_{CL}, α_{CF}, α_{CS}, α_{CP}, etc.) can be determined by preparing binary borate - calcium oxide and ternary borate - calcium oxide - iron(III) oxide, borate - calcium oxide - silicon dioxide, borate - calcium oxide - phosphorus(V) oxide standards, etc.

For brevity, only the determination of the factors for the influence of oxides on iron(III) oxide will be described as being typical of the method developed, although up to the present time the mutual interferences of lithium tetraborate, iron(III) oxide, manganese(II) oxide, calcium oxide, phosphorus(V) oxide, silicon dioxide, aluminium oxide and magnesium oxide have been determined.

PROCEDURE—

Synthetic samples were prepared by using lithium tetraborate, iron(III) oxide (>99·995 per cent. pure), trimanganese tetroxide (99·995 per cent. pure), AnalaR calcium carbonate, AnalaR diammonium hydrogen orthophosphate, silicon dioxide (99·9999 per cent. pure), aluminium oxide (99·99 per cent. pure) and AnalaR magnesium oxide.

The correct proportions to make 20 g of sample were weighed into crucibles (platinum 80 per cent., rhodium 20 per cent.) and fused for 15 minutes at 1100 °C. The glasses formed on quenching were removed and ground for 1 minute in a Tema mill with a tungsten carbide pot. Suitable specimens for X-ray measurements were prepared by compressing the powders into pellets, 38 mm in diameter, in lead rings at a total pressure of 20 320 kg (50 tons).

A 1-kW Philips PW 1540 X-ray spectrometer was used for the X-ray measurements and a ratio technique was also used.

RESULTS—

Fig. 1 illustrates the linear relationship obtained between $\left[\frac{C_F}{R_F}\right]$ and C_F for the binary specimens of iron(III) oxide. The values of both β_F and α_{FL} calculated from the slope and intercept are shown in detail in Fig. 1. The 95 per cent. confidence limit for iron(III) oxide calculated from counting statistics only is 0·2 per cent.

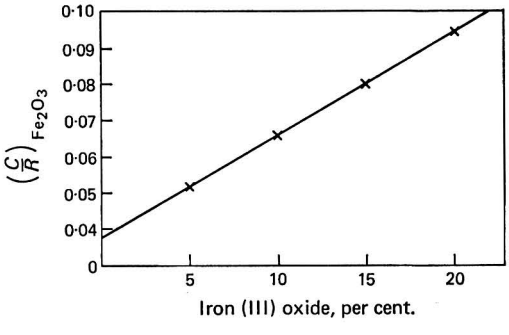

Fig. 1. $\left[\frac{C}{R}\right]_{Fe_2O_3}$ plotted against concentration for iron(III) oxide in borate - iron(III) oxide mixtures: $\frac{1 + \alpha_{FL}}{\beta} = 0{\cdot}0377$; $\frac{\alpha_{FL}}{\beta} = -0{\cdot}283$; $\beta = 3{\cdot}118$; and $\alpha_{FL} = -0{\cdot}88$

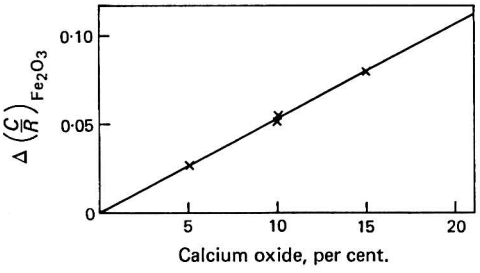

Fig. 2. Changes in $\left[\frac{C}{R}\right]_{Fe_2O_3}$ plotted against concentration of calcium oxide in borate - iron(III) oxide - calcium oxide mixtures: $\frac{\alpha_{FC} - \alpha_{FL}}{\beta} = 0{\cdot}5273$; $\frac{\alpha_{FC}}{\beta} = 0{\cdot}2443$; and $\alpha_{FC} = +0{\cdot}76$

Fig. 2 illustrates the linear relationship between changes in $\left[\frac{C_F}{R_F}\right]$ (for the same value of C_F) and the concentration of calcium oxide in ternary specimens. The 95 per cent. confidence limit calculated from counting statistics only is 0·28 per cent. The agreement with the predicted behaviour allows the determination of α_{FC} and this is shown in detail in Fig. 2. A similar procedure was followed with manganese(II) oxide, phosphorus(V) oxide, aluminium oxide, silicon dioxide and magnesium oxide but the results are omitted. The interference (α) factors of the various oxides on iron(III) oxide are given below.

Interfering oxide	$Li_2B_4O_7$	MgO	Al_2O_3	SiO_2	P_2O_5	CaO	MnO
α-Factor	−0·88 (α_{FL})	−0·58 (α_{FMg})	−0·55 (α_{FA})	−0·50 (α_{FS})	−0·46 (α_{FP})	+0·76 (α_{FC})	−0·03 (α_{FMn})

Other factors similarly determined for other oxides will be used in the second section of the paper.

DISCUSSION OF RESULTS—

The validity of Lachance and Traill's modification of Claisse's relationship has been confirmed as the linear relationships predicted by theory have been obtained in practice over the range examined. Second-order terms that were ignored by Lachance and Traill have, in fact, been shown to be negligible.

Reference to the original papers[3–5] shows that α_{AB} can be expressed in terms of the absorption of the primary and secondary (fluorescent) beam by A and B.

Factors could, of course, be calculated from mass-absorption data but for the difficulty in assessing the correct value to use for the mass-absorption coefficients of A and B for the primary beam. This problem arises because the primary beam of interest is not limited to a fixed wavelength. However, in general, it is possible to compare experimentally determined α-factors with mass-absorption values.

The α-factors for the effects of other oxides on iron(III) oxide have been plotted against the atomic number of the metal in the oxide in Fig. 3, and it can be seen that the values of the α-factors obtained are sensibly related to mass-absorption values.

Lithium tetraborate is a very light matrix giving an α_{FL} value of −0·88 (approaching the minimum value of −1·0, the α-factor for a hypothetical matrix with zero absorption); α-values then increase with the atomic number of the metal in the oxide and become significantly positive as this metal approaches the absorption edge for iron. At the absorption edge there is a discontinuous change to a lower α-value.

The work described thus far has shown that Lachance and Traill's equation can be used to relate X-ray fluorescence intensity with the concentration of the constituent determined and the concentration of other constituents present, and that the method developed to determine factors to correct for inter-element effects has produced values that are sensibly related to mass-absorption values.

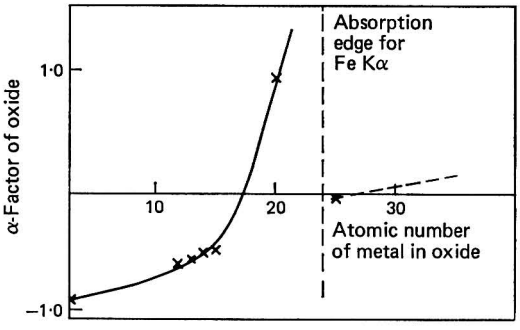

Fig. 3. Variation of α-factors for iron(III) oxide with atomic number of metal in interfering oxide

X-RAY ANALYSIS OF LOW-GRADE IRON ORES

METHOD—

A fusion technique for sample preparation was adopted as it is well established that difficulties arise because of heterogeneity effects in "mineral"-type samples such as those under investigation. These difficulties are virtually impossible to overcome without carrying out a fusion before making X-ray measurements.

It had previously been established in the author's laboratory that a standard dilution ratio of lithium tetraborate to sample of 5:1 is the most satisfactory compromise for the fusion of the wide variety of oxide materials involved in the production of iron and steel, and this ratio was used in the present work.

Lachance and Traill's basic equation relating X-ray ratio to concentration can be shortened to $R = \frac{\beta C_i}{\mu}$, where μ (which is equal to $1 + \Sigma\alpha_{ij}C_j$) is representative of the absorption of the matrix for the X-radiation measured in the determination of a particular oxide.

With the correction procedure this term can be calculated for the actual samples examined (μ_E) by using the α-factors described in the first section of the paper, and also a similar term (μ_{SM}) can be calculated for a defined (hypothetical) standard matrix. Then, if R_E is the experimental X-ray ratio—

$$R_E = \frac{\beta C}{\mu_E} \text{ and } R_{SM} = \frac{\beta C}{\mu_{SM}}$$

$$\text{and therefore } R_{SM} = \frac{R_E \mu_E}{\mu_{SM}}$$

R_{SM} is the corrected X-ray ratio and is the ratio that would have been obtained if the same oxide content had been measured in the hypothetical "average" matrix, rather than in the real matrix.

There are two other important points to be observed in the correction procedure—

(*a*) Apart from the six constituents for which factors have been determined, the other major constituents in the low-grade type of iron ore mined in Lincolnshire and Northamptonshire are carbon dioxide and water, which are present in the sample when weighed for analysis, but are evolved during the fusion process. The total percentage content of the volatile constituents is approximately 100 — total of six major oxides. This is treated as a constituent, allowance being made in the correction procedure by assuming this content to be lost during the fusion process and, therefore, to have zero absorption (α-factor — 1·0) irrespective of the oxide determined.

(*b*) The only element of doubtful valency in iron ores is iron. Whatever its original form, it is converted into iron(III) oxide during the fusion process and, consequently, α-factors based on iron(III) oxide are realistic, even though the analytical results obtained will relate strictly to iron and give no indication of the actual oxide present.

The procedure is described in detail in the Appendix.

PROCEDURE—

Twenty-four samples from the Lincolnshire and Northamptonshire ore mines were used as calibration standards. They were standardised in a co-operative programme of work by four laboratories within the British Steel Corporation. The values for iron, calcium oxide, silicon dioxide and aluminium oxide are the mean of determinations made by the four laboratories. The values for manganese(II) oxide are the mean of duplicate determinations by one laboratory only and the magnesium oxide values are also those determined by one laboratory by using an atomic-absorption technique.

Seven other samples, two B.C.S. iron ore standards and five samples standardised at the Appleby-Frodingham Works of the British Steel Corporation, were also used in the present work.

Samples were dried for 1 hour at 110 °C and then mixed with anhydrous lithium tetraborate in crucibles (platinum 80 per cent., rhodium 20 per cent.) in the proportions of 15 g of borate to 3 g of sample prior to fusion for 15 minutes at 1100 °C. Specimens for X-ray examination were prepared as for the synthetic samples described in the first section.

TABLE I

EXPERIMENTAL SETTINGS FOR X-RAY MEASUREMENTS

Oxide	Tube	kV	mA	Crystal	Collimator	Counts s^{-1} on reference	Counting time, s, on reference
Fe_2O_3	Au	15	6	LiF	Fine	3500	128
MnO	Au	44	20	LiF	Fine	1150	128
CaO	Cr	22	8	LiF	Fine	2000	128
SiO_2	Cr	44	20	PE	Coarse	1150	128
Al_2O_3	Cr	44	20	PE	Coarse	300	128
*MgO	Cr	50	40	ADP	Coarse	50	184

* Examined on PW1212; background measurements also made on each sample at 132·0° 2θ (count-rate approximately 20 counts s^{-1}).

A ratio technique was used for X-ray measurements with ore No. 3 from the calibration series as the reference. Table I gives in detail the experimental conditions used in the X-ray measurements. Iron, manganese(II) oxide, calcium oxide, silicon dioxide and aluminium oxide ratios were determined on the manual PW 1540 instrument, but the magnesium oxide values were obtained on a PW 1212 instrument, with the kind co-operation of the Applications Laboratory of Pye Unicam Limited, as the PW 1540 instrument does not have sufficient sensitivity to measure magnesium adequately at the levels involved. In addition, in this last instance, a background measurement was also made on each sample at a suitable point adjacent to the magnesium peak to correct for significant background changes that occur from sample to sample, depending on the amount of calcium present in the sample.

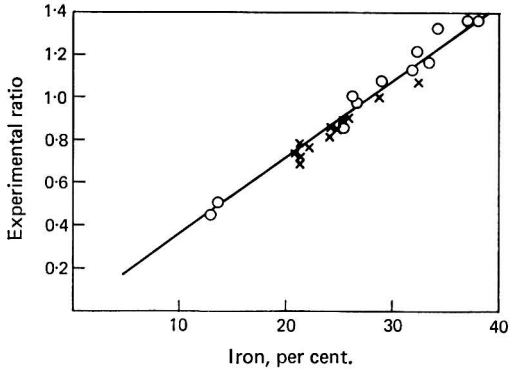

Fig. 4. Experimental ratios for iron (corrected for dead-time losses) plotted against concentration: ×, Lincolnshire ore; and O, Northamptonshire ore

RESULTS—

Experimental ratios were obtained for iron, manganese(II) oxide, calcium oxide, silicon dioxide, aluminium oxide (all after correction for dead-time losses) and magnesium oxide (after correction for dead-time losses and special background correction) and have been plotted against chemical analyses for the twenty-four calibration standards in Figs. 4 and 5 for iron and silicon dioxide, respectively, with 95 per cent. confidence limits, calculated from counting statistics only, of 0·2 and 0·5 per cent., respectively.

These uncorrected calibration graphs show considerable scatter of points and illustrate clearly the effect of the wide variation in composition of the matrices on X-ray ratios.

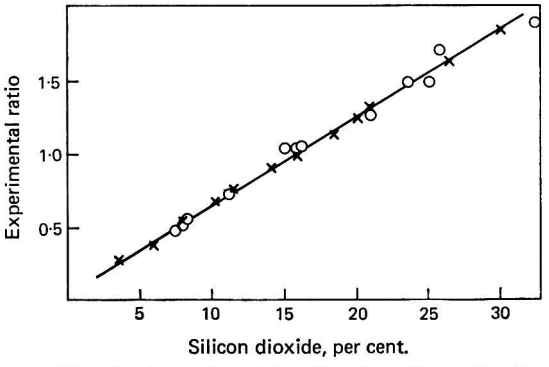

Fig. 5. Experimental ratios for silicon dioxide (corrected for dead-time losses) plotted against concentration: ×, Lincolnshire ore; and O, Northamptonshire ore

All X-ray ratios were corrected for inter-element effects by the procedure already referred to briefly, but described in more detail in the Appendix, and the calibration graphs after matrix corrections are illustrated in Figs. 6, 7, 8, 9, 10 and 11, with 95 per cent. confidence limits, calculated from counting statistics only, of 0·2, 0·5, 0·5, 0·3, 0·8 and 7 per cent., respectively. Figs. 6 and 7 clearly show the improvement in the graphs for iron and silicon dioxide, respectively.

The seven samples referred to were then analysed, by using the corrected calibration graphs. Table II summarises the values obtained and the differences between X-ray and chemical values.

DISCUSSION OF RESULTS—

Uncorrected calibration graphs illustrate that a correction procedure is essential to convert the inherent precision of the X-ray method into accurate analysis because of the wide range of compositions in the samples examined. The correction procedure, developed by using factors determined from synthetic standards, has been rigorously tested and has produced excellent calibration graphs (as illustrated in Figs. 6, 9, 7 and 8) for the twenty-four calibration standards for the important constituents iron, calcium oxide and silicon dioxide, and also for manganese(II) oxide. The scatter of points is within 0·5 per cent. of the content. The procedure has also produced improved calibration graphs for aluminium oxide and magnesium oxide (Figs. 10 and 11), although the improvement is not as good as for the other four constituents because of the lower confidence limit of the X-ray measurement and because the standardised figures for aluminium oxide and magnesium oxide may not be as reliable. A further small refinement to the correction would be possible if the mutual interference factors for the six oxides determined and the minor constituents titanium(IV) oxide, sulphur and sodium and potassium oxides were known.

Further confirmation of the performance of the correction procedure has been obtained by the analysis of other standardised samples, when very good agreement was obtained between X-ray and chemical values, as shown in detail in Table II.

The advantage of the present method is considered to be that the correction factors are determined on accurately prepared synthetic standards and are independent of chemically

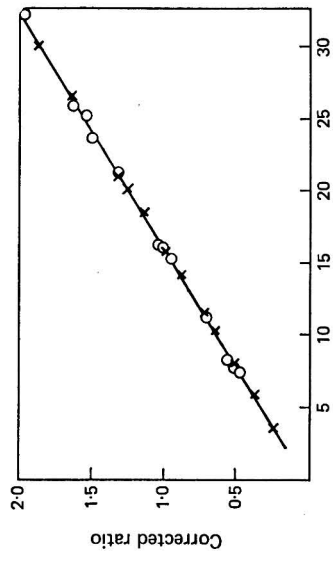

Fig. 7. Ratios for silicon dioxide (corrected for matrix effects) plotted against concentration: ×, Lincolnshire ore; and O, Northamptonshire ore

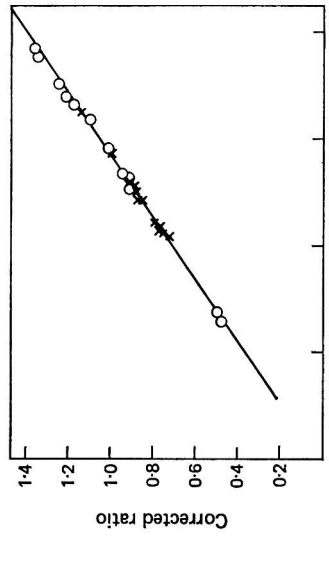

Fig. 6. Ratios for iron (corrected for matrix effects) plotted against concentration: ×, Lincolnshire ore; and O, Northamptonshire ore

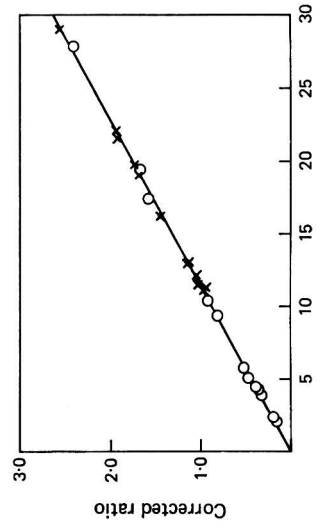

Fig. 9. Ratios for calcium oxide (corrected for matrix effects) plotted against concentration; ×, Lincolnshire ore; and O, Northamptonshire ore

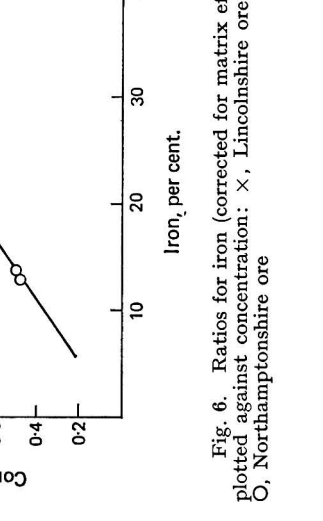

Fig. 8. Ratios for manganese(II) oxide (corrected for matrix effects) plotted against concentration: ×, Lincolnshire ore; and O, Northamptonshire ore

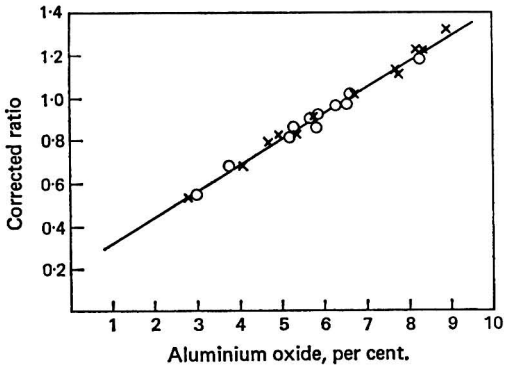

Fig. 10. Ratios for aluminium oxide (corrected for matrix effects) plotted against concentration: ×, Lincolnshire ore; and O, Northamptonshire ore

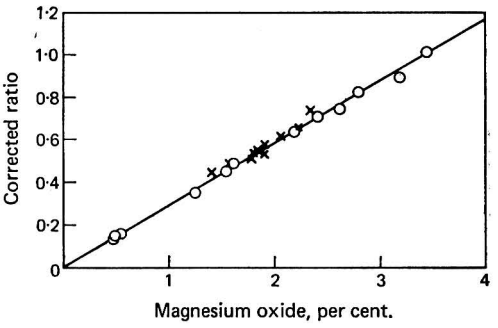

Fig. 11. Ratios for magnesium oxide (corrected for matrix effects) plotted against concentration: ×, Lincolnshire ore; and O, Northamptonshire ore

standardised samples. If the accuracy of standardised values is in doubt, then meaningless factors can be obtained by regression analysis, which, while apparently correcting matrix effects in the samples from which the results are obtained, are not as efficient in correcting results from other samples.

It is obvious that the correction procedure is complex and the use of a computer is essential. Once programmes have been prepared, however, few problems would arise. This approach fits in with the modern idea that sophisticated physical instruments should be directly linked to computers.

The throughput of samples with this technique is naturally of considerable interest. Use of a computerised multi-channel instrument should enable an analysis to be completed in less than 1 minute with satisfactory precision and, with automated sample preparation, a total analysis time of 10 minutes from receipt of sample should be possible. This time-scale for oxide analysis may well be required for production control of iron and steel-making plants in the not very distant future.

Further developments should aim at reducing the types analysed to the minimum number possible (with their own calibration graphs). For example, iron ores, sinters and

TABLE II

X-RAY ANALYSIS OF IRON ORE SAMPLES AND THE DIFFERENCE FROM RESULTS OF CHEMICAL DETERMINATIONS

	Fe, per cent.		MnO, per cent.		CaO, per cent.	
Ore	X-ray value	Difference	X-ray value	Difference	X-ray value	Difference
B.C.S. 301	25·0	+0·3	1·31	—	22·45	+0·45
B.C.S. 302	35·5	—	0·22	+0·01	3·10	−0·20
Colsterworth 2	34·2	+0·2	0·20	+0·02	4·20	+0·10
Exton Park	34·4	−0·1	0·23	+0·02	4·90	—
Cottesmore North	37·35	−0·0$_5$	0·28	+0·04	2·00	−0·30
Nassington	32·1	+0·2	0·19	+0·03	8·55	+0·15
B.I.S.R.A. Northants ..	34·1	+0·3	0·25	—	10·40	+0·29

	SiO_2, per cent.		Al_2O_3, per cent.		MgO, per cent.	
Ore	X-ray value	Difference	X-ray value	Difference	X-ray value	Difference
B.C.S. 301	7·05	−0·15	4·40	+0·14	1·75	−0·12
B.C.S. 302	20·50	+0·50	7·65	+0·41	0·93	−0·14
Colsterworth 2	15·80	−0·18	6·50	+0·10	2·23	−0·31
Exton Park	21·25	—	7·00	−0·05	0·70	−0·23
Cottesmore North	21·00	−0·12	7·95	+0·40	0·42	−0·17
Nassington	8·80	−0·36	5·40	+0·10	1·07	−0·32
B.I.S.R.A. Northants ..	8·00	−0·36	5·35	+0·10	2·04	+0·15

sinter mixes would be treated as one type and all slags, from various iron and steel-making processes, as another. Such an approach would reduce the number of standards required and increase the efficiency of the X-ray technique by reducing to a minimum the ratio of the number of calibration tests to analysis tests.

The author thanks Dr. F. H. Saniter, O.B.E., Director of Research, for permission to publish this paper. He also thanks Mr. M. Crookes for his meticulous practical work in preparing the samples.

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Appendix

Correction of experimental ratios to compensate for matrix variations

It is required to calculate a corrected X-ray ratio (R_{SM}) for an oxide constituent in a diluted sample (1 + 5) from the relationship

$$R_{SM} = \frac{R_E \mu_E}{\mu_{SM}}$$

where R_E is the experimental X-ray ratio, μ_E represents the absorption by the diluted (experimental) sample of the fluorescent radiation measured to determine the oxide and μ_{SM} represents the absorption in a theoretical matrix whose composition is defined.

The calculation of μ_E is straightforward from the α-factors concerned and the composition of the (fused) diluted sample for standards of known composition. For example, in the determination of iron—

$$\mu_E = 1 + \alpha_{FL} C_L + \sum \frac{\alpha_{FX} C_X}{6}$$

where C_L is the lithium tetraborate concentration in the sample, which is equal to 5/6 or 0·8333, and $\frac{C_X}{6}$ is the concentration of oxide X in the sample after the 1 + 5 dilution. ("D", the difference in the total from 100 per cent., is treated as a constituent.)

Before calculating μ_{SM}, it is necessary to define the composition of the standard matrix. It will be noted that some of the α-factors for a particular oxide are positive and some negative. A matrix can therefore be defined for each constituent when the proportions of the other constituents are such that $\sum \frac{\alpha_{ij} C_j}{6} = 0$. These matrices will be different for each constituent, but their compositions are immaterial and need not be known.

This simplifies the determination of μ_{SM} for each constituent—

$$\mu_{SM} = 1 + \alpha_{FL} C_L \text{ for iron}$$
$$= 1 + \alpha_{SL} C_L \text{ for silicon dioxide, etc.}$$

and is a constant for each constituent.

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The Use of Elastic Particle Scattering for Preliminary Survey Examinations in Positive-ion Microprobe Analysis

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The intensity of particles scattered elastically from a sample during bombardment with positive ions provides a useful complement to existing positive-ion microprobe techniques. This new approach can be used to give a rapid preliminary indication of gross elemental distributions, which is particularly valuable for identifying areas that require more detailed examination.

INFORMATION about the micro-scale composition of sample surfaces can be obtained by measurement of the products of nuclear interaction when the surfaces are irradiated with positive-ion beams of small diameter with energies of up to a few million electron volts.[1,2] Reaction products may be either photons,[3] or charged particles[4,5] emitted promptly as a result of nuclear interaction of the primary particle beam with elements in the sample, or X-rays resulting from charged-particle induced electronic excitation.[6] By careful choice of type and energy of incident particles, and of the radiation measured, positive-ion microprobe techniques are able to provide analytical information about the elemental composition or the nature of surface layers within the volume of sample interacting. Nuclear methods and X-ray techniques based on charged-particle irradiation complement one another, as nuclear methods are available for determining light elements that are less satisfactorily measured by counting X-rays.

While adequate information can sometimes be obtained from single irradiations of very small samples or of small areas of larger samples, several irradiations with the beam incident on different areas of a sample surface are usually necessary to contrast changes in composition. However, detailed analysis of the entire surface is rarely either practicable or necessary, and irradiations will usually be restricted to limited regions that are believed to be of special interest. Because, in general, the time taken for an analysis will be longer the more complete is the information required from each location irradiated, a method of initially surveying the sample, which could rapidly yield qualitative information about the location of regions in the sample of different composition, would be of value, as these areas of special interest could be selected for a more detailed study by the more selective charged-particle methods. A suitable survey technique should (*a*) be sensitive to changes in those characteristics of importance to the operating analyst, (*b*) be very rapid so that a comprehensive survey can be completed quickly and (*c*) must not require very special conditions for operation that are likely to complicate the use of other methods of positive-ion microanalysis that may be necessary for the more detailed studies. Use of the positive-ion microprobe provides a way of examining variations in elemental composition so that a suitable survey technique should be sensitive to over-all changes in elemental content in order to give a preliminary guide to the position of different zones in the sample. Measurement of particles that are elastically scattered from the sample fulfils conditions (*b*) and (*c*) and is sensitive to major changes in elemental composition, provided that layering does not complicate interpretation of results. Results reported here to demonstrate the technique have been obtained by scattering 2-MeV α-particles, but the method can be equally well applied to different particles accelerated to other energies.

EXPERIMENTAL

α-Particles were accelerated to 2 MeV in a 3-MeV electrostatic generator and were focused to form a small beam spot on the target. Beam diameters varied from 100 to 500 μm, depending on the size of the sample to be examined, and the position of the beam on the target was controlled either by mechanically moving the table to which the target was secured, or by electrostatic deflection of the ion beam. In all instances, the scanning movement of the beam with respect to the target was discontinuous, that is to say, movement occurred

between irradiations, but during each irradiation the beam was maintained on the same spot on the target.

Scattered α-particles were detected with a silicon semiconductor detector subtending a solid angle of 0·1 sr. After amplification, pulses were fed to a single-channel pulse height analyser, whose thresholds were initially set with a pulse generator, which was calibrated with the aid of a multi-channel pulse height analyser. During irradiation, those pulses from the counting system the heights of which fell between the analyser thresholds were fed to a multi-channel analyser operating in multi-scaler mode and with channel stepping controlled from the system governing the movement of the ion-beam on the target, so that all counts from irradiation of a specific area of the sample were accumulated in a single channel. Read-out of the successive channels then gave the count from successive irradiations of the sample. The flux of charged particles falling on the target was monitored by beam-current integration, all irradiations in any scan being carried out to the same total particle dose.

Most consistent results were obtained when the surfaces of samples to be examined were flat so that trapping of particles was avoided. Consequently samples were mounted in resin, cut and then polished with a succession of progressively finer grinding papers until a smooth finish was obtained.

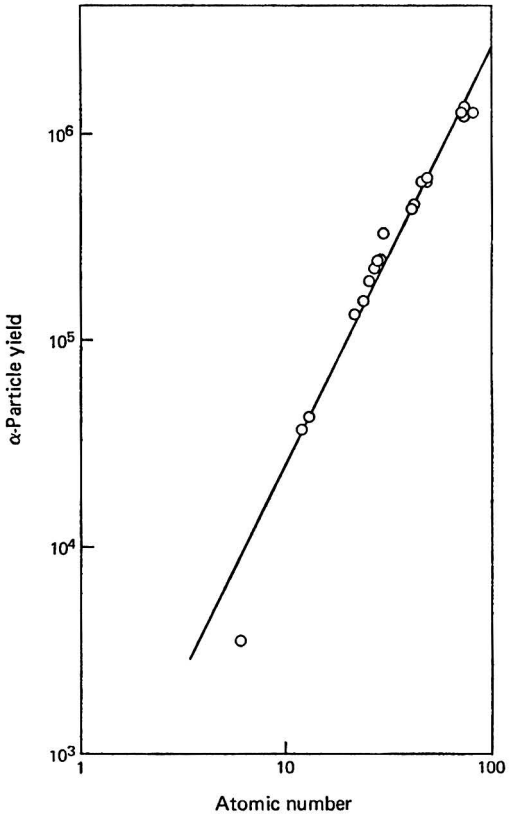
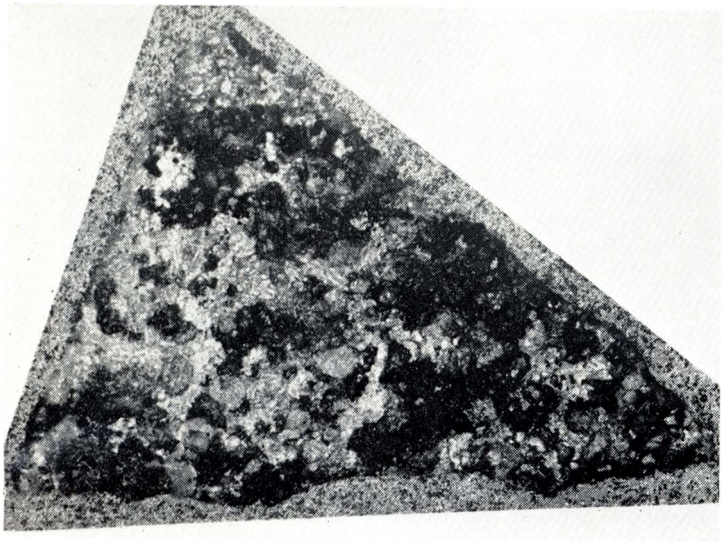


Fig. 1. Graph of α-particle yield against atomic number recorded for some thick targets of pure materials

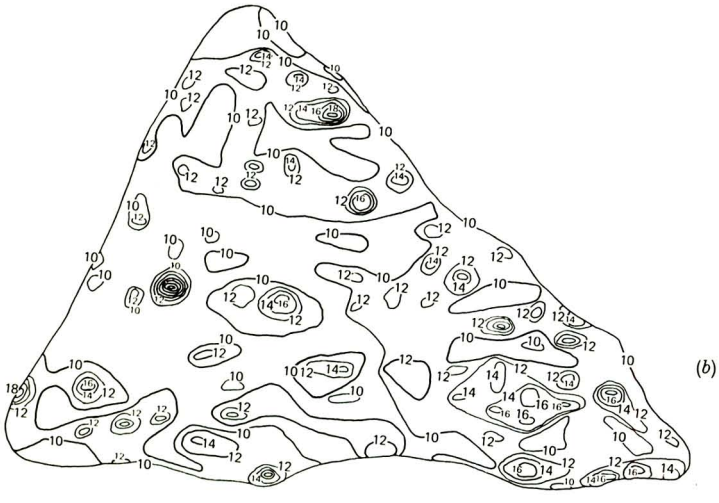
Results and discussion

The intensity of particles scattered from a film that is thin enough for the energy loss of particles in the film to be negligible is given by the equation—

$$q = Qnt\omega \left(\frac{Zze^2}{mv^2}\right)^2 \operatorname{cosec}^3\phi \frac{[\cot\phi \pm \sqrt{\operatorname{cosec}^2\phi - (m/M)^2}]^2}{\sqrt{\operatorname{cosec}^2\phi - (m/M)^2}}$$



(*a*)



(*b*)

Fig. 2. (*a*), Section of stony meteorite; and (*b*), contour plan of intensity of α-particles scattered from stony meteorite sample. Numbers given are thousands of counts recorded

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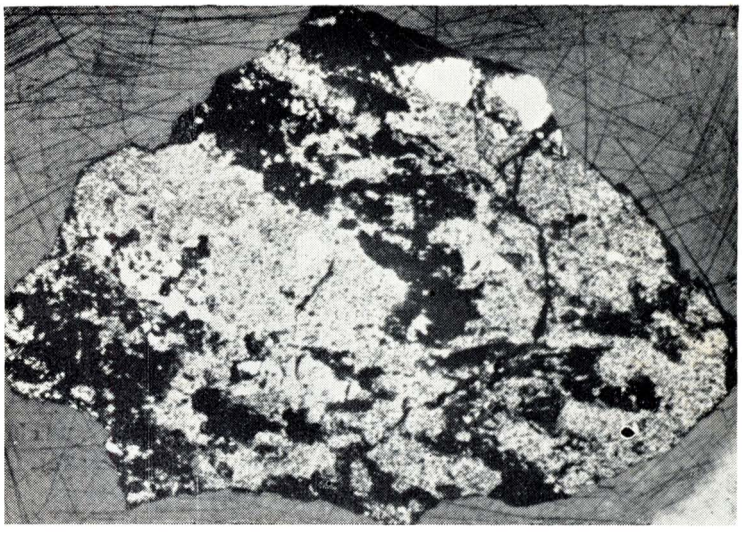

(*a*)

(*b*)

Fig. 3. (*a*), Section of copper pyrites sample; and (*b*), contour plan of intensity of α-particles scattered from copper pyrites sample. Numbers given are thousands of counts recorded

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where q is the number of particles of charge z, mass m and initial velocity v, scattered through an angle ϕ into a solid angle ω from a layer containing n atoms per cubic centimetre and of thickness t, Z and M are the charge and mass of the scattering nucleus, respectively, and Q is the number of particles falling on the film per second. Therefore the intensity of the scattered particles is proportional to the square of the atomic number of the scattering element. For a thick target, particles scattered from nuclei below the sample surface will lose energy on passing through the sample both before and after scattering, thus arriving at the detector with a wide spread of energies, so that the count registered will be critically dependent on the threshold settings of the counting system. Moreover, as the maximum energy of a particle scattered at the sample surface is dependent on the mass of the scatterer according to the equation—

$$E_s = E_0 \frac{(m \cos\theta \pm \sqrt{M^2 + m^2 \sin^2\theta})^2}{(m + M)^2}$$

where E_0 and E_s are the energies of the incident and scattered particles, respectively, variation of the counter threshold will not have the same effect for all elements in their contribution to the total count. In order to obtain the high count-rate necessary to permit rapid scanning, a low threshold was set on a single-channel analyser so that pulses corresponding to a wide range of particle energies could be recorded. Counts were usually accumulated for 10 or 100 ms per point irradiated and the time taken for examination of a sample depended on the spot and sample size and on the detail required. Typically, a 1000-s scan will permit relatively detailed examination of samples, as shown in Figs. 2 (*b*) and 3 (*b*). The total particle yield from thick targets is dependent on the stopping power of the matrix, but Fig. 1 shows the strong dependence of yield on atomic number obtained for thick targets of several elements. In practice, samples for scanning are unlikely to be pure materials, so that the total yields recorded will be a function of elemental composition and stopping power of the surface layers over the depth, which contribute to scattered-particle yield counted by the detector. However, in general, a high count-rate will indicate a region of materials of high atomic number and hence correlation of count-rate with position is likely to give a measure of elemental distribution.

Application of the technique is demonstrated by two examples, in which the samples scanned with beams of 2·0-MeV α-particles have been chosen so that regions of different composition can be distinguished visually although, of course, the particle yield measured was a function of elemental composition, and application of the technique did not require visual identification of the different regions of the sample. Fig. 2 (*a*) shows a polished section of a stony meteorite with the dark-coloured regions containing relatively heavy elements such as iron clearly visible, while a contour plan of the total yield of α-particles scattered into the detector is given in Fig. 2 (*b*). Good correlation of high count-rate with the dark area of the sample can be seen to exist, with the highest counts being obtained when the particle beam was incident on nickel - iron inclusions. Variations in the iron content of silicon at levels of less than 1 per cent. could easily be followed. The polished geological sample shown in Fig. 3 (*a*) is copper pyrites (light-coloured phase) in a host mineral made up mainly of light elements. Results from particle scanning, summarised in the contour plan for this sample in Fig. 3 (*b*), again emphasise that measurement of the yield of scattered particles at different positions on the sample surface gives a useful indication of the position of regions of different elemental composition.

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The Spectrofluorimetric Determination of Orthophosphate as Quinine Molybdophosphate

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Between 0·02 and 1·2 μg of phosphorus is determined as orthophosphate via the formation of a complex between a molybdophosphate and the alkaloid quinine. Quinine molybdophosphate is precipitated in 0·5 M sulphuric acid, the excess of quinine reagent is removed by washing the precipitate with 0·5 M sulphuric acid, and the complex is dissolved in the solvent mixture acetone - 0·5 M sulphuric acid (9 + 1 v/v). The fluorescence intensity of this solution is measured at 445 nm with excitation at 352 nm. Optimum conditions for the determination have been established, and the effects of 100-fold weight excesses of each of thirty-eight foreign ions have been examined. The determination is selective; large amounts of silicate do not interfere, and arsenic(III) and tungsten(VI) are tolerated at 20 and 50-fold weight excesses, respectively. The nature of the quinine molybdophosphate complex has been examined.

MOST of the commonly used methods for the determination of orthophosphate are based on the absorption spectrophotometry in solution of molybdophosphoric or molybdovanadophosphoric acid, or of their reduction products in aqueous or organic media. These methods are neither highly sensitive nor selective, although the selectivity can be improved by solvent extraction of the heteropoly acid or by the use of masking agents.[1–6] Indirect amplification methods have been described for the determination of orthophosphate, in which the twelve molybdate ions associated with each phosphate ion in molybdophosphoric acid are determined by atomic or molecular-absorption spectrophotometry.[7–10] These methods may be quite selective and can show high sensitivity. The application of the ion-association complexes formed between molybdophosphoric acid and various organic basic dyestuffs to the molecular spectrophotometric determination of orthophosphate has been studied by several workers. From a study of the use of a range of dyestuffs in this way, Babko, Shkaravskii and Kulik[11] recommended the use of iodine green or crystal violet for the extraction - spectrophotometric determination of phosphate. Spectrophotometric methods based on the molybdophosphate complexes of malachite green[12] and safranine[13] have also been reported.

Several types of method for the determination of orthophosphate exploit the high sensitivity of fluorimetric techniques. The quenching by orthophosphate of the fluorescence of the complex formed between aluminium and morin has been used for the determination of orthophosphate.[14] Sensitive spectrofluorimetric methods for phosphate based on the conversion of glycogen to glucose 6-phosphate in the presence of inorganic phosphate, activated phosphorylase and phosphoglucomutase have been described. The glucose 6-phosphate then reacts with the oxidised form of triphosphopyridine nucleotide[15] or nicotinamideadenosine diphosphate[16] to yield their reduced forms whose fluorescence is measured. In an attempt to combine in one method the advantages of the methods based on the formation of ion-association complexes between organic bases and molybdophosphate with those of a spectrofluorimetric finish to the determination, we have previously reported a sensitive and selective procedure for the determination of orthophosphate based on the formation of the complex formed between rhodamine B and molybdophosphate.[17] In this paper is reported a similar method that has the additional advantage that the final fluorescence measured is that of the organic base quinine; by virtue of its high fluorescence intensity and stability in solutions of sulphuric acid, quinine is frequently used as a standard for the calibration of spectrofluorimeters. The alkaloids strychnine[18] and quinine[19] have been used previously for the determination of orthophosphate in methods in which their insoluble molybdophosphates are precipitated; the absorbance of the solution[18] or the volume of the precipitate[19] is then

measured. In the procedure described here quinine molybdophosphate is precipitated in an acidic medium, washed free from excess of quinine and dissolved in acidified acetone. The fluorescence of the solution is then measured at 445 nm with an excitation wavelength of 352 nm to provide a sensitive and selective method for the determination of orthophosphate.

Experimental

Apparatus—

A double-monochromator spectrofluorimeter (Aminco-Bowman, American Instrument Co., Silver Spring, Md., U.S.A.), fitted with a 150-W high-pressure xenon arc lamp and an IP 28 photomultiplier, was used in conjunction with an X - Y recorder (Bryans, Model 21000). Fused quartz cells (10 × 10 × 50 mm) were used. Slits of width 3 mm (Aminco slit arrangement No. 3, spectral band width 30 nm) were used in both the excitation and analysing monochromators.

Pyrex glass centrifuge tubes of 10-ml capacity were used throughout. These were cleaned thoroughly in a 4 per cent. solution of Decon (Decon Laboratories Ltd., Brighton) after each use.

Reagents—

Phosphate solution—Dissolve 0·1258 g of sodium dihydrogen orthophosphate dihydrate (AnalaR grade) in distilled water and dilute to 1 litre. This solution contains 25 μg ml^{-1} of phosphorus and was diluted when necessary to 0·1 or 1 μg ml^{-1} with distilled water.

Molybdate solution—Dissolve 30 g of sodium molybdate dihydrate (AnalaR grade) in distilled water and dilute the solution to 1 litre with distilled water. From this solution, prepare a reagent solution by the following procedure. To 250 ml of the molybdate solution, add an equal volume of distilled water and sufficient concentrated sulphuric acid (AnalaR grade) to make the solution approximately 0·5 M with respect to the acid. Allow the solution to stand for 10 minutes, and extract with two 100-ml volumes of a chloroform - butanol mixture (4 + 1 v/v). Allow the phases to separate each time and discard the organic phase. Finally, shake the solution several times with 100-ml volumes of chloroform to remove dissolved butanol from the aqueous solution. Store the aqueous molybdate reagent in a polythene container.

Quinine sulphate solution—Dissolve 7·83 g of quinine sulphate, $(C_{20}H_{24}N_2O_2)_2.H_2SO_4.2H_2O$, (BDH Chemicals Ltd., Poole, Dorset) in a few drops of concentrated sulphuric acid and dilute the solution to 1 litre with distilled water. The over-all acid concentration should be approximately 0·05 M with respect to sulphuric acid.

Acetone - 0·5 M sulphuric acid solvent (9 + 1 *v/v*)—Use AnalaR grade acetone.

Foreign ions—Prepare stock solutions, each containing 1000 p.p.m. of a foreign ion, by dissolution of their salts (analytical-reagent grade) in distilled water or about 0·02 M hydrochloric acid.

Wash solution—A 0·5 M solution of sulphuric acid in distilled water is used.

Calibration graph for phosphorus—

To a series of eight 10-ml centrifuge tubes add 2 ml of molybdate reagent solution and 1 ml of 2·4 M sulphuric acid. Add 2-ml volumes of solution containing 0, 0·02, 0·05, 0·2, 0·4, 0·6, 0·9 and 1·2 μg of phosphorus in distilled water to the tubes. Mix each solution and allow the mixtures to stand for 5 minutes. Add 1 ml of quinine sulphate reagent solution to each tube, mix each solution again and place the tubes in a beaker of boiling water for about 5 minutes. Place the tubes in a laboratory centrifuge and centrifuge the solutions for a sufficient time (1 to 2 minutes) to ensure that a closely packed precipitate is produced at the base of each tube. Decant the clear solution. Wash the precipitate in each tube with 5 ml of 0·5 M sulphuric acid and again centrifuge and decant the wash liquid. Add 5 ml of acetone - sulphuric acid reagent to each tube to dissolve the precipitate. Transfer suitable volumes of each solution to the fused silica sample cell and measure the fluorescence of each solution in turn at 445 nm with an excitation wavelength of 352 nm. After subtraction of the observed fluorescence from that of the reagent blank (prepared simultaneously), the calibration graph is linear and passes through the origin.

ANALYSIS OF SAMPLE—

When analysing a sample, proceed as directed above, omitting the addition of phosphate solution but adding 2 ml of sample solution containing not more than 1·0 μg of phosphorus.

RESULTS AND DISCUSSION

During the initial qualitative examination of the procedure for the detection of orthophosphate as quinine molybdophosphate, various organic solvents were tested for their suitability for the dissolution of the quinine molybdophosphate precipitate and as solvents for the fluorescence measurement. We observed that while several solvents were suitable for the dissolution step, including acetone and ethanol, the fluorescence intensity observed after dissolution was low. The addition of dilute sulphuric acid to these solutions, however, resulted in considerable enhancement of the fluorescence of the dissolved material in the organic solvent. We preferred to use acetone, to which the dilute sulphuric acid had been added initially, as the solvent for the precipitate, and this mixture was used in all the experiments described below. During the initial qualitative studies, it was also realised that in order to achieve low blank fluorescence signals when small volumes of precipitate were to be handled, it would be necessary to wash the precipitate free from excess of reagent before its dissolution. This was undertaken in all subsequent quantitative work by washing the precipitate with 0·5 M sulphuric acid before its dissolution.

SPECTRAL CHARACTERISTICS—

Curves B in Fig. 1 show the fluorescence excitation and emission spectra obtained after formation of the quinine molybdophosphate precipitate from 0·5 μg of phosphorus and dissolution of the precipitate in the acetone - sulphuric acid solvent. The wavelengths of maximum excitation and emission observed in this solvent were 352 and 445 nm, respectively. It is apparent that the spectral characteristics are identical with those exhibited by quinine sulphate itself in this solvent; Curves A show the excitation and emission spectra for a 6×10^{-7} M solution of quinine sulphate in the same solvent. This suggests that the fluorescence measured is that of free quinine sulphate itself, and that the acetone - sulphuric acid mixture serves to decompose the quinine molybdophosphate. There is no evidence to suggest that the quinine forms a stable complex with molybdophosphate rather than a simple insoluble salt in aqueous medium. The similarity between the spectral excitation and emission

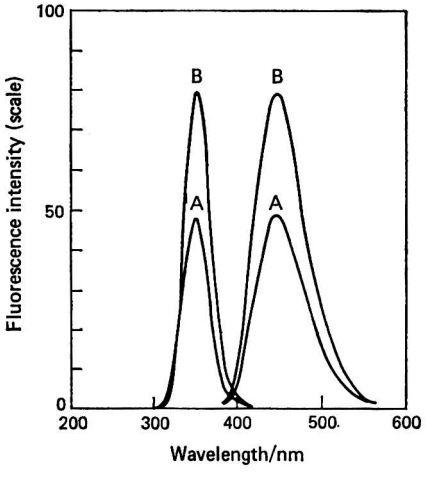

Fig. 1. Excitation and emission spectra (uncorrected) for: A, 6×10^{-7} M quinine sulphate solution (0·1 scale); and B, fluorescence produced by dissolution of quinine molybdophosphate from 0·5 μg of phosphorus (0·3 scale). Both spectra were recorded in acetone - 0·5 M H_2SO_4 (9 + 1 v/v)

characteristics of the quinine sulphate and the sample fluorescence does not constitute a disadvantage provided that the excess of reagent used to precipitate the quinine molybdophosphate is removed by washing before dissolution of the precipitate.

EFFECTS OF THE CONCENTRATIONS OF SULPHURIC ACID, QUININE SULPHATE AND MOLYBDATE REAGENT—

The conditions initially used for the formation of molybdophosphoric acid were similar to those used by Wadelin and Mellon.[1] The effect of the concentration of the sulphuric acid in the aqueous phase on the formation of quinine molybdophosphate was studied by using the procedure described above. The experiments were carried out by using 2 μg of phosphorus in 2 ml of aqueous solution to which 2 ml of molybdate reagent and 1 ml of sulphuric acid of various molarities were added. The effect of the sulphuric acid concentration, in the initial solution, on the fluorescence intensity at 445 nm measured in the final acetone - sulphuric acid solvent is shown in Fig. 2 (*a*). A concentration of 1 ml of 2·4 M sulphuric acid in the initial solution was chosen as the optimum and was used in all further work.

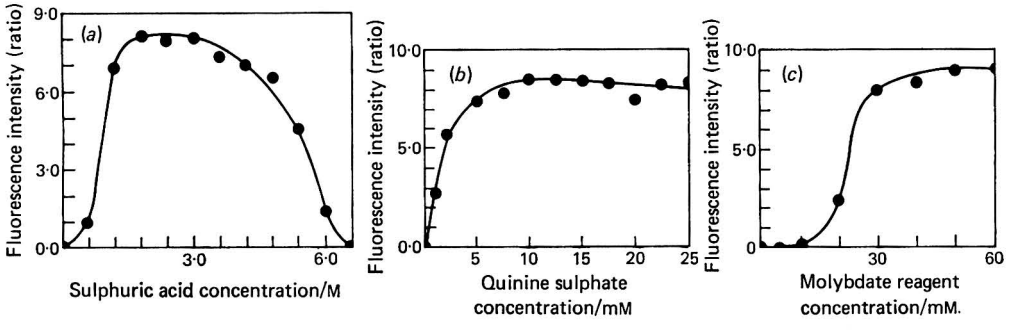

Fig. 2. (*a*) Effect of sulphuric acid concentration on net fluorescence produced for 2 μg of phosphorus (after subtraction of blank fluorescence); (*b*) effect of quinine sulphate reagent concentration on net fluorescence produced for 2 μg of phosphorus; and (*c*) effect of molybdate reagent concentration on net fluorescence for 2 μg of phosphorus. In (*a*), (*b*) and (*c*) the fluorescence measured at 352/445 nm is expressed as ratio of intensity *versus* fluorescence intensity of standard aqueous quinine sulphate solution at 350/450 nm

The optimum quinine sulphate concentration was determined in a similar way by using 2 μg of phosphorus in 2 ml of aqueous solution, 2 ml of molybdate reagent and 1 ml of 2·4 M sulphuric acid, to which various amounts of quinine sulphate reagent in a constant volume of aqueous solution were added. The precipitate was washed with 5 ml of 0·5 M sulphuric acid before dissolving it in 5 ml of acetone - sulphuric acid solvent for measurement of the fluorescence. As shown in Fig. 2 (*b*), the fluorescence intensity increased for 1 ml of quinine sulphate up to a concentration of 10 mM and then remained constant as the reagent concentration was increased further. This concentration, 10 mM, was chosen for use in all further work; the use of a larger amount of quinine sulphate than necessary serves only to increase the magnitude of the reagent blank and to decrease the precision.

In order to ensure a low blank, the molybdate reagent solution was pre-stripped of any phosphate impurities before use; this procedure, which involves the formation of molybdophosphoric acid and its extraction from any phosphate present in the reagents used, is described in the Experimental section. The optimum concentration of the molybdate reagent was determined by using 2 μg of phosphorus in 2 ml of solution. This solution was added to a mixture of 1 ml of 2·4 M sulphuric acid and 2 ml of molybdate solution that had various concentrations. Quinine molybdophosphate was then precipitated by the addition of 1 ml of 10 mM quinine sulphate reagent solution. The effect on the final fluorescence intensity of variations in the concentration of the molybdate reagent solution in this way is shown in Fig. 2 (*c*); 2 ml of 60 mM molybdate reagent solution was chosen as the optimum volume and was used in all further work.

EFFECT OF WASHING THE QUININE MOLYBDOPHOSPHATE PRECIPITATE—

The effects of the final fluorescence intensity of the acidity of the solution used to wash the precipitate free from excess of reagent and of the number of washes used are shown in Fig. 3. The precipitate formed from 2 μg of phosphorus was washed with 5-ml volumes of sulphuric acid solutions having various concentrations between 0·05 and 2 M, and also with water. It is evident that a single wash with 5 ml of dilute acid was sufficient to produce a low blank fluorescence after the removal of excess of quinine, and also to retain the quinine molybdophosphate precipitate without significant dissolution. The concentration of the acid used did not significantly affect the fluorescence signal-to-blank intensity ratio after only a single wash step, although when the concentration of the acid was greater than 0·5 M a significant decrease in the fluorescence signal was produced on further washing of the precipitate.

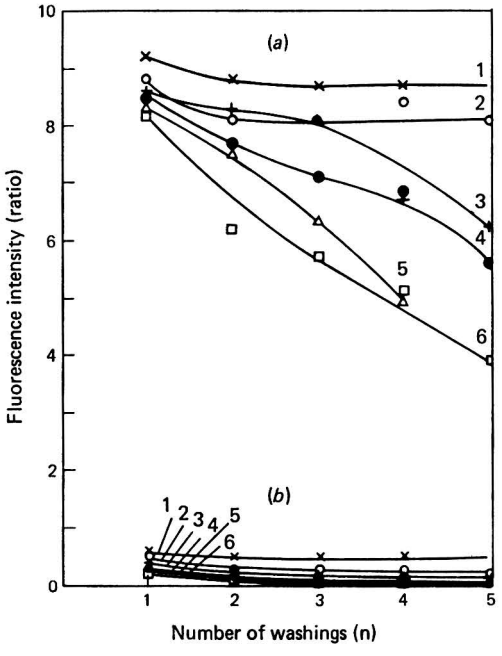

Fig. 3. Effect of number of washes with 5-ml volumes of wash liquid for (*a*), quinine molybdophosphate from 2 μg of phosphorus and (*b*), blank (no phosphorus): 1, water; 2, 0·05 M H_2SO_4; 3, 0·5 M H_2SO_4; 4, 1·0 M H_2SO_4; 5, 1·5 M H_2SO_4; and 6, 2·0 M H_2SO_4

ORDER OF ADDITION OF REAGENTS—

The order of addition of the reagents used must ensure that the molybdophosphoric acid is formed in acidic medium before the quinine sulphate reagent is added. Although the rate of formation of molybdophosphoric acid was quite rapid under the conditions used, we preferred to allow the solution to stand for 5 minutes before adding the quinine sulphate reagent. The order of addition used was therefore molybdate reagent, acid, sample and quinine sulphate reagent.

CALIBRATION GRAPH, SENSITIVITY AND PRECISION—

With the operating conditions described, the calibration graph for phosphorus was linear in the range 0·02 to 1·2 μg of phosphorus in the original 2 ml of aqueous sample solution taken, *i.e.*, 0·01 to 0·6 p.p.m. in the sample solution. Appreciable curvature of the calibration graph towards the concentration axis occurred when more than 1·2 μg of phosphorus was determined under the recommended conditions. This is not caused by the presence of

insufficient quinine or molybdate reagent to allow the quantitative recovery of the larger amounts of quinine molybdophosphate, but is caused by concentration quenching of the quinine fluorescence at higher concentrations. This was demonstrated by the construction of a calibration graph for quinine sulphate alone in acetone - sulphuric acid solution; the graph of fluorescence intensity against quinine concentration became curved towards the concentration axis at concentrations similar to those produced when more than 1 μg of phosphorus was determined.

The limit of the sensitivity of the procedure is determined by the magnitude and reproducibility of the fluorescence of the procedure blank. By using the procedure described here, the limiting detectable phosphorus concentration was 0·01 μg in 2 ml of aqueous solution.

When 1 μg of phosphorus in 2 ml of sample solution was determined ten times by the recommended procedure, a coefficient of variation of 4·7 per cent. was obtained.

Effects of foreign ions—

The effects of 100-fold weight excesses of thirty-eight foreign ions on the fluorescence intensity obtained in the final acetone solution for 1 μg of phosphorus determined by the recommended procedure were investigated. An ion was considered to interfere when it produced an error in fluorescence intensity compared with that produced by 1 μg of phosphorus alone of greater than the coefficient of variation (*i.e.*, about 5 per cent.). The following ions did not interfere under these conditions: aluminium, antimony(III), bismuth, cadmium, calcium, cerium(IV), cobalt(II), chromium(III), copper(II), gold, iron(III), lead, magnesium, manganese(II), mercury(II), nickel, selenium(IV), silicon, silver, tellurium(IV), thallium(III), tin(IV), titanium(IV), vanadium(IV), vanadium(V), zinc, zirconium, fluoride, chloride, nitrate, perchlorate and ethylenediaminetetraacetate. The presence of a 100-fold weight excess of the following ions produced the errors in fluorescence intensity given in parentheses: arsenic(III) (+28 per cent.), chromium(VI) (−11 per cent.), germanium(IV) (+155 per cent.), thorium (+7 per cent.) and tungsten(VI) (+13 per cent.). The presence of a 50-fold weight excess of arsenic(V) caused an error in fluorescence intensity of +97 per cent. Arsenic(III) and tungsten(VI) can be tolerated in 20 and 50-fold weight excesses, respectively, whereas germanium(IV) in only a 2-fold weight excess and arsenic(V) present in the same amount as phosphorus interfere seriously. Silicon as silicate does not interfere; under the conditions used for the formation of molybdophosphoric acid, the formation of molybdosilicate is inefficient, and an insoluble quinine molybdosilicate does not appear to be formed.

Accuracy—

An estimate of the accuracy of the procedure was obtained by the determination of phosphorus in synthetic solutions containing foreign ions that were treated as unknown samples. The results of these analyses are shown in Table I.

Table I

Determination of phosphorus as orthophosphate in synthetic mixtures treated as unknowns

Phosphorus taken/μg	Phosphorus found*/μg	Error, per cent.	Foreign ions present. Values in parentheses are the weight excess ratios of the ions over phosphorus
0·40	$0{\cdot}37_5$	−6·25	Zn (30); Pb (20)
0·20	$0{\cdot}18_5$	−7·50	Cu(II) (15); Ni (15)
0·08	$0{\cdot}08_1$	+1·25	Al (15); Si (10)
0·25	0·26	+4·0	As(III) (5); Co (10)
0·14	0·13	−5·7	Bi (10); Mg (15)
0·45	0·46	+2·2	Ca (20); Fe(III) (10)
0·30	$0{\cdot}28_5$	−5·0	F (10); ClO_4 (10)
0·22	0·22	0	Cr(III) (10); Fe(III) (15)

* Each result represents the average of three determinations in good agreement.

Nature of the precipitate—

Several attempts were made to isolate and dry the quinine molybdophosphate precipitate so that complete elemental analysis could be carried out. Each time, however, it was apparent that decomposition of the compound occurred during drying, even at room temperature.

The decomposition was evident from the change in colour of the precipitate from very pale green to blue. The precipitate was therefore prepared wet from known amounts of orthophosphate, centrifuged off and washed with water or dilute sulphuric acid. The molybdenum content of the precipitate was determined by atomic-absorption spectroscopy after dissolution in concentrated sulphuric acid. This determination showed that the added orthophosphate was completely recovered, as 12 mole of molybdate were recovered for each mole of orthophosphate taken. The quinine content of the precipitate was determined similarly by fluorimetric measurement and comparison with standards prepared from quinine sulphate alone in acetone - sulphuric acid medium. This determination indicated a combining ratio of quinine to molybdophosphate of 1·8:1, a ratio which is probably slightly high owing to the difficulty of removing the last traces of excess of reagent from the precipitate. Potentiometric titration of the precipitate, obtained from 150 μg of phosphorus precipitated by the recommended procedure and washed with distilled water to free it from acid, against standard 0·01 M sodium hydroxide solution gave the results shown in Fig. 4. The results indicate that there are two titratable protons present in the compound formed. The pH values at the points corresponding to these two points on the titration curves are similar to the pH values corresponding to the two protonation steps for quinine itself, which suggests that quinine (Q) is precipitated as QH_2^{2+} to form the insoluble molybdophosphate. The titre from these experiments and the results of the fluorimetric determination of the quinine suggest that the precipitate corresponds to the composition $(C_{20}H_{24}O_2N_2H_2)_3(PMo_{12}O_{40})_2$, which is an uncharged species. The structure may be similar to that of quinine molybdovanadophosphate proposed by Ripan and Suteu.[20]

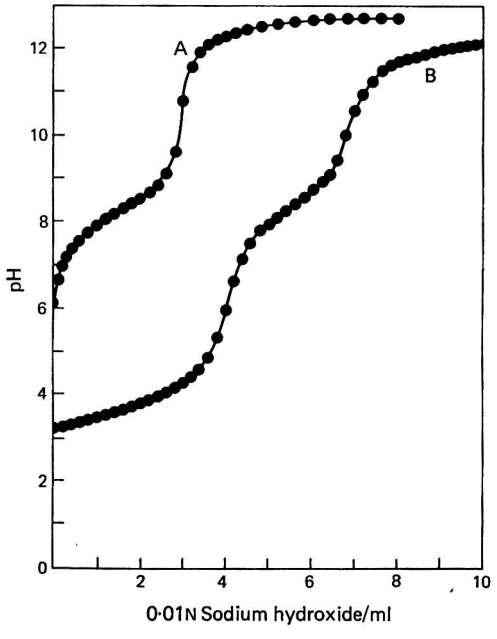

Fig. 4. Potentiometric titration curves for: A, quinine sulphate (2·5 ml of 0·005 M solution) and B, quinine molybdophosphate (from 150 μg of phosphorus). Titration with 0·01 N NaOH

Conclusions

High sensitivity is obtained for the determination of phosphorus by the method developed. The sensitivity achieved is higher than that for most other methods based on the formation of molybdophosphoric acid, and compares favourably with that of more complex enzymatic

methods. The sensitivity is slightly higher than that obtained in the determination of orthophosphate as the rhodamine B - molybdophosphate complex described in earlier work from this laboratory.[17] Of the foreign ions investigated, only arsenic(V) and germanium(IV) cause serious interference as a result of the formation and extraction of the corresponding quinine molybdoarsenate and molybdogermanate, respectively. Arsenic(III), however, can be tolerated when it is present in moderate excess over phosphorus. Silicate, the elimination of interference from which can be troublesome in other procedures involving the use of molybdophosphoric acid for the determination of orthophosphate, does not interfere in the present procedure. A particular advantage of the method described here is the use of the fluorescent base quinine, which is often used as a standard for fluorescence intensity measurements, as the component whose fluorescence is monitored for analytical purposes.

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The Direct Determination of Calcium in Zirconium and Zircaloy-2 by Flame-emission Spectrophotometry with the Nitrous Oxide - Acetylene Flame*

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A method is proposed for the direct determination of calcium impurities in nuclear-grade zirconium and Zircaloy-2 by flame-emission spectrophotometry with the nitrous oxide - acetylene flame. Because chemical manipulations are limited to the dissolution of the sample, the method is very simple and rapid, and it gives a precision that compares favourably with that of other available methods. By the method described, 6 to 20 p.p.m. of calcium in zirconium and Zircaloy-2 have been determined with standard deviations ranging from 0·8 to 1·8 p.p.m.

The spectral interference from zirconium, the choice of instrumental conditions and the procedures for zirconium background correction are discussed.

THE amount of calcium present as an impurity in zirconium and its alloys is known to be directly related to the rate of aqueous corrosion of such materials.[1] Nuclear specifications for zirconium generally require that the calcium content should not exceed 20 to 30 p.p.m., this limit being mainly defined by the sensitivity of the analytical methods available. These methods include direct spectrographic analysis with different excitation and working conditions,[2–11] and separation of calcium from zirconium, followed by its determination by different techniques.[12–18] Precision, calculated as the coefficient of variation, in the direct procedures is seldom better than 20 per cent., and is not significantly improved in the other procedures in spite of chemical separations, which are often very complicated because of contamination problems.

The attractive features of the nitrous oxide - acetylene flame in emission spectrometry have been recently pointed out.[19,20] Although its analytical sensitivity has been extensively investigated, the flame has seldom been used in practical analytical procedures.[21–23] In this paper, a simple method is proposed for the direct determination of calcium in zirconium and Zircaloy-2 by flame-emission spectrophotometry with the nitrous oxide - acetylene flame. With this method, 6 to 20 p.p.m. of calcium in zirconium and Zircaloy-2 were determined with standard deviations ranging from 0·8 to 1·8 p.p.m.

Experimental

A maximum versatility Jarrell-Ash atomic-absorption and flame-emission spectrophotometer was used, with a water-cooled nitrous oxide - acetylene burner head. Calcium emission was measured at the atomic line 422·673 nm. Detailed descriptions of the instrument and of its operating conditions are given in Table I.

Zirconium and Zircaloy-2 samples were dissolved in the minimum volume of acids necessary, to avoid the possibility of damaging the burner head. For 0·5-g samples, 2 ml of 0·6 M hydrochloric acid, 1 ml of 50 per cent. hydrofluoric acid and 5 drops of concentrated nitric acid proved to be satisfactory.

Calcium standard solutions were obtained by suitable dilution of calcium stock solutions containing 5 mg ml^{-1} of calcium. The concentrations of the stock solutions were checked by complexometric titration. Potassium was used as an ionisation buffer and was added as potassium chloride; this reagent, and the acids used to dissolve the samples, were of Merck Suprapur grade; all other chemicals used were C. Erba RP grade reagents. Freshly de-ionised distilled water was used for all dilutions.

Other experimental details are given under Recommended procedure.

* Presented at the Third SAC Conference, Durham, July 12th to 16th, 1971.

TABLE I

INSTRUMENTS AND OPERATING CONDITIONS

Flame	Reducing flame (red cone of about 1·5 cm height); Nippon Jarrell-Ash 5-cm slot water-cooled laminar flow burner; Jarrell-Ash gas-flow distribution unit; nitrous oxide and acetylene pressures at the tank gauges 3·5 and 1 atmosphere, respectively; flow-rates optimised for maximum sensitivity.
External optics	Spherical quartz lens, 3-cm diameter, 10-cm focal length.
Spectrophotometer	Jarrell-Ash, Model 82000, 0·5-m Ebert mounting, scanning spectrophotometer with 1180 grooves mm^{-1} grating, blazed at 300 nm; effective aperture *f*8·6; reciprocal linear dispersion at the exit slit 1·6 nm mm^{-1} in the first order; automatic scanning speeds from 50 to 0·2 nm min^{-1}; entrance and exit slits continuously variable from 0 to 0·4 mm; slit height, variable from 0 to 20 mm, was fixed at 4 mm.
Electronics and read-out ..	RCA 1P28 photomultiplier; signal fed into a Jarrell-Ash a.c. amplifier and displayed on a Leeds and Northrup AZAR recorder with scale adjustable from 0 to 100 mV; time constants up to 5 s are available.

RESULTS AND DISCUSSION

SENSITIVITY—

Zirconium has a depressive effect on both the emission and absorption of calcium, probably because of the formation of compounds such as calcium zirconate.[24] This effect limits the use of air - acetylene flames for the spectrometric determination of calcium in the presence of zirconium so that the determination of trace amounts of calcium in zirconium materials by using these flames is possible only after a preliminary chemical separation.

The high temperature and the reducing character of the nitrous oxide - acetylene flame overcome the zirconium depressive effect and give good sensitivity for calcium even in the presence of large amounts of zirconium. The sensitivity can be further enhanced by suppressing calcium ionisation by the addition of suitable amounts of potassium.[20]

The attractive features of the nitrous oxide - acetylene flame[19,20] were checked by preliminary experiments with solutions containing calcium alone or calcium *plus* zirconium, the ionisation buffer being potassium at a concentration of 1 mg ml^{-1}. The results showed that the analytical sensitivity for calcium was as high as 0·005 $\mu g\ ml^{-1}$, and that zirconium-to-calcium ratios up to 10^5:1 had no significant effect on the calcium emission. In spite of this high sensitivity, 0·05 to 0·5 $\mu g\ ml^{-1}$ of calcium was chosen as the analytical range so as to avoid significant contamination of samples during the experiments. To cover calcium impurity levels normally encountered in nuclear-grade zirconium alloys, the standard procedure was then based on 0·5 g of sample dissolved in 50 ml of solvent mixture, *i.e.*, on 10 mg ml^{-1} of zirconium and 0·05 to 0·5 $\mu g\ ml^{-1}$ of calcium. All the experiments described were carried out at these concentration levels; 1 mg ml^{-1} of potassium was used as the ionisation buffer, unless otherwise specified.

CALIBRATION GRAPH—

The unbroken line in Fig. 1 shows the calibration graph obtained with solutions containing known amounts of calcium, together with reagents necessary to dissolve the metal samples and also 1 mg ml^{-1} of potassium to act as the ionisation buffer.

The peculiar shape of the lower portion of the graph can be attributed to a certain degree of calcium ionisation,[25] although no detailed investigation was undertaken to explain why the phenomenon still occurs in spite of the presence of the buffer. In fact, this effect appears to be reduced, but still observable, when the buffer concentration is increased ten-fold (closed circles in Fig. 1).

In the recommended procedure the original potassium concentration of 1 mg ml^{-1} was maintained to limit contamination from calcium, which is occasionally present in the buffer reagent.

SPECTRAL INTERFERENCES—

Large amounts of zirconium nebulised in the nitrous oxide - acetylene flame emit intense atomic lines superimposed on a relatively constant continuum. One of these lines occurs at 422·776 nm, which is very near to the calcium analytical line at 422·763 nm. The feasibility of the analysis, therefore, is dependent on satisfactory resolution between these two lines.

In order to investigate the extent of overlap of these lines scans were made in the region of interest, with the monochromator slit width set at different values. Fig. 2 shows representative spectra obtained with solutions containing 10 mg ml^{-1} of zirconium and 0·4 or 0·08 μg ml^{-1} of calcium. Optimum resolution was obviously obtained with the narrowest band width (spectrum A in Fig. 2), implying, however, a considerable loss in intensity of the signals, as is apparent from the figure. The best compromise for analysis was found to be a theoretical band width of 0·026 nm (16 μm nominal value on the slit-adjusting knob of the monochromator used by us), where satisfactory resolution is obtained together with a still adequate intensity of the calcium signal (see spectra B and F in Fig. 2).

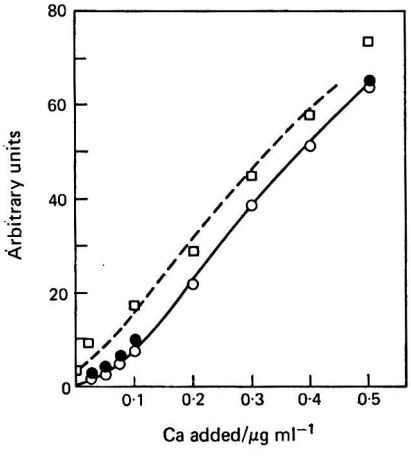

Fig. 1. Calibration graph obtained with solutions containing only calcium, dissolution reagents and 1 mg ml^{-1} of potassium (open circles); closed circles refer to analogous solutions containing 10 mg ml^{-1} of potassium. Squares refer to 0·5-g Zircaloy-2 sample solutions with calcium added. The dotted line represents the calibration graph suitably shifted to the left to account for calcium originally present in the alloy (see text)

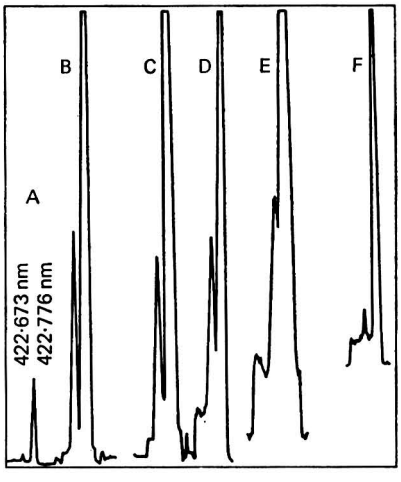

Fig. 2. Effect of monochromator band width on the resolution of the Zr 422·776 nm and Ca 422·673 nm peaks. Zirconium concentration: 10 mg ml^{-1}. Calcium concentration: 0·4 μg ml^{-1} (peaks A to E) and 0·08 μg ml^{-1} (F). Theoretical band widths: 0·016 nm (A); 0·026 nm (B and F); 0·032 nm (C); 0·048 nm (D); and 0·064 nm (E). Recorder scales: 2 mV (A); 5 mV (B and F); 10 mV (C); 25 mV (D and E). Scanning speed: 0·2 nm min^{-1}

To investigate the possible interference by the other alloying metals in Zircaloy-2 on the emission of calcium and zirconium, experiments were carried out with solutions containing 0·1 μg ml^{-1} of calcium together with tin, iron, chromium and nickel at the concentrations corresponding to the maximum amount of each usually specified for Zircaloy-2 (170, 20, 15 and 8 μg ml^{-1}, respectively). The metals were considered both separately and together. Analogous solutions were prepared containing 10 mg ml^{-1} of zirconium in addition to these other metals. The spectra obtained by scanning the wavelength region of interest, with a theoretical band width of 0·026 nm, were compared with those obtained for solutions containing calcium, both alone and with zirconium. No apparent difference was found in any instance.

Similar experiments were carried out to check the possible effect of varying the amounts of reagents used to dissolve the sample. No effect was detected for a two-fold variation of the reagent concentrations.

BACKGROUND CORRECTION—

After checking that the instrumental conditions chosen eliminated interference by the neighbouring zirconium peak on the calcium analytical line, the possibility of carrying out the analysis by setting the monochromator at fixed wavelengths was investigated. This procedure was attractive because of its simplicity and rapidity compared with individual scanning for each sample solution; it implied, however, the finding of a simple correction to the over-all signal for the zirconium continuum background (clearly visible in all spectra in Fig. 2).

Experiments carried out by scanning the emissions of solutions obtained from Zircaloy-2 samples of different weights (0·25 to 0·6 g) showed that the continuum background signal could be taken to be roughly proportional to the amount of zirconium present, and that its value could be reliably measured at a fixed wavelength in the vicinity of the calcium peak towards the shorter wavelengths. Analyses could be performed at fixed wavelengths with no loss of precision or accuracy. The calcium concentrations in different samples of the same Zircaloy-2 specimen were found to be 8·2 $\pm$ 0·7 p.p.m. when the calcium peak heights were evaluated by scanning, and 7·4 $\pm$ 0·8 p.p.m. when the signals obtained at the calcium analytical line were corrected for their respective backgrounds (evaluated at a fixed wavelength near the calcium peak).

By using the fixed wavelength procedure, the analyses of different samples of the same Zircaloy-2 specimen gave an average calcium concentration of 7·00 $\pm$ 0·48 p.p.m. when each signal at the calcium line was corrected for the corresponding background value, and 7·25 $\pm$ 0·51 p.p.m. when signals were corrected for the average background, after taking into account the weight of each sample. The standard deviations of results were 1·3 and 1·2 p.p.m., respectively, while the standard deviation of the background was 0·4 p.p.m. These last results demonstrated that background deviations had little effect on the precision of the over-all results. In a given run of measurements backgrounds can be evaluated for a few samples, and the resulting average value can be used to correct all signals obtained at the calcium line without any loss in precision.

This simplification was included in the recommended procedure as the concentration of calcium in zirconium and Zircaloy-2 usually exceeds 7 p.p.m. In fact, the above average value would not be representative for lower calcium concentrations, as the calcium value corresponding to the standard deviation of the background signal strongly depends on the amount of calcium whose signal has to be corrected, because of the peculiar shape of the calibration graph. The same standard deviation of the background corresponding to 0·4 p.p.m. at the 7 p.p.m. of calcium level varies from 1·3 p.p.m., when no calcium is present, to 0·25 p.p.m., when more than 10 p.p.m. of calcium is present, *i.e.*, in the steepest portion of the calibration graph. Therefore, when very small amounts of calcium have to be determined, individual correction, *i.e.*, correction of each signal for the corresponding background, is recommended.

The reliability of fixed wavelength measurements is strictly dependent on the good resolution of the calcium *versus* zirconium peaks. When the available instrument does not allow a satisfactory resolution, the background on the calcium signal is due not only to the continuum but also to the tail of the zirconium peak, the shape of which is greatly affected by unavoidable small fluctuations in the excitation conditions. In such instances reliable determinations can still be obtained, but only by scanning the region of interest; the analysis is more cumbersome and the background correction more difficult.

With band widths greater than 0·048 nm, direct analysis should not be attempted.

RECOMMENDED PROCEDURE

The following procedure is intended for the routine analysis of samples containing more than 6 p.p.m. of calcium. For lower calcium contents individual background corrections are preferred.

Calibration with calcium solutions run through the entire procedure is recommended to allow for possible contamination during the dissolution steps (see under Accuracy and precision). These solutions should be used to check the calibration for each run of measurements. In routine work, however, a calcium reference solution, prepared by simple dilution of a stock solution as described below, can be used with advantage.

Throughout the whole procedure, only plastics laboratory ware should be used.

CALIBRATION—

The following solutions were used.

Calcium stock solution, 5 mg ml^{-1}—Dissolve 1·25 g of calcium carbonate in about 50 ml of water containing an appropriate amount of hydrochloric acid, make the volume up to 100 ml with water and check the calcium concentration by means of a suitable titration.

Potassium stock solution, 10 mg ml^{-1}—Dissolve 3·82 g of potassium chloride in 200 ml of water.

Calcium reference solution, 0·1 μg ml^{-1}—Dilute the stock solutions so as to obtain 250 ml of solution containing 0·1 μg ml^{-1} of calcium and 1 mg ml^{-1} of potassium.

Prepare solutions containing 1·25, 2·5, 5·0, 7·5 and 12·5 μg ml^{-1} of calcium by suitable dilutions of the calcium stock solution. These solutions should be prepared by independent dilutions of the stock solution.

Place 2 ml of each solution into small polytetrafluoroethylene beakers. To each beaker, and to an additional one containing 2 ml of water, add 2 ml of 0·6 M hydrochloric acid, 1 ml of 50 per cent. hydrofluoric acid and 5 drops of concentrated nitric acid. Boil the mixtures in the beakers gently for about half an hour. Then transfer the cooled solutions into 50-ml calibrated plastics containers, add to each of them 5 ml of the potassium stock solution, and dilute to volume with water.

By using an instrument that possesses characteristics comparable with those reported in Table I, set the monochromator to a band width of 0·026 nm. Set the instrument at the calcium analytical line at 422·673 nm and nebulise each of the calibration solutions and the calcium reference solution in turn, and record the signals. Plot the signals on a graph as a function of the amount of calcium added.

ANALYSIS OF ZIRCONIUM AND ZIRCALOY-2 SAMPLES—

Weigh accurately samples of about 0·5 g, after suitable de-greasing and cleaning; Zircaloy-2 samples can be conveniently de-greased with trichloroethylene and pickled by immersion for 2 minutes in an aqueous solution containing 32·5 per cent. w/v of nitric acid and 2·5 per cent. w/v of hydrofluoric acid.

Place the samples into small polytetrafluoroethylene beakers together with 2 ml of 0·6 M hydrochloric acid, adding to each beaker sufficient water to immerse the sample completely. Add 1 ml of 50 per cent. hydrofluoric acid in 3 or 4 portions, gently warming the beaker to assist dissolution. When the sample is completely dissolved, add 5 drops of concentrated nitric acid to clear the solution. Transfer the cooled solutions into 50-ml calibrated plastics containers, add 5 ml of the potassium stock solution and dilute to volume with water.

Control the instrumental conditions, checking that the signal for the reference solution at the calcium analytical line is as close as possible to the signal obtained when deriving the calibration graph.

Set the instrument to a wavelength in the vicinity of the calcium peak towards the shorter wavelengths, and record the signals obtained, nebulising two or three sample solutions.

Set the instrument back to the calcium analytical line and record the signal obtained by nebulising the reference and sample solutions. When a large number of determinations are carried out, periodically check the constancy of the conditions with the reference solution (a shift of the peak positioning on the monochromator is sometimes observed, probably caused by small mechanical vibrations or slight temperature changes).

CALCULATIONS—

Subtract the zirconium background to each signal obtained at the calcium analytical line. For a sample of weight W_S, this background is $\bar{S}_B W_S / \bar{W}_B$, where $\bar{S}_B$ is the average of the signals obtained in the vicinity of the calcium peak and $\bar{W}_B$ is the average weight of the corresponding samples. Multiply the resulting figures by the ratio between the signals obtained for the calcium reference solution when deriving the calibration graph and those obtained when performing the actual run of analyses. Read off the resulting figures from the calibration graph to obtain the amounts of calcium present in the samples.

ACCURACY AND PRECISION—

The accuracy of the proposed method strongly depends on the possible calcium contamination of the sample solutions. Great care must be taken during chemical manipulations;

only plastics laboratory ware can be used and reagents must be carefully chosen for their purity and freedom from calcium.

However much care is taken, a small degree of contamination by calcium is unavoidable, and accurate determinations must allow for the average contamination of the solutions. This allowance is best made by plotting the calibration graph with results from calcium solutions that have been taken through the entire procedure, unless a suitable statistical analysis demonstrates that this can be simplified. In our experiments, for example, involving the use of solutions with known amounts of added calcium, no difference in average calcium content was detected between solutions run through the entire procedure and others that had not undergone the treatment relating to the dissolution of actual samples. Statistical tests demonstrated that the standard deviations of the results were also comparable.

To verify that the calibration graph obtained with synthetic solutions containing only calcium was also valid for solutions of actual samples, known amounts of calcium were added to solutions in which 0·5 g of zirconium or Zircaloy-2 was dissolved; because of the difficulty of obtaining metallic samples of the same weight, and to minimise contamination, suitable aliquots of the same original solution were used in each run. Results obtained with nuclear-grade Zircaloy-2 are plotted in Fig. 1 (square symbols). It appears that the signals obtained fit well the dotted line shown in the figure. This is the calibration graph obtained with calcium alone, shifted to the left to allow for the amount of calcium originally present in the samples, as found with the solutions to which no calcium was added. This demonstrates the good accuracy of the over-all method, and in particular, the reliability of the lower portion of the calibration graph. Analogous results were obtained with spectrographically pure zirconium. Taking into account the amount of calcium found to be present in the samples (10 p.p.m. on average, *i.e.*, 0·1 μg ml^{-1} in the analysed solutions), averages of 0·025 and 0·49 μg ml^{-1} of calcium were found when 0·025 and 0·50 μg ml^{-1} had been added, respectively.

The precision of the proposed method depends essentially on the level of calcium contamination. Deviations between different sample solutions were found to be much greater than deviations between results obtained when a given sample solution was analysed several times, either in the same run of measurements or in different independent runs. For example, in three instances at the 7 p.p.m. of calcium level, standard deviations were 1·3 (27 degrees of freedom), 0·26 (7 degrees of freedom) and less than 0·01 p.p.m. (8 degrees of freedom).

TABLE II

PRECISION OF THE PROPOSED METHOD

Theoretical band width/nm	Material	Calcium level, p.p.m.	Standard deviation, p.p.m.	Coefficient of variation, per cent.	Degrees of freedom
0·026	Zircaloy-2	7	1·3	19	27
	Zirconium	10	1·8	18	3
	Zircaloy-2	12	1·5	13	8
	Zircaloy-2	22	0·8	3·5	2
0·032	Zircaloy-2	10	1·0	10	7
	Zircaloy-2	17	0·8	5	7
0·048	Zirconium	7	0·5	7·4	2
	Zircaloy-2	10	$0·3_5$	3·5	1
	Zircaloy-2	26	1·2	4·5	3

Standard deviations obtained with 0·026 nm band width refer to the recommended procedure; otherwise, actual calcium peak heights were evaluated by scanning. Figures referring to calcium levels higher than 12 p.p.m. were obtained with samples to which calcium was added during dissolution.

In Table II, representative precision figures are given as the standard deviations of independent determinations, calculated from sets of results obtained in a particular run of measurements. Different specimens of Zircaloy-2 and spectrographically pure zirconium were considered. Results for calcium concentrations greater than 12 p.p.m. refer to samples to which were added suitable amounts of calcium during dissolution. Owing to the unusual character of the source of deviations, the hypothesis of the normal distribution of results was statistically checked by χ^2 tests on several series of values, satisfactory results being obtained in all instances.

TABLE III

PRECISION OF ALTERNATIVE METHODS

Method	Calcium level, p.p.m.	Coefficient of variation, per cent.	Reference
Direct spectrography, Ga_2O_3 carrier distillation in d.c. arc ..	>10	13 to 20	5
Direct spectrography, arc excitation of oxidised samples mixed with resin	30	20	9
Ion-exchange separation, flame spectrophotometry	30	17	17
Ion-exchange separation, atomic-absorption spectrophotometry	30	17	17
Separation by $ZrCl_4$ distillation, flame photometry	20	10	15
	10	28	18

The best precision figures for the direct spectrographic determination of calcium in zirconium and its alloys are given in Table III, together with all those available in the literature for methods involving a preliminary separation of calcium from zirconium. The 1 p.p.m. standard deviation for the spectrographic determination of 30 p.p.m. of calcium in Zircaloy by the rotating graphite disc technique is not included in Table III because this value refers to aliquots of an original solution and not to independent samples.[6]

It appears that the precision of the proposed method compares favourably with all the figures reported in Table III.

CONCLUSIONS

The use of the nitrous oxide - acetylene flame in emission spectrophotometry provides a simple and rapid method for the direct determination of calcium in zirconium and Zircaloy-2, with improved precision compared with that of other available methods, either direct or involving a preliminary separation of calcium from the bulk of zirconium.

The method is likely to be extended to the determination of calcium present in other zirconium alloys.

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The Use of a Mixed-solvent System for the Determination of Calcium and Zinc in Petroleum Products by Atomic-absorption Spectroscopy*

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A mixed-solvent system that permits the use of inorganic compounds as standards has been applied to the determination of calcium and zinc in unused lubricating oils and automatic transmission fluids by atomic-absorption spectroscopy. By the incorporation of hydrochloric acid into the solvent system, it has been found possible to eliminate the systematic errors that may occur when an air - acetylene flame is used in the determination of calcium.

Results have been obtained for a wide range of unused lubricating oils and automatic transmission fluids and there is good agreement with those obtained by X-ray fluorescence and established Institute of Petroleum chemical procedures (I.P. 111/49T Method B and I.P. 117/66T Method B).

FLAME methods of analysis are a means of enabling solutions of petroleum products in organic solvents to be analysed directly for metallic constituents. The use of organic solutions offers a rapid means of analysis compared with conventional atomic-absorption spectroscopic techniques, which require a time-consuming wet-oxidation stage.

Previously, when atomic-absorption spectroscopy was used in conjunction with simple solvent systems, metal naphthenate standards and the air - acetylene flame, the accuracy of the results was always suspect because the metal additive could differ from the metal standards in either chemical or physical form.

We have therefore concentrated on applying and improving two advances in technique that were described previously,[1] *viz.*, the use of a 90 + 10 mixed-solvent system of isopropyl alcohol - toluene, by which a small amount of oil could be rendered compatible with an aqueous inorganic salt solution, and the incorporation of glacial acetic acid into the solvent, to eliminate the systematic errors that may arise when an air - acetylene flame is used in the determination of calcium in lubricating oils.

Our complexometric work had indicated that it was possible to devise solvent mixtures that were superior to the isopropyl alcohol - toluene mixture, and we based our new mixed solvent on cyclohexanone because of its desirable burning qualities. After a number of simple compatibility tests, we selected a mixture consisting of 50 volumes of cyclohexanone, 30 volumes of butyl alcohol and 20 volumes of industrial methylated spirit. This improved solvent will accommodate, as a homogeneous solution, 10 ml of an aqueous solution of an inorganic salt together with up to 300 mg of lubricating oil per 100 ml of solution. In addition, to eliminate systematic errors, we are now able to incorporate hydrochloric acid into the system instead of the glacial acetic acid mentioned above.

The advantage of being able to incorporate inorganic salt solutions into the mixture is illustrated by considering current practice. Thus, for samples of unknown composition it has been necessary either to carry out a wet-oxidation stage or to use organic salts (naphthenates, cyclohexanebutyrates, etc.) of metals as standards. These organic compounds, although soluble in simple solvents, are not always readily available, and may be expensive or require expensive accurate analysis. On the other hand, inorganic salts are cheap and readily available and can be checked for purity conveniently and quickly.

The presence of acid is necessary to match the metal compounds in the sample solutions with those in the reference solutions. Metal-based additives can vary widely in composition and physical form and, when standards and sample are dissimilar, the differences may lead to

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systematic errors. Our earlier work showed that the discrepancy between the result for a calcium determination obtained, for instance, by atomic-absorption spectroscopy (with an air - acetylene flame, cyclohexanone as solvent and calcium naphthenate as standard), and that obtained by an established chemical procedure, can be as much as 50 per cent. of the required chemical value (see results for sample 8 in Table I). Investigations showed that the interference was not caused by the matrix of the sample (as would normally be expected), but by differences between the calcium compounds. We concluded that the low result was obtained either because the calcium additive had a larger particle size than normal or because it was shielded in some manner that prevented complete atomisation during its brief residence time in the flame. By incorporating a high concentration of glacial acetic acid into the solvent, this systematic error was minimised and the result then agreed with that obtained by chemical analysis.

To achieve the necessary high concentration of acetic acid, 40 ml of glacial acetic acid had to be added to each 100 ml of sample and standard. This operation had to be carried out in a fume cupboard and gave a rather obnoxious solution. Advantage was taken of the change from a purely organic solvent system to the new, partially aqueous, one to replace the acetic acid with hydrochloric acid; this had the additional advantage that only 5 ml of acid had to be added to each 100 ml of solution. The final solution was approximately 0·5 N in hydrochloric acid. The nebuliser could have been prone to corrosion but to minimise any tendency for this to occur we flushed the burner system thoroughly with mixed solvent or distilled water at frequent intervals. Extensive use of this system has not caused any noticeable deterioration in the spraying qualities of the nebuliser, or the need for its frequent replacement. The burner heads on most commercial atomic-absorption spectrophotometers are made from stainless steel and the spray chambers are lined either with glass or a corrosion-resistant coating. We have not experienced any corrosion problems caused by using the acidic mixed solvent.

The work described in the present paper is concerned with the determination of zinc and calcium in unused lubricating oils and automatic transmission fluids by atomic-absorption spectroscopy with an air - acetylene flame, and was carried out in order to assess the usefulness of inorganic salts as standards and the general applicability of the acidic mixed-solvent system in obviating systematic errors.

Determination of calcium in unused lubricating oils and automatic transmission fluids by an atomic-absorption spectroscopic procedure with an inorganic salt - acidic mixed solvent

Apparatus—

The instrument used was a Techtron AA3, equipped with a digital indicator unit. The fuel used was an air - acetylene mixture, the wavelength was 422·7 nm and the slit width was 25 μm.

Preparation of sample—

Weigh into a 50-ml beaker an amount of lubricating oil containing approximately 0·5 mg of calcium. To this add approximately 10 ml of a mixed solvent consisting of 50 volumes of cyclohexanone, 30 volumes of butyl alcohol and 20 volumes of industrial methylated spirit. Add 5 ml of concentrated hydrochloric acid with a pipette and shake the mixture. Heat the mixture to approximately 40 °C and transfer it into a 100-ml calibrated flask by use of approximately 60 ml of mixed solvent, add 5 ml of water by means of a pipette, and shake it again. Cool the mixture to room temperature and make it up to the calibration mark with the mixed solvent.

Preparation of standards—

By means of an Agla syringe-burette, add 0, 0·1, 0·2, 0·3 and 0·4 ml of a 2000 mg l^{-1} standard aqueous solution of calcium to successive 100-ml calibrated flasks. This standard calcium solution is prepared by dissolving calcium carbonate, which has been oven-dried for at least 1 hour at 105 °C, in 100 ml of 2 N hydrochloric acid, and standardised by complexometric titration. Add 5 ml of water and 5 ml of concentrated hydrochloric acid to each flask with a pipette and make up to the calibration mark with mixed solvent.

Determine the calcium content of the sample by atomic-absorption spectroscopy, comparing the sample with standards in the usual manner.

A fuel-lean air - acetylene flame is used for the determination; this flame gives lower sensitivity than an acetylene-rich flame but is necessary to obtain good precision. For the most concentrated standard, an absorbance approximating to 50 per cent. transmission is obtained under these conditions.

Determination of zinc in unused lubricating oils and automatic transmission fluids by an atomic-absorption spectroscopic procedure with an inorganic salt - acidic mixed solvent

Apparatus—

The instrument used was a Techtron AA3, equipped with a digital indicator unit. The fuel used was an air - acetylene mixture, the wavelength was 213·9 nm and the slit width was 300 μm.

Preparation of sample—

Weigh directly into a 100-ml calibrated flask an amount of oil containing approximately 0·1 mg of zinc. Add approximately 60 ml of the mixed solvent, consisting of 50 volumes of cyclohexanone, 30 volumes of butyl alcohol and 20 volumes of industrial methylated spirit. Add 5 ml of concentrated hydrochloric acid with a pipette and shake the mixture thoroughly, then add 5 ml of water by means of a pipette and shake it again. Finally, make up to the calibration mark with the mixed solvent. No heating is required.

Preparation of standards—

From an Agla syringe-burette, add 0, 0·1, 0·2, 0·3 and 0·4 ml of a 500 mg l^{-1} standard aqueous solution of zinc (prepared by dissolving metallic zinc in 20 ml of 6 N hydrochloric acid) to successive 100-ml calibrated flasks. Add 5 ml of water and 5 ml of concentrated hydrochloric acid with a pipette. Make up to the calibration mark with mixed solvent.

Determine the zinc content of the sample by atomic-absorption spectroscopy, comparing the sample with standards in the usual manner.

A fuel-lean air - acetylene flame is used for the determination to give good precision. For the most concentrated standard, an absorbance approximating to 50 per cent. transmission is obtained under these conditions.

For certain blends the concentrations may be such as to make it possible to determine calcium and zinc on the same aliquot. In this situation the dissolution procedure for the calcium determination should be used; the heating stage does not affect the recovery of zinc.

Results

Determination of calcium in unused lubricating oils and automatic transmission fluids—

We applied the procedure with the acidic mixed solvent - inorganic salt standards to the analysis of twenty-three samples of lubricating oils and automatic transmission fluids from various sources.

For the preparation of standard solutions, an Agla-type hypodermic syringe was used to deliver small but repeatable volumes of a 2000 mg l^{-1} standard calcium solution. Although this last process entailed the addition of 0·4 ml of water to the standard solution in excess of that added to the sample (*i.e.*, 5·0 ml to the sample solution and 5·4 ml to the standard), no significant difference was evident in the spraying rate or in the results obtained, and this volume discrepancy was therefore ignored in subsequent work.

Two oils, samples 1 and 3, gave slightly low results when compared with the figures obtained by the use of established procedures (Table I). However, in subsequent work, heating the solution of the sample to 40 °C in the pre-treatment procedure was effective in obtaining full recoveries. The heating stage is now recommended as part of the preparation of all the samples as no adverse effect was observed in those instances in which full recovery had been obtained without heating.

The results obtained by this procedure are given in Table I and also, for comparison, the results obtained by established procedures.

TABLE I

DETERMINATION OF CALCIUM IN LUBRICATING OILS

Sample number	Elements of interest present	Calcium, per cent. w/w: Results by established procedures and/or specification values	Calcium, per cent. w/w: A.A.S. method—inorganic standard and acidic mixed solvent, no heating	Calcium, per cent. w/w: A.A.S. method—inorganic standard and acidic mixed solvent after heating
1	Zn, Ca and P	0·130 (IP) 0·134 (AAS/D) 0·125 (AAS/DOA)	0·123, 0·125	0·125, 0·124
2	Zn, Ca, Mg and P	0·093 (IP) 0·094 (AAS/D) 0·095 (AAS/DOA)	0·088, 0·087	0·093, 0·090
3	Zn, Ca and P	0·145 (IP) 0·145 (AAS/D) 0·136 (AAS/DOA)	0·134, 0·135	0·140, 0·143
4	Zn, Ca, Ba and P	0·198 (Spec.)	0·182, 0·186	0·181, 0·183
5	Zn, Ca, Ba and P	0·242 (X)	—	0·231, 0·227
6	Zn, Ca, Ba and P	0·076 (IP) 0·080 (AAS/DOA)	—	0·073, 0·075
7	Ca and P	0·069 (IP) 0·066 (Complexo.)	—	0·064, 0·064
8	Zn, Ba, Ca and P	0·230 (IP) 0·108 (AAS/D) 0·236 (AAS/DOA)	—	0·234, 0·237
9	Ca, Mg and P	0·058 (Calc.)	—	0·058, 0·058
10	Ca, Mg and P	0·122 (Calc.)	—	0·114, 0·116
11	Ca and P	0·167 (X)	—	0·161, 0·166
12	Zn, Ca and P	0·130 (X) 0·135 (Complexo.)	—	0·135, 0·134
13	Zn, Ca and P	0·480 (X) 0·483 (Complexo.)	—	0·476
14	Ca and P	0·277 (IP) 0·268 (X)	—	0·263, 0·262
15	Zn, Ca, Ba and P	0·271 (Complexo.) 0·265 (X)	—	0·259, 0·261
16	Zn, Ca, Ba and P	0·401 (IP)	—	0·398, 0·399
17	Ca + unknown	0·287 (IP)	—	0·271, 0·269
18	Ca + unknown	0·159 (IP)	—	0·159, 0·159
19	Ca + unknown	0·169 (IP)	—	0·163, 0·165
20	Ca + unknown	0·167 (IP)	—	0·167
21	Zn, Ba, Ca and P	0·089 (X) 0·090 (IP) 0·12 (ES)	—	0·089, 0·087
22	Zn, P and Ca	0·183 (X) 0·190 (IP) 0·18 (ES)	—	0·179, 0·183
23	Zn, P, Ca and Ba	0·218 (X) 0·230 (IP) 0·29 (ES)	—	0·217, 0·221

IP: I.P. 111/49T Method B.
AAS/D: atomic-absorption spectroscopy after direct dilution with cyclohexanone; naphthenate standards.
AAS/DOA: atomic-absorption spectroscopy after direct dilution with cyclohexanone; glacial acetic acid and naphthenate standards.
Spec.: mid-point specification.
X: X-ray fluorescence.
Complexo.: complexometric titration in organic media.
Calc.: calculated from the calcium additive content.
ES: emission spectrography.

DETERMINATION OF ZINC IN UNUSED LUBRICATING OILS AND AUTOMATIC TRANSMISSION FLUIDS—

Sixteen samples of lubricating oils and automatic transmission fluids were analysed with the acidic mixed-solvent system and with inorganic salt standards and the results are shown in Table II.

It was still found necessary for the acid to be present in the solvent mixture as, when the samples and standards were analysed without hydrochloric acid, low zinc concentrations were obtained (Table II). Heating during sample preparation was not necessary for any of the oils that were analysed.

TABLE II

DETERMINATION OF ZINC IN LUBRICATING OILS

Sample number	Elements of interest present	Zinc, per cent. w/w: Results by established procedures	Zinc, per cent. w/w: A.A.S. method—inorganic standard and mixed solvent but without hydrochloric acid	Zinc, per cent. w/w: A.A.S. method—inorganic standard and acidic mixed solvent
1	Zn, Ca and P	0·043 (IP)	—	0·044, 0·042
2	Zn, Ca, Ba, P and W	0·074 (IP)	—	0·071, 0·073
3	Zn, Ca and P	0·068 (IP)	—	0·069, 0·069
4	Zn, Ca, Ba and P	0·162 (X) 0·164 (Complexo.)	—	0·178, 0·179
5	Zn, Ca and P	0·103 (IP)	0·056	0·106, 0·106
6	Zn, Ca, Ba and P	0·055 (Complexo.)	—	0·054, 0·054
7	Zn, Ca and P	0·080 (IP) 0·082 (AAS/D)	—	0·080, 0·080
8	Zn, Ca, Mg and P	0·099 (IP)	—	0·096, 0·096
9	Zn + unknown	0·036 (IP)	—	0·036, 0·036
10	Zn and P (?)	0·306 (IP) 0·290 (Complexo.)	0·221	0·312, 0·313
11	Zn, Ca, Ba and P	0·050 (IP)	—	0·052, 0·052
12	Zn, Ca and P	0·094 (X) 0·106 (Complexo.)	—	0·106, 0·107
13	Zn, Ca, Ba and P	0·136 (X) 0·130 (IP) 0·14 (ES)	—	0·144, 0·145
14	Zn, Ca and P	0·074 (X) 0·080 (IP) 0·07 (ES)	—	0·078, 0·079
15	Zn, Ca and P	0·036 (X) 0·040 (IP) 0·04 (ES)	—	0·041, 0·040
16	Zn, Ca, Ba and P	0·143 (X) 0·150 (IP) 0·16 (ES)	—	0·155, 0·154

IP: I.P. 117/66T Method B.
AAS/D: atomic-absorption spectroscopy after direct dilution with cyclohexanone; naphthenate standards.
X: X-ray fluorescence.
Complexo.: complexometric titration in organic media.
ES: emission spectrography.

DISCUSSION

It is evident that the results for the determination of both zinc and calcium with the inorganic salt - acidic mixed-solvent system used with an air - acetylene flame are in good agreement with the values obtained by use of established procedures. It is also evident that

there is no inter-element effect in the determination of calcium in this work. When an air - acetylene flame is used in the determination of calcium in aqueous solution, phosphate causes interference owing to the formation of refractory calcium phosphate. However, in purely organic solutions organophosphates, *e.g.*, arylphosphates or dialkyldithiophosphates, have no effect on the determination of calcium. Similarly, in the mixed-solvent system there is no interference from organophosphates during the determination of calcium.

A slight discrepancy can be seen between the values for the zinc content of sample 4 (Table II) as given by the different techniques. The X-ray fluorescence result, which is supported by the complexometric value, is lower than the result obtained by use of the technique under discussion. In our previous work there were indications that the result for the complexometric determination of zinc in automatic transmission fluids containing significant amounts of barium and polymer (as sample 4 does) can be approximately 5 per cent. lower than results obtained with the Institute of Petroleum (I.P.) procedure. We intend to investigate this discrepancy, which, although significant, is not too serious when it is considered that we are comparing results obtained by different techniques and different operators.

The procedure recommended by the Atomic Absorption Panel of the Institute of Petroleum covers the determination of barium, calcium and zinc in unused lubricating oils. White spirit is used as the solvent, with metal naphthenates as standards and potassium naphthenate as the ionisation suppressant. The nitrous oxide - acetylene flame, which is much hotter than the air - acetylene flame, is used for the determination of all three metals. The use of this flame is essential for the determination of barium and it minimises the type of systematic error encountered in our earlier work on the determination of calcium in which an air - acetylene flame was used. The hotter flame is also used in the determination of zinc because it is convenient to continue to use the same flame. However, there are disadvantages with the nitrous oxide - acetylene flame as its use is rather hazardous and should therefore be avoided for routine applications. It is also a particularly "noisy" flame, so that even with careful operation the precision can be inadequate. In addition, the burners at present in general use are subject to carbon build-up along the slit, and even the latest burner heads do not eliminate this problem but only provide longer burning periods between frequent and necessary shutdowns for cleaning.

Our work, in which the air - acetylene flame was used, showed that particular attention must be given to fine and steady control of the gas flow in order to minimise small, but significant, flame fluctuations. The population of calcium atoms in the flame is particularly dependent on the flame temperature, and we found that very slight fluctuations in gas flow markedly affected readings. It is also desirable for an experienced operator to carry out the work when good precision is required, as a critical attitude must be maintained throughout the operation.

Conclusions

Previously, when the simple solvent systems, metal naphthenate standards and air - acetylene flame were used, the accuracy of the results for samples containing additives of unknown chemical or physical form were often suspect. Now, in addition to permitting cheap inorganic standards to be used, the acidic mixed-solvent system has been shown to be effective in standardising solutions so that systematic errors are obviated.

Our work was initially carried out to establish the usefulness of the inorganic salt - acidic mixed-solvent system with samples of unknown chemical or physical composition. However, its successful outcome offers further promise. Hitherto, we have had doubts concerning the precision of the atomic-absorption spectroscopic technique for the analysis of lubricating oils, but our results now indicate that the precision of the technique when an air - acetylene flame is used can be adequate for the control of blending of calcium and zinc in unused lubricating oils and automatic transmission fluids.

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Silver Contamination from an Electric Furnace

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Silver contamination encountered in a synthetic base, which is used in the preparation of standards for the spectrographic analysis of rocks and soils, was successfully traced to the silver thermal fuse of an electric furnace used during the sintering process. The degree of contamination is related to temperature.

SPECTROGRAPHIC-EMISSION techniques applied to the trace determination of elements in rocks and soils frequently involve the use of synthetic standard bases. In order to reduce matrix effects to the minimum, it is strongly recommended that the chemical compositions of the standard and the sample be as similar as possible. Furthermore, sintering of the synthetic bases is desirable in order to simulate the physical characteristics of the samples and to improve arcing quality.

There are many sources of contamination inherent in the preparation of such a synthetic mixture. Ahrens and Taylor[1] and Mitchell[2] drew attention to the risk of impurities in such base ingredients, and Mitchell suggested methods of purification. Thompson and Bankston[3] dealt fully with contamination in the grinding and mixing processes. There is little reference in the literature to the electric furnace as a source of contamination. Mitchell[4] recommended the use of a silica lining and Sandell[5] indicated the danger of contamination by atmospheric dust or by substances that have been volatilised from the interior of the furnace during dry ashing; the latter effect was observed by Williams and Vlamis[6,7] for boron. Maxwell[8] pointed out the risk of the occurrence of metallic dust in the heating chamber, originating from covered heating elements.

In the preparation of a synthetic standard base for use in the analysis of rocks and soils we encountered contamination by silver and copper. In terms of the normal range of silver (0·05 to 0·50 p.p.m.) and copper (2 to 100 p.p.m.) contents in silicates, the level of contamination observed was regarded as serious. The high levels of silver were traced to the silver thermal fuse of an electric furnace that was used for sintering at 900 °C. Such fuses melt at about 1000 °C. This specific type of contamination has not, to our knowledge, been previously reported.

EXPERIMENTAL AND RESULTS

In the investigation of this contamination by silver, 0·5-g portions of Specpure aluminium oxide (Johnson Matthey) were heated at 500, 600, 700, 800 and 900 °C for 30 minutes. The heating was carried out in platinum basins in a Vitreosil D-shaped muffle closed at the rear in an electric fireclay-lined muffle furnace with the thermal fuse fitted internally. The fuse was enclosed in a twin-bore fireclay sleeve, surrounded by an open-ended refractory sheath. About 6 mm of the fuse was exposed. The assembly is shown in Fig. 1. The process was repeated with the fuse fitted externally. The aluminium oxide was then examined spectrographically by using a modification of the method of Tennant.[9] The results are shown in Table I. The process was repeated in a second furnace and similar results were obtained.

The contamination by silver resulting from heating at 900 °C with the fuse fitted internally clearly identifies the fuse as the main source of the contamination by silver. The contamination in the other instances seems to indicate that the silver was volatilised from the interior of the furnace, which itself had been previously contaminated. Such contamination is assumed to have arisen during previous operations at temperatures of the order of 800 °C, which is not surprising as silver begins to volatilise at about 850 °C. The contamination by copper appears to be derived only from the interior of the furnace as there is only a trace amount of copper present in the thermal fuse. The possibility that some contamination arises

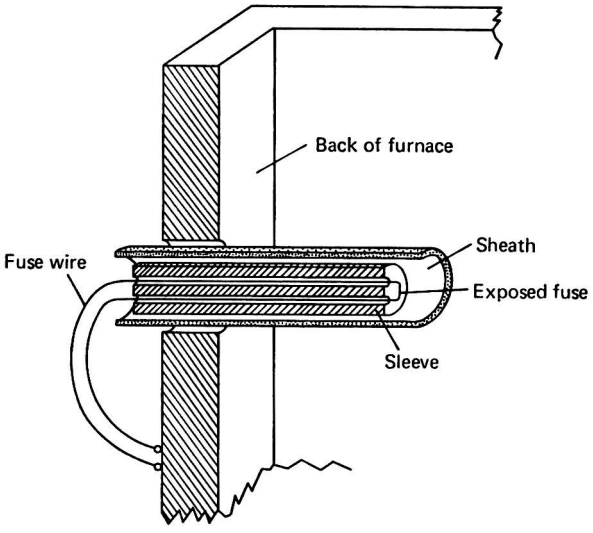

Fig. 1. Thermal fuse assembly

from the Vitreosil muffle can be ruled out as a new muffle gave similar results. Heating at 900 °C without the use of the Vitreosil muffle resulted in severe contamination by copper and silver, with nickel, lead, zinc and vanadium as trace contaminants. The risk of loss of these elements during dry-ashing procedures is well known.[10,11]

Under the above conditions, the sintering at temperatures above 700 °C of base material for use in the preparation of standards for the determination of silver (and copper) cannot be recommended. Although a cover on the platinum vessel should reduce contamination, the condensation of silver on the outside of the vessel would still present risks.

When sintering was carried out in a covered platinum crucible over a large Meker burner at a temperature of about 1200 °C no contamination occurred.

TABLE I

CONTAMINATION OF ALUMINIUM OXIDE DURING SINTERING

Temperature of sintering/°C	Thermal fuse inside furnace		Thermal fuse outside furnace	
	Contaminant	Amount present, p.p.m.	Contaminant	Amount present, p.p.m.
500	Silver	N.D.	Silver	N.D.
	Copper	N.D.	Copper	N.D.
600	Silver	N.D.	Silver	N.D.
	Copper	N.D.	Copper	N.D.
700	Silver	N.D.	Silver	N.D.
	Copper	N.D.	Copper	N.D.
800	Silver	0·5 to 0·8	Silver	0·5 to 0·8
	Copper	2·0 to 5·0	Copper	2·0 to 5·0
900	Silver	1·5 to 2·0	Silver	0·5 to 0·8
	Copper	2·0 to 5·0	Copper	2·0 to 5·0

N.D. Not detectable.

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The Continuous Determination of Sodium in High Purity Water by Using a Sodium Monitor Incorporating a Sodium-responsive Glass Electrode

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An Electronic Instruments Limited sodium monitor, Model 89C, incorporating a sodium-responsive glass electrode, has been shown to be basically suitable for use in power stations to determine continuously the sodium contents of steam and boiler feed and make-up waters, except when filming amines (*e.g.*, octadecylamine) are present.

Details are given of the tests required to show the essentially Nernstian response of the electrode to changes in sodium concentrations; a regular standardisation procedure is also described.

WEBBER and Wilson[1] have shown that a GEA 33 (Electronic Instruments Limited) sodium-responsive glass electrode was satisfactory for measuring sodium concentrations down to 1 μg l^{-1} in high purity water. They found that the electrode potential obeyed the Nernst equation down to this concentration when the electrode was used in a continuously flowing sample system, with ammonia vapour injected to raise the pH of the sample sufficiently to eliminate interferences from hydrogen ions, *i.e.*, to pH 10·5 to 11·0. This method was developed for the analysis of discrete samples, but it was envisaged that there would be many applications for a continuous on-stream sodium monitor. This type of instrument was developed by Electronic Instruments Limited and a collaborative testing programme was arranged with the Central Electricity Research Laboratories at Leatherhead. The instrument was installed at Croydon "B" Power Station, and this paper describes the instrument and the tests carried out to show its basic suitability for power-station applications.

EXPERIMENTAL

THE ANALYSER—

The basic system consists of a panel carrying the flow cell, electrodes and associated sampling equipment (shown in Fig. 1), the main control chassis and the electronic modules. The modules, which are sealed in a case above the panel, include an amplifier (No. 9914), an "Auto-Compensation" unit (No. 9902) and a meter calibrated to show the concentration of sodium ions. A galvanometric strip-chart recorder is also provided.

A thermostatically controlled 3-kW fan heater is installed inside the instrument cabinet to maintain stable temperature conditions. Suitable reagent containers are situated on a shelf inside the cabinet.

The sample is pumped continuously at a flow-rate of 4 ml min^{-1} by a peristaltic pump through a mixing chamber that contains a sodium-responsive glass electrode (GEA 33) and a liquid junction tube connected to the reference electrode (CZ 68). A separate pump feeds ammonia vapour [obtained by aspirating air through ammonia solution (sp. gr. 0·88) contained in a Drechsel flask] into the sample line to maintain the alkaline pH necessary during measurement of the concentration of sodium ions. The potential developed between the sodium-responsive electrode and the reference electrode is logarithmic with respect to the concentration of sodium ions. This potential is amplified and the output signal is displayed on the meter and recorder.

The sodium monitor is normally calibrated over two concentration ranges: Range 1, 0·0001 to 0·1 p.p.m., and Range 2, 0·1 to 10 p.p.m.

Modifications—The monitor automatically standardises on Range 2 irrespective of the range selected for measurement. To enable the test programme to be performed throughout

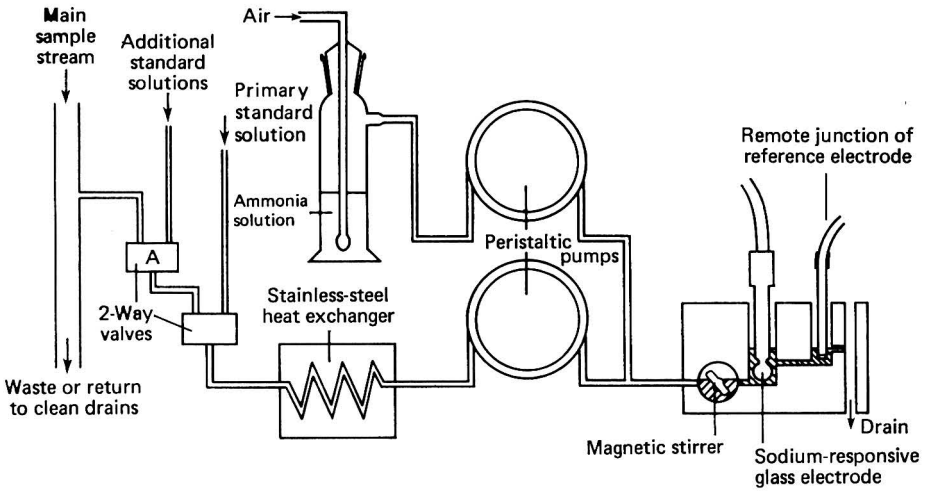

Fig. 1. Schematic diagram of the analytical system of the E.I.L. sodium monitor (89C). Valve A was added for testing purposes by Central Electricity Research Laboratories

on a single range, the instrument was modified to standardise automatically on Range 1, which itself was altered to read 0·0005 to 0·5 p.p.m. To facilitate the introduction of standard sodium solutions, a two-way valve was inserted into the sample line immediately before the heat exchanger.

Standard sodium solutions—A stock sodium solution containing 23 μg ml^{-1} of sodium was prepared by dissolving 0·117 g of dry sodium chloride in a suitable volume of water and diluting the solution with water to a volume of 2 litres in a calibrated flask. This solution was stored in a polythene bottle and more dilute standard sodium solutions were prepared from it by dilution with de-ionised water when required. All the solutions were stored in cleaned polythene containers and were found to be stable, within experimental error, for several weeks.

PRELIMINARY TESTS—

The instrument was installed in a laboratory with the ambient temperature controlled at 21 °C, and the instrument cabinet was thermostatically controlled at 32 °C. The response of the instrument to standard solutions of sodium was tested as follows.

A series of solutions was prepared to contain the following amounts of added sodium: 0, 11·1, 27·8, 55·5 and 222 μg l^{-1}; the concentrations were checked flame photometrically by the method of Webber and Wilson.[2] Subsequently, on three occasions over a period of 4 days, the concentrations of the first four solutions were measured on the sodium monitor, the 222 μg l^{-1} sodium solution being used for standardising the instrument. The results obtained are given in Table I, which shows that there was reasonable agreement between the flame-photometric and electrode results, the average difference between them being 1·6 μg l^{-1}.

TABLE I

RESPONSE TO SOLUTIONS OF KNOWN SODIUM CONCENTRATIONS

Concentration of sodium added to water/μg l^{-1}	Concentration of sodium/μg l^{-1}				
	Determined by flame photometry*	Determined by sodium electrode			
		Day 1	Day 2	Day 3	Mean
0	0·9 (0·5)	2·8	3·3	2·6	2·9
11·1	11·9 (0·7)	13·1	13·5	13·7	13·4
27·8	28·4 (0·9)	30·0	30·0	28·4	29·5
55·5	55·7 (1·1)	56·2	52·0	53·2	53·8

* The values in parentheses are standard deviations of individual results.

The response of the electrode depends on the temperature of the sample solution, and to ensure that precise results are obtained it is necessary to control this temperature. To test the efficiency of the stainless-steel heat exchanger in the instrument (at 32 °C), samples were presented to the instrument at three temperatures (1, 32 and 50 °C) and the temperatures in the electrode compartment were measured; these were found to be 29, 32 and 32·7 °C, respectively. In a second experiment, the temperature of the cabinet was increased to 39 °C and, after standardisation, solutions with sodium concentrations of 20 and 5 μg l^{-1} (both at 21 °C) were analysed, firstly with temperature equilibration through the heat exchanger and secondly with the heat exchanger being by-passed. The temperatures of the water passing through the cell were 38 and 35 °C for the first and second analyses, respectively. No significant changes were observed in the response of the electrode to either solution at the two temperatures.

TESTS IN A POWER STATION—

The instrument was sited adjacent to the condensate extraction pump of turbo-generator No. 1 at Croydon "B" Power Station and a continuous flow of feed-water was supplied to the instrument. To remove the larger debris from the sample, an in-line filter containing a porous polythene disc with a pore size of 25 μm was used.

The instrument was operated continuously at this site for 9 months, during which period a number of long-term tests were carried out and the over-all availability and maintenance requirements were assessed.

Availability and maintenance—Apart from routine maintenance, which consisted of replacing the ammonia solution (every 4 weeks) and the pump tubing (every 8 weeks), and occasions when the instrument was being standardised (on average four times per week, requiring 30 minutes per standardisation), the instrument was fully available.

However, on a few occasions, the validity of the results was suspected and tests revealed that the errors were due to the non-theoretical (non-Nernstian) response of the electrode. When these errors occurred, the sensitivity invariably decreased and falsely high values for sodium concentration were obtained, *e.g.*, a solution containing 11·6 μg l^{-1} of sodium (determined by flame photometry[2]) gave a result of 15 μg l^{-1} after the instrument had been standardised by using a standard solution containing 200 μg l^{-1} of sodium.

Because the change from Nernstian to non-Nerstian response can occur rapidly (and normally irreversibly) after several weeks of satisfactory operation, it is recommended that for precise work the instrument should be standardised regularly at two widely different sodium concentrations (this recommendation is discussed fully later). If erroneous results are obtained it is advisable to replace the electrode.

Performance of the instrument—A test programme was carried out on the instrument over a period of 30 days to evaluate the precision, bias, stability and response times. During this time the instrument was standardised seventeen times with intervals of at least 24 hours

TABLE II

REPRODUCIBILITY OF CALIBRATIONS OVER A 30-DAY PERIOD

Sodium concentration/μg l^{-1}				Mean response time†/ minutes	
Nominal	By flame photometry	By E.I.L. monitor, mean result*	Degrees of freedom	90 per cent.	Equilibrium
$8 + x$‡	10·2 (0·7)§	10·8 (0·8)§	16	4·5	20
$4 + x$	6·3 (0·6)	6·4 (0·8)	12	4·5	15
x	2·0 (0·5)	2·4 (0·4)	12	5	30
$20 + x$	22·3 (0·9)	21·0 (1·3)	12	3	9
$200 + x$		195 (7·0)‖	12	3	8

* Reading taken after 30 minutes.

† The solutions were analysed in the order described in the text and the response times given are times from the initial response of the electrode after changing the solution. The total response time of the instrument will be greater than these times owing to the time required for the sample to be pumped to the electrode compartment; in the present tests this time-lag was 2 minutes.

‡ x = sodium content of the blank water/μg l^{-1}.

§ The values in parentheses are the standard deviations of individual results.

‖ This result was obtained from readings taken immediately before the automatic compensation unit was activated during the standardisation procedure.

between standardisations, and immediately after the standardisation (at a sodium concentration of 200 μg l^{-1}) a second standard solution nominally containing 8 μg l^{-1} of sodium was analysed for 30 minutes. On thirteen of these occasions, a further four standard solutions (nominally containing 200, 20, 4 and 0 μg l^{-1} of sodium) were analysed consecutively for 30-minute periods. At all other times during this test the instrument was used to analyse power-station feed-water.

The results summarised in Table II show that in the 2-hour period immediately following standardisation both the precision and bias of the results for concentrations of sodium between 2 and 25 μg l^{-1} were reasonably good, and indicate adherence to the Nernstian equation. Both the 90 per cent. and equilibrium response times were slightly quicker than those reported by Webber and Wilson[1] during laboratory tests on an Electronic Instruments Limited sodium electrode, although at very low sodium concentrations (about 3 μg l^{-1}) equilibrium response was obtained only after continuous analysis of the solution for several hours.

Stability over 24-hour periods—In each of four approximately 24-hour periods the instrument was first standardised and then one of four synthetic sodium solutions was analysed for the remainder of the period. Readings were taken every hour and a summary of the results is given in Table III.

All the measurements decreased throughout the 24-hour period and re-standardisation indicated that this decrease was due to drift in the amplifier. The amplifier unit used in this test is no longer used in the Electronic Instruments Limited monitor; it has been replaced by a more stable F.E.T. amplifier.

TABLE III

PRECISION OF RESULTS OVER 24-HOUR PERIODS

Sodium concentration/μg l^{-1}				
Nominal	Mean of hourly results*	Highest result	Lowest result	Degrees of freedom
17	16·75 (1·2)	19·0	14·0	23
8	8·0 (0·6)	9·0	7·0	21
3	3·3 (0·5)	4·3	2·8	23
1	0·86 (0·15)	1·3	0·61	24

* The values in parentheses are the standard deviations of individual results.

Effect of octadecylamine—Earlier work by Webber and Wilson[1] had shown that the use of a 10-p.p.m. solution of octadecylamine caused a rapid deterioration in the response of the sodium electrode. It was therefore decided to test the effect on the instrument of octadecylamine at a concentration normally used in practice, *i.e.*, about 0·1 p.p.m. The results, summarised in Table IV, show that after 6 hours the observed concentration of sodium in the octadecylamine solution had nearly doubled, and after a further 16 hours it had increased by a factor of almost ten compared with the original value. The response of the electrode to the solution containing 200 μg l^{-1} of sodium was very sluggish and indicated an observed sodium concentration of only 70 μg l^{-1}.

TABLE IV

EFFECT OF OCTADECYLAMINE

Solution analysed	Time after addition of octadecylamine/ minutes	Observed sodium concentration/ μg l^{-1}
Water	0	0·75
Water + 0·1 p.p.m. of octadecylamine	10	1·6
	300	2·0
	360	3·0
	1300	17·0
	1360	8·0
Water + 200 μg l^{-1} of sodium	1400	70·0

Effects of other substances—As the effects of many of the other substances likely to be present in power-station waters had already been established,[1] no further tests were made. However, although the pre-filter in the sample line removed particles with diameters greater than 25 μm, a large amount of particulate matter did accumulate in the electrode compartment and some was deposited on the surface of the sodium electrode and on the sintered

disc of the remote junction of the reference electrode. During the whole of the test period no changes in the characteristics of the sodium electrode could be attributed to these deposits.

Errors introduced by the automatic standardisation procedure—The automatic compensation unit, which re-sets the instrument during the standardisation procedure, is actuated by a high - low alarm system set at either side of the standard concentration level. Between these two alarms there is a dead-space of about 3 mV, equivalent to $\pm 5{\cdot}5$ per cent. of the standard concentration, and bias of this magnitude may be introduced at each standardisation and persist at least until a fresh standardisation is carried out.

Discussion

The main aim of the present work was to test the applicability of the Electronic Instruments Limited Model 98C sodium monitor for measuring very low ($\ll$1 p.p.m.) concentrations of sodium in steam and boiler feed and make-up waters in modern power stations. For this purpose, it was unnecessary to use the two ranges of the instrument and therefore two minor modifications were made—the three-decade range was calibrated from 0·5 to 500 $\mu g\ l^{-1}$ of sodium, and the automatic standardisation system was changed so that it operated on this three-decade range rather than on the higher, two-decade, range. It is thought that these modifications will in no way invalidate the results obtained.

Precision and bias—

The results in Table II show that the measured sodium concentrations in standard solutions (2 to 20 $\mu g\ l^{-1}$ of sodium) agreed well with independent flame-photometric determinations and with the expected concentrations. This confirms the essentially Nernstian behaviour of the sodium electrode. The standard deviation of the analytical results varied between 0·4 and 1·3 $\mu g\ l^{-1}$ of sodium for sodium concentrations of about 2 to 20 $\mu g\ l^{-1}$; these standard deviations are in excellent agreement with those obtained by Webber and Wilson[1] by using a sodium-responsive glass electrode in the laboratory.

Response time—

Although the response time is dependent to a certain extent on the individual sodium electrode, the results in Table II indicate a general trend towards longer equilibrium response times at lower sodium concentrations. In all instances, the time taken to give a 90 per cent. response was 7 minutes or less and, except for the solution containing 2 $\mu g\ l^{-1}$ of sodium, the equilibrium response was established within about 20 minutes.

Effect of temperature—

From theoretical considerations, changes in temperature that occur in the instrument or sample would be expected to affect the response. With the cabinet thermostatically controlled at 32 °C it was shown that influent sample temperatures between 1 and 50 °C had only a small effect on the temperature of the sample passing through the electrode compartment and any resultant effects on the electrode response were small compared with calibration and other random errors.

Maintenance requirements—

The instrument was inspected for faults three or four times per week on average and, apart from the few occasions when erroneous results due to faulty electrodes were obtained, no maintenance was necessary other than that of a routine and scheduled nature, such as replacing the ammonia solution and topping-up the potassium chloride solution in the reference electrode (monthly), and replacing the pump-tubing (every two months). Although particulate matter did accumulate in the electrode compartment and on the electrode it was not found necessary to clean the components; however, this may become necessary after longer periods or with samples that contain relatively large amounts of particulate or colloidal material.

Standardisation—

The automatic standardisation procedure incorporated in the instrument relies on a single standard solution and an assumption that the sodium-responsive glass electrode gives theoretical Nernstian response to changes in sodium concentration. Although this procedure is subject to small errors (about ± 5 per cent.) due to the electronic circuit dead-space at the

point of standardisation, it gave good reproducibility. However, we showed that on a number of occasions the electrode did not give theoretical Nernstian response; this would not have been detected by using only the automatic standardisation procedure. We recommend, therefore, that when an electrode is first installed in the instrument it should be tested to prove that the response is Nernstian over a wide range of sodium concentrations, and a convenient method of doing this is described below. After each automatic standardisation, a second standard, different from the primary standard by at least one pNa unit, should be analysed.

Confirmation of Nernstian response—This can be achieved in the following way. Prepare a series of standard solutions by diluting a concentrated sodium standard with de-ionised water expected to contain less than 5 μg l^{-1} of sodium. Recommended nominal sodium concentrations for this series are 200, 90, 20, 8 and 4 μg l^{-1}. Calibrate the instrument with the standard solution containing 200 μg l^{-1} of sodium (Note) and analyse all the other solutions; compare the results obtained for the concentrations with the nominal concentrations. If the instrument is working satisfactorily, the differences between each observed concentration and nominal concentration of the solution will be constant (within experimental error) and will agree with the observed concentration for the de-ionised water. Systematic deviations from the result obtained for the concentration of sodium in the de-ionised water will indicate probable errors either in the response of the electrode or in the scale-length of the concentration indicator on the instrument.

NOTE—

To enable the instrument to be set at 200 μg l^{-1} of sodium when Range 1 of the standard instrument is being used, multiply the range scale by 10 so that it corresponds to 1 to 1000 μg l^{-1} of sodium. If the reading for sodium concentration in the de-ionised water is less than 1 μg l^{-1} during this test and the other estimates of its concentration from the results obtained from the other solutions are less than or equal to 1 μg l^{-1}, the instrument can be considered to be working satisfactorily.

Automatic standardisation—Ideally, the standard solution used in conjunction with the automatic compensation unit of the instrument should have a concentration approximately the same as that of the sample solution and it is recommended that a second standard solution should be analysed for 20 minutes immediately after the completion of automatic standardisation. The results from this analysis will show whether or not any systematic changes are occurring in the electrode response and they will also give an estimate of the precision of the determinations. However, if samples contain an extremely low concentration of sodium it is not possible to prepare with sufficient accuracy a standard solution at a similar concentration because of the errors resulting from measurement of the sodium content in the de-ionised water used to prepare the standard. Even if the concentration of sodium in the standard solution was known, the time taken for the sodium electrode to equilibrate with such a solution would preclude its use as a standard. The minimum recommended concentration for this standard is, therefore, about 10 μg l^{-1} of sodium. The solution with a nominal sodium concentration of 8 μg l^{-1} prepared for the confirmation of Nernstian response can be conveniently used and the solution with a sodium concentration of 90 μg l^{-1} is suitable for use as the second standard.

CONCLUSIONS

The standard instrument is designed to determine sodium concentrations down to 0·0001 p.p.m. and is calibrated up to a maximum value of 10 p.p.m. of sodium. This work proves that the instrument will measure satisfactorily the very low concentrations of sodium that are found in steam and boiler feed and make-up waters, except when octadecylamine (and presumably other filming amines) are present. Moreover, there is no fundamental reason why higher concentrations should not be measured satisfactorily; from the general performance characteristics observed it is probable that results will usually lie within ± 10 per cent. of their true values.

This paper is published by permission of the Central Electricity Generating Board. We thank Mr. A. L. Wilson for valuable discussions, and the Station Superintendent and staff of Croydon "B" Power Station where the instrument was installed for the duration of these tests.

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Electrochemical Studies of Copper Lactate and Glycollate Complexes at the Mercury Cathode

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Polarographic studies of the copper complexes formed with lactic and glycollic acids have been undertaken using water and mixtures of water and organic solvents. The stability constants have been determined. The effects of pH, concentration of ligand, non-aqueous solvents and surface-active substances have also been investigated. The values of ΔH, ΔF and ΔS have been calculated.

As part of a series of polarographic investigations into the complexes of transition metals[1–5] we investigated the complexes of copper(II) with lactic and glycollic acids at a dropping mercury electrode in aqueous and non-aqueous media, because the only relevant literature available concerns a few potentiometric studies.[6,7]

All the reagents used were of analytical-reagent grade and, with the sodium salts of lactic and glycollic acids, were prepared with conductivity water. Potassium nitrate was used as a supporting electrolyte and also to maintain a constant ionic strength (1·0). Polarographic waves were plotted manually in each case by means of an Adept potentiometer and a Scalamp galvanometer. An H-type cell was used with a saturated calomel reference electrode and a temperature of 30 ± 1 °C was maintained during the investigations. The dropping-mercury electrode had the following characteristics: $m = 2{\cdot}700$ mg s^{-1} and $t = 2{\cdot}81$ s.

It is known that on complexation the half-wave potential of a simple metal ion becomes more negative. In cases when only one complex species is formed in solution with a ligand X, the graph of $-\log C_X$ *versus* $E_{\frac{1}{2}}$ is a straight line. For a system in which stepwise complex formation takes place, the above graph may be generally a smooth curve.

With copper glycollate and lactate systems, graphs resulted that indicated the presence of more than one complex species in the solutions. Hence, the technique developed by DeFord and Hume[8] has been applied. These workers derived the following relationship between the half-wave potential shift ($E_{\frac{1}{2}}$) and the free ligand concentration [X] for a reversible metal deposition as follows:

$$\text{antilog}_{10}\left[\frac{0{\cdot}4343\,nF}{RT}\cdot\Delta E_{\frac{1}{2}}+\log\frac{I_M}{I_C}\right]=f_M\sum_0^N\frac{\beta_j[X]^j}{f_{MX_j}}$$

$$=1+\beta_1[X]\frac{f_M\cdot f_X}{f_{MX_1}}+\beta_2\frac{[X]^2f_M(f_X)^2}{f_{MX_2}}$$

In the above equation I_M and I_C are the diffusion current constants for simple and complex metal ions, respectively, R and F have their usual significance, n denotes the number of electrons required for reduction, β_j is the over-all formation constant of the jth complex, f_X is the activity coefficient of the complexing ligand and f_M and f_{MXj} are the activity coefficients at the electrode surface of the metal and of the jth complex species, respectively

Irving[9] pointed out that activity coefficient terms could be omitted without serious loss of accuracy for uncharged ligands. Values of $f_o[X]$ can be determined from the experimental results, and when the graph of $f_o[X]$ against C_X is extrapolated to $C_X = 0$, the intercept on the f_X axis for $C_X = 0$ indicates the value of K_o. Another function, $f_1[X]$, may be introduced as—

$$f_1[X]=\frac{f_o[X]-K_o}{C_X\cdot f_X}$$

and, where K_o is taken to be unity,

$$f_1[X] = \frac{f_o[X] - 1}{C_X \cdot f_X}$$

The graph of $f_1[X]$ against C_X, extrapolated to $C_X = 0$, gives the value of K_1. In a similar manner other functions are introduced as—

$$f_2[X] = \frac{f_1[X] - \beta_1}{C_X \cdot f_X}$$

$$f_j[X] = \frac{f_{j-1}[X] - \beta_{j-1}}{C_X \cdot f_X}$$

By plotting each f[X] function against the free ligand concentration, the stability constants and co-ordination numbers of all complex species present can therefore be evaluated.

Results and discussion

Polarographic studies of the reduction of copper in the presence of glycollate and lactate ions reveal that a single and well defined wave is obtained in each case. The waves have been found to be diffusion controlled and may also be reversible, but the degree of reversibility has been found to decrease with increase in pH and ligand concentration. This conclusion has been drawn on the basis of the gradients of the graphs of log $(i/i_d - i)$ *versus* E_{de}, which increase from 30 to 50 mV. The half-wave potentials for the reduction of copper glycollate and copper lactate complexes have been found to be —0·0140 V *versus* S.C.E. and —0·0280 V *versus* S.C.E. when the glycollate and lactate ions are 0·05 M (each) at pH 5·4 and 6·4, respectively.

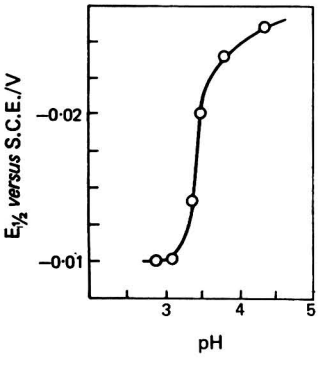

Fig. 1. Effect on copper glycollate system of varying the pH

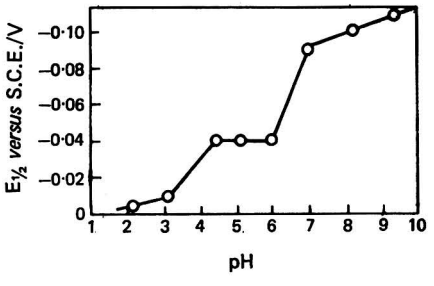

Fig. 2. Effect on copper lactate system of varying the pH

Effect of pH—

Copper glycollate—The electrochemical reactions taking place at the dropping-mercury electrode are influenced to a large extent by the hydrogen-ion concentration. In order to establish the pH ranges over which the formation of the complexes is dependent, or otherwise, on pH, it was decided to observe the effect on both ions of varying the pH and thus attempt to discover the electrode reaction mechanism.

For copper glycollate, from pH 2·8 to 3·0 the half-wave potential remains unaffected, on increasing the pH from 3·0 to 7·0 the half-wave potential increases and at high pH values the wave becomes obscured. Therefore it can be concluded that the process of chelation is affected by the hydrogen-ion concentration above pH 3·0, as shown by the changes in half-wave potential (Fig. 1).

It was observed that as the pH increased, the degree of irreversibility also increased, as shown by the gradient values of the graph of log $(i/i_d - i)$ *versus* E_{de}. The increase in $(E_{\frac{3}{4}} - E_{\frac{1}{4}})$ values from 45 to 72 mV further supports this conclusion.

A decrease in diffusion current was also observed as the pH increased from 2·9 to 7·0.

Copper lactate—With the copper lactate system, pH again has an effect on the reduction process of the complex. The $E_{\frac{1}{2}}$ value increases with pH over the range 2·1 to 4·4 from −0·0020 V to −0·0400 V *versus* S.C.E., after which it is constant up to pH 6·0. Above pH 6·0, $E_{\frac{1}{2}}$ becomes more negative, increasing from −0·0400 V to −0·2800 V *versus* S.C.E.

A graph of, $E_{\frac{1}{2}}$ *versus* pH shows that the half-wave potential is not affected by the hydrogen-ion concentration in the pH range 4·4 to 6·0, but that in the range 2·1 to 4·4 $E_{\frac{1}{2}}$ shifts in a negative direction, showing that hydrogen ions are involved in the electrode process. Above pH 6·0, $E_{\frac{1}{2}}$ becomes even more negative showing that hydroxyl ions are also involved in the electrode process, as is clear from Fig. 2.

The reduction process is found to be reversible up to pH 4·4, but above pH 6·0 the process is irreversible, as is shown by the gradient of the graph (Fig. 2) and $(E_{\frac{1}{2}} - E_{\frac{1}{4}})$ values.

EFFECT OF LIGAND CONCENTRATION—

Copper glycollate—A cathodic shift in half-wave potential on increasing the glycollate-ion concentration from zero to 0·7 M indicates the formation of the complex. The graph of $-E_{\frac{1}{2}}$ *versus* $-\log C_X$ is curved, suggesting the formation of more than one complex species.

The method of DeFord and Hume,[8] as improved by Irving,[9] was used for the calculation of the stability constants of the various complex species formed.

TABLE I

f[X] AND OTHER RESULTS FOR SOLUTIONS CONTAINING COPPER GLYCOLLATE COMPLEXES

Concentration of Cu(II), 9×10^{-4} M; $\mu = 1$ (KNO_3)

Concentration of glycollate ion/M	$E_{\frac{1}{2}}$ *versus* S.C.E./V	Gradient of the graph of E_{de} *versus* $i(i_d - i)$/mV	i_d in divisions	$f_0[X]$	$f_1[X]$	$f_2[X]$	$f_3[X]$
Temperature 20 °C—							
0·00	+0·015	38	76·5	—	—	—	—
0·05	−0·014	31	70·5	10·68	193·6	—	—
0·10	−0·026	32	70·0	27·16	261·6	516	—
0·15	−0·034	37	69·5	49·10	320·6	737·33	1148·7
0·20	−0·040	37	69·0	81·98	404·7	—	—
0·30	−0·046	41	67·5	139·2	460·6	835·3	—
0·40	−0·054	45	66·5	247·0	615·0	1012·5	1118·7
0·50	−0·058	47	66·0	351·0	700·0	—	—
0·60	−0·064	50	65·5	570·6	949·3	1232·16	—
0·70	−0·068	52	62·5	817·6	1468·0	1797·1	1111·9
Temperature 35 °C—							
0·00	+0·015	38	87·5	—	—	—	—
0·05	−0·007	30	86·5	6·092	101·84	—	—
0·10	−0·018	30	86	13·49	124·9	—	—
0·15	−0·028	35	84	31·99	206·6	—	—
0·20	−0·030	35	83·5	36·73	178·65	293·25	366
0·30	−0·038	40	83	66·49	218·3	327·66	358·8
0·40	−0·044	47	82·5	104·7	258·75	346·84	—
0·50	−0·048	47	82	145·5	289·0	—	—
0·60	−0·054	50	80·5	233·1	386·83	444·7	374·5
0·70	−0·058	50	80	315·3	449·0	470·0	342

Various f[X] values and other results for the glycollate system are shown in Table I.

The number of ligand molecules bound to the metal ion at pH 5·4 and at a temperature of 20 °C has been found to be one in the concentration range 0·05 to 0·10 M, two from 0·1 to 0·3 M and three from 0·3 to 0·7 M. It is probable that the following electrode reactions take place at pH 5·4—

$$Cu(L)^+ + 2e^- + Hg = Cu(Hg) + L^-$$ (concentration range 0·05 to 0·1 M)

$$Cu(L)_2^+ + 2e^- + Hg = Cu(Hg) + 2L^-$$ (concentration range 0·1 to 0·3 M)

$$Cu(L)_3^+ + 2e^- + Hg = Cu(Hg) + 3L^-$$ (concentration range 0·3 to 0·7 M)

The values of the over-all stability constants were found to be: $\beta_1 = 210$, $\beta_2 = 565$ and $\beta_3 = 118 \pm 2$ at 20 °C; and 120, 220, 370 ± 2, respectively, at 35 °C.

The change in free energy (ΔF) and enthalpy (ΔH) can be calculated from the following equation—

$$F = 2{\cdot}303\ RT \log \beta$$

$$\text{and } \log \beta_2/\beta_1 = \Delta H\ (T_2 - T_1)/4{\cdot}576\ T_1T_2.$$

The values were found to be $\Delta H = -6691$ cal and $\Delta F = -761{\cdot}9$ cal for the glycollate complex at a temperature of 20 °C.

The change in entropy was calculated to be $-20{\cdot}24$ cal °C^{-1} mol^{-1} from the relationship $\Delta S = (\Delta H - \Delta F)/T$.

TABLE II

f[X] AND OTHER RESULTS FOR SOLUTIONS CONTAINING COPPER LACTATE COMPLEXES

Concentration of Cu(II), 9×10^{-4} M; $\mu = 1$ (KNO_3)

Concentration of lactate ion/M	$E_{\frac{1}{2}}$ *versus* S.C.E./V	Gradient of the graph of E_{de} *versus* $i(i_d - i)$ mV	i_d in divisions	$f_0[X]$	$f_1[X]$	$f_2[X]$	$f_3[X]$
Temperature 20 °C—							
0·00	+0·015	31	69·5	—	—	—	—
0·05	−0·028	33	68·5	29·63	572·6	—	—
0·10	−0·042	33	68·0	88·98	889·8	3698	9380
0·15	−0·050	33	64·5	175·6	1164·0	4293	10220
0·20	−0·056	33	61·5	294·7	1468·5	4742	9910
0·30	−0·064	31	60·5	544·1	1810·33	4300	—
0·40	−0·072	31	60·0	1050·0	2622·5	5256	—
0·50	−0·078	31	57·5	1767·0	3532·0	6024	—
0·60	−0·084	31	56·5	2828·0	4711·6	6986	—
0·70	−0·088	31	55·5	3170·0	4527·1*	—	—
Temperature 35 °C—							
0·00	+0·015	31	82	—	—	—	—
0·05	−0·018	33	81	13·75	255·0	—	—
0·10	−0·032	33	80	41·24	402·4	1524	5540
0·15	−0·040	34	76	81·21	534·7	1898	6186
0·20	−0·046	34	73·5	134·3	666·5	2082·5	5560
0·30	−0·058	34	73	341·7	1135·6	—	—
0·40	−0·064	35	72	546·8	1364·5	2786·25	—
0·50	−0·070	35	71	840·4	1675·8	—	—

* Omitted from Fig. 4.

Copper lactate—As with the glycollate, the number of ligand molecules bound depends on the concentration of the ligand and it is evident from the graph of $E_{\frac{1}{2}}$ *versus* −log [X] that the number of ligand molecules bound to the metal ion at pH 5·6 and 20 °C is one in the concentration range 0·05 to 0·4 M and two in the range 0·4 to 0·7 M.

The value of ΔH was -8758 cal, of $\Delta F - 1099$ cal, and the change in entropy was $-26{\cdot}14$ cal °C^{-1} mol^{-1}. The electrode reaction is similar to that of the glycollate system.

The values of the over-all stability constants were found to be: $\beta_1 = 520$, $\beta_2 = 2760$ and $\beta_3 = 9977$ at 20 °C; and 250, 970, 5540, respectively, at 35 °C.

Various f[X] values are shown in Table II and the graphs of the various functions for the two systems are shown in Figs. 3 and 4.

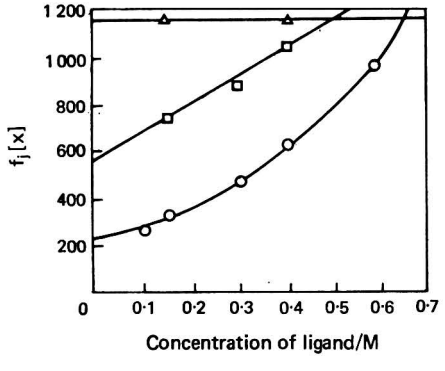

Fig. 3. Variation of $f_j[X]$ with concentration of ligand for copper glycollate at 20 °C: o, $f_1[X]$; □, $f_2[X]$; and △, $f_3[X]$

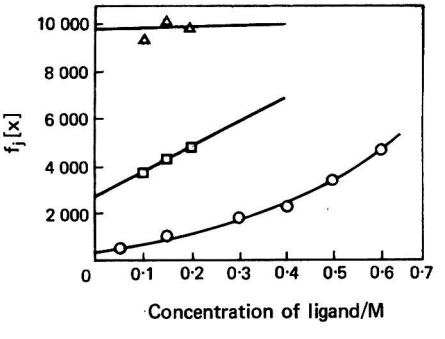

Fig. 4. Variation of $f_j[X]$ with concentration of ligand for copper lactate at 20 °C: o, $f_1[X]$; □, $f_2[X]$; and △, $f_3[X]$

Studies of various surface-active substances (1-naphthol, 2-naphthol, gelatin and camphor) revealed that they did not have any marked influence on the reduction process or the diffusion current, the process simply tending towards irreversibility in the above order of surfactants as indicated by the slope values.

EFFECT OF NON-AQUEOUS MEDIA—

Copper glycollate—The investigations on the copper glycollate system in the methanol-water mixture reveal that $E_{\frac{1}{2}}$ is shifted from −0·3370 V to −0·3100 V *versus* S.C.E. up to 10 per cent. of methanol. From 20 to 50 per cent. of methanol the value remains constant and at 60 per cent. of methanol, $E_{\frac{1}{2}}$ decreases suddenly to −0·3075 V *versus* S.C.E. A decrease in the diffusion current is also noticed. The reduction tends to be more reversible as the concentration is increased from 0 to 40 per cent., and a 60 per cent. methanol concentration actually reverses the reduction process. The probable explanation for the variation in $E_{\frac{1}{2}}$ values is that the interionic attraction is large in solvents of low dielectric constant so that polarographic waves are influenced significantly by the supporting electrolyte even if complex formation is not involved.

The decrease in diffusion current may be partly caused by an increase in the viscosity of the medium and partly by ion-pair formation. The ion-pair factor must be considered because a continuous decrease in the diffusion current is observed. This phenomenon would account for the fact that there is first a decrease and then an increase in current in the solution.

Ethanol causes a shift in $E_{\frac{1}{2}}$ value from −0·3375V to −0·3850 V as the concentration is increased from 0 to 60 per cent. The gradient of the graph of log $(i/i_d - i)$ *versus* E_{de} changes from 66 mV to 47 mV, and hence it may be concluded that the reaction becomes faster and more reversible in the presence of increasing amounts of ethanol.

With propan-2-ol, it was not possible to study the effect at a concentration above 20 per cent. of the alcohol, as the wave became distorted. At lower concentrations a shift in $E_{\frac{1}{2}}$ takes place, becoming more positive from −0·3375 V to −0·3250 V *versus* S.C.E.

The effect of 2-methylpropan-2-ol could not be studied, as the wave becomes ill-defined when this solvent is present.

With dimethylformamide as solvent, a positive shift in $E_{\frac{1}{2}}$ takes place between 0 and 20 per cent. of dimethylformamide. From 20 to 40 per cent. it remains constant at −0·2700 V *versus* S.C.E. and then shifts in a negative direction as the concentration of dimethylformamide is increased beyond 40 per cent. The reduction becomes more irreversible with increasing concentration of dimethylformamide, being reversible below 40 per cent.

Similar behaviour to the above was observed with copper lactate in non-aqueous media such as methanol, ethanol, propanol, butanol and dimethyl sulphoxide.

The $E_{\frac{1}{2}}$ value shifts in a negative direction with increase in concentration of the solvents, while the slope values remain unaffected in all media, except in methanol, when they vary from 33 to 62 mV. In concentrations of methanol above 40 per cent., ill-defined waves are obtained. Shifts in $E_{\frac{1}{2}}$, decrease in diffusion current and variation in reversibility can be explained as for the copper glycollate system.

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A Semi-automated Method for the Determination of the Available Carbohydrate Content of Poultry Feeds

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A rapid method is described for the determination of the sugar content of takadiastase digests of poultry feeds. The reproducibility of the method has been assessed and the results of recovery tests on pure carbohydrates are presented. The method gives results in reasonable agreement with those obtained by a previously published titrimetric method involving Fehling's reaction.

THE metabolisable energy content of a diet for poultry is controlled by its digestible protein, fat and carbohydrate contents. The fat and protein contents of a diet can be readily determined by conventional methods[1] and factors[2] can be applied to ascertain their contribution to the over-all metabolisable energy of the diet.

However, of the three components referred to, the digestible carbohydrate content is the major determinant of the metabolisable energy of a normal poultry diet. Bolton[3] described a method for the chemical determination of the available carbohydrate content of poultry feeds and showed that the amount thus determined was highly correlated with the digestible carbohydrate content as determined by balance experiments with chicks and hens. Bolton's method[3] involves incubation of a gelatinised sample of the feed with takadiastase at 37 °C overnight, hydrolysis of the resultant mixture of oligosaccharides with 1·5 N sulphuric acid and determination of the reducing sugars in the protein-free hydrolysate by Fehling's reaction, with methylene blue as indicator.[4] The sulphuric acid hydrolysis takes 2 hours to complete and Fehling's titration is time consuming and inaccurate, except when carried out by an experienced analyst. In the method described in this paper the hydrolysis procedure is automated and an automated version[5] of the orcinol - sulphuric acid reaction is used instead of Fehling's titration. It is estimated that with a batch of twenty samples the use of the automated method would reduce the analysis time to 25 per cent. of that required for the manual method.

EXPERIMENTAL

APPARATUS—

The components of the Technicon AutoAnalyzer assembly were as follows: voltage stabiliser; sampler II; proportioning pump; heating bath operating at 95 °C; colorimeter with 15-mm flow cell and 420-nm filters; and single-pen recorder.

REAGENT—

Orcinol, 0·1 per cent., in 70 per cent. v/v sulphuric acid—Place 2 g of orcinol* in a light-protected, 2-litre reagent flask. Add carefully 2 litres of cold (20 °C) 70 per cent. v/v analytical-reagent grade sulphuric acid, cover the flask top with aluminium foil and stir the flask gently with a PTFE-coated follower on a magnetic stirrer until the orcinol has dissolved (about half an hour). The follower is removed before the reagent is used. This reagent is light-sensitive but, if carefully protected, is stable for at least 4 weeks. The acid must be cooled to 20 °C before it is added to the orcinol. Further purification of the grade recommended has not been found necessary.

METHOD

The first stage of the analysis is as described by Bolton,[3] but is included for the sake of completeness. A weighed sample of the feed, containing about 0·5 g of starch, is transferred to a 100-ml flask [the flasks used in this laboratory were 100-ml conical flasks without lips and fitted with metal caps (Oxoid Ltd.), and were calibrated at 110 ml]. The feed is moistened with a few drops of ethanol, about 20 ml of water are added and, with continuous swirling, the contents of the flask are brought to boiling-point over a naked flame, cooled slightly

* Pure grade supplied by Koch-Light Ltd.

and again brought to boiling-point to gelatinise the starches. The flask is cooled and 0·5 ml of 5 per cent. v/v acetic acid and 0·1 to 0·2 g of takadiastase* are added. The contents of the flask are mixed and the walls are washed down with the minimum amount of water. Toluene (0·5 ml) is added and the flasks, covered with metal caps, are incubated overnight at 37 °C. The incubated contents are heated to boiling, clarified by the addition of 5 ml of 5 per cent. w/v zinc sulphate solution and 5 ml of 3·67 per cent. w/v potassium ferrocyanide solution and made up to volume (110 ml) with water. The flasks are inverted gently several times and left to stand for a few minutes to allow the precipitate to settle. A 1-ml aliquot of the residue-free solution is diluted to 100 ml with water to give the concentration required for analysis with the AutoAnalyzer. As the precipitate and residue from a 1-g sample of normal poultry feeds occupy a volume of about 5 ml, it is assumed when calculating the results that the sample was made up to 105 ml. The need for this correction can be avoided by filtering the clarified solution before making up the standard volume.

The manifold used on the AutoAnalyzer, shown in Fig. 1, is a modification of that used by Catravas.[5] A combined orcinol - sulphuric acid reagent is used in preference to adding the orcinol as an aqueous solution. The Technicon sampler is set to give sampling rates of twenty or thirty samples per hour, depending on the accuracy required, and a sample-to-wash (water) ratio of 1 : 5. The AutoAnalyzer should be switched on for 1 hour before any samples are introduced so as to allow the pump tubes to become adjusted to the reagents. Standards containing between 0·01 and 0·10 mg ml^{-1} of glucose in saturated aqueous benzoic acid solution, diluted takadiastase digests and blanks are read in duplicate. All the standards are read before, and a few after, the samples, so that corrections can be made for changes in the delivery rates of the pump tubes with time.

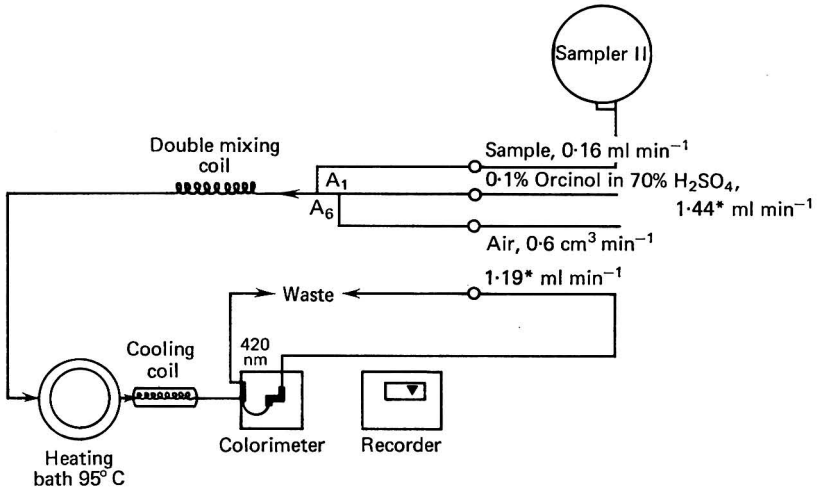

Fig. 1. Manifold used for the determination of available carbohydrate (*Acidflex tubing)

CALCULATION OF RESULTS—

A standard graph relating optical density to glucose concentration is constructed for the first set of standards. The "glucose" concentration of the diluted sample can be obtained from this standard graph. Despite allowing 1 hour at the start of each day it has been found that the same standard read at the end of an 8-hour period will give about a 10 per cent. higher optical density reading than at the beginning; the effect is common in AutoAnalyzer systems in which Acidflex pump tubes are used. A correction can be made if several standards are run at intervals. From these standards the percentage change in sensitivity over the period can be calculated and corrections made for samples read at different positions along the trace. As in the method of Bolton,[3] the percentage of glucose multiplied by 0·91 is reported as the percentage of available carbohydrate.

* Parke, Davis and Co., Ltd.; standardised with talc.

$$\text{Available carbohydrate, per cent.} = \frac{\text{Glucose (mg ml}^{-1}) \times 105 \times 100 \times 0{\cdot}91 \times 100}{\text{Sample weight (g)} \times 1000}$$

$$= \frac{\text{Glucose (mg ml}^{-1}) \times 955{\cdot}5}{\text{Sample weight (g)}}$$

Results

Reproducibility of measurement of standard solutions—

To test the reproducibility of the method, several standards were determined in quadruplicate in increasing and decreasing order of concentration. The results shown in Table I are for samples read at a sampling rate of twenty per hour. The effect of increasing the sampling rate to thirty per hour was tested by reading a glucose standard, containing 0·06 mg ml^{-1}, ten times at sampling rates of both twenty and thirty per hour; the coefficients of variation were 0·90 and 1·40 per cent., respectively. The carry-over from sample to sample was also greater when samples were read at a rate of thirty per hour.

Table I

Reproducibility of determination of glucose standards

Standard concentration/μg ml^{-1}	10	20	40	60	80	100
Number of observations (read at rate of 20 samples per hour)	8	8	8	8	8	4
Mean optical density	0·090	0·172	0·356	0·544	0·738	0·941
Standard deviation	0·0032	0·0032	0·0083	0·0076	0·0107	0·0048
Coefficient of variation, per cent.	3·51	1·84	2·33	1·40	1·45	0·52

Digestion procedure—

The takadiastase digestion procedure was tested by measuring the recovery of various sugars and starches as glucose; the mean results of duplicate analyses are given in Table II. It can be seen that the results for hexose oligosaccharides and polysaccharides are close to the theoretically expected values. The results for pentose sugars are more than twice the expected values, because the colour produced with this reagent by pentoses was greater than that produced by hexoses.[6]

Table II

Recovery as glucose of some standard sugars and starches

	Recovery, per cent.								
Glucose	Maltose	Cellobiose	Xylose	Arabinose	Maize starch	Wheat starch	Soluble starch	Maize dextrin	Rice starch
100·0	101·6	101·0	207·7	221·9	98·2	100·6	100·8	100·7	100·5

Comparison with titrimetric method—

The method was further tested by comparing the results obtained by the AutoAnalyzer procedure with those obtained by Fehling's titration method.[3] The results for some feedstuffs and mixed diets are shown in Table III. The results obtained with the AutoAnalyzer procedure are about 2 per cent. higher than those obtained with Fehling's method, but otherwise are in reasonable agreement. The results for mixed diets tended to differ more than those for feedstuffs. The effects of some possible interfering substances on the available carbohydrate content of maize starch were tested and results are shown in Table IV. A low value was obtained for the available carbohydrate content of maize starch by using the titrimetric method[3]; it can only be assumed that this was because of destruction during acid hydrolysis. None of the substances tested seriously affected the results obtained by the AutoAnalyzer procedure.

Discussion

The method presented permits the rapid assessment of the available carbohydrate content of poultry feeds. As takadiastase has been shown[7] to be specific for the hydrolysis of α-linked glucose polymer, the method determines the starch *plus* free sugars present in the feed. In the calculation of the available carbohydrate it is assumed that all the sugar originated from starch; this assumption will be seriously in error only for feeds containing

Table III

THE AVAILABLE CARBOHYDRATE CONTENTS OF A RANGE OF FEEDSTUFFS, DETERMINED BY USING THE AUTOANALYZER AND FEHLINGS' TITRATION METHODS

	Available carbohydrate content, per cent., by	
Feedstuffs—	Fehling's titration[a]	AutoAnalyzer
Maize starch	93·9	97·9
Maltose	93·3	100·5
Glucose	102·4	100·5
Wheat meal	62·4	62·2
Barley meal	55·0	54·0
Maize meal	61·5	63·1
Triticale	57·2	58·0
Naked barley (15 per cent. of water)	61·1	63·7
Rice, ground	79·3	80·3
Field bean meal (1)	34·4	32·9
Field bean meal (2)	47·8	48·9
Wheat middlings	34·7	36·9
Maize bran	30·5	34·5
Maize grits	52·7	53·7
Mixed diets—		
Chick mash	47·8	52·8
Layer's mash 1	42·1	42·1
Layer's mash 2	42·2	40·2
Broiler mash	42·6	46·4
Semi-synthetic diet	30·1	35·1

Table IV

EFFECT OF SOME INTERFERING SUBSTANCES ON DETERMINATION OF THE AVAILABLE CARBOHYDRATE CONTENT OF MAIZE STARCH

	Available carbohydrate content, per cent., by	
	Fehling's titration	AutoAnalyzer
Starch (maize)	85·8	102·3
Starch + cellulose powder	97·0	101·9
Starch + minerals + vitamins	97·4	100·5
Starch + fish meal	99·5	99·7
Starch + maize oil	105·1	100·8

considerable amounts of free sugar. All water-soluble carbohydrates are assumed to be completely available; while this is likely to be true for normal dietary ingredients it is not true for some dextrins (Shannon, D. W. F., McNab, J. M., and Pritchard, P. J., unpublished work) and for lactose.[8]

From the results shown in Table II it can be seen that pentoses will be over-estimated by at least 100 per cent. However, because of the specificity of takadiastase and the low levels of free pentoses likely to be found in most poultry feeds it is not considered that this over-estimation will lead to serious error.

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An Enzymic Method for the Determination of Skimmed Milk Powder in Soup and Sauce Mixes

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An enzymic method for the determination of skimmed milk powder in soup and sauce mixes is described. The method is based on the determination of free lactose by its hydrolysis with β-galactosidase to galactose and glucose, the latter being determined by the hexokinase method. The determination is free of interference from reducing sugars and other substances present in soup and sauce mixes. The method is more rapid, accurate and reliable than other methods in current use.

Existing methods used for the determination of skimmed milk powder in soup and sauce mixes rely upon the chemical determination of total nitrogen or lactose.

The determination of total nitrogen can be unreliable because it will include unknown amounts of nitrogen from wheat flour and other ingredients used in the manufacture of soup and sauce mixes. On the other hand, the direct determination of lactose content by the Lane and Eynon[1,2] and other chemical methods[3,4] is liable to interference from other reducing sugars present in soup and sauce mixes. Selective fermentation with yeast[5–7] to eliminate these interfering sugars is a tedious process and is not practical for routine laboratory use.

This paper describes a method based on the enzymic determination of free lactose which is similar to that previously developed for the determination of skimmed milk powder in raw sausages[8] and mashed potatoes.[9] The lactose is hydrolysed with β-galactosidase to glucose, which is determined by the hexokinase method.[10]

The method is rapid and simple provided the usual precautions necessary with enzymic assays are strictly observed.

Experimental

The experimental work consisted of two parts, the preparation of an aqueous extract of soup and sauce mixes (described below), and the determination of the lactose content of the aqueous extract (described in an earlier paper[8]).

Reagents—

Sodium sulphate solution—Dissolve 200 g of laboratory-reagent grade sodium sulphate decahydrate in distilled water and make the volume up to 1 litre.

Dialysed iron solution, about 5 per cent. iron(III) oxide—Laboratory-reagent grade.

Procedure—

Weigh accurately 5 g of the well powdered and thoroughly mixed sample and macerate it at high speed with 200 ml of distilled water heated to 40 to 50 °C and 25 ml each of the sodium sulphate and dialysed iron solutions. Filter the mixture through a 15-cm Whatman No. 4 filter-paper. Dilute an appropriate volume of the filtrate to 100 ml with glass-distilled water to adjust the lactose concentration of the final solution to within the range 40 to 240 μg ml^{-1}.

Results

To test the suitability of this method for the determination of skimmed milk powder in soup and sauce mixes, recovery tests were carried out on samples that had known concentrations of skimmed milk powder of known lactose content. The results, given as the average of six determinations for each sample, are presented in Tables I and II.

The results show satisfactory recoveries for the different types of soup and sauce mixes.

It has been established previously that various supplies of skimmed milk powder available

TABLE I

RECOVERY OF SKIMMED MILK POWDER FROM SOUP MIXES

Type of soup mix	Skimmed milk powder added, per cent. w/w	Skimmed milk powder found, per cent. w/w	Standard deviation	Recovery, per cent.
Mushroom	25·00	24·92	±0·20	99·7
Chicken	25·00	25·04	±0·12	100·2
Chicken and leek	25·00	24·36	±0·15	97·4
Thick onion	20·00	19·96	±0·11	99·8
Asparagus	5·00	4·90	±0·02	98·0
Minestrone	0·00	0·00	0·00	0·0

TABLE II

RECOVERY OF SKIMMED MILK POWDER FROM SAUCE MIXES

Type of sauce mix	Skimmed milk powder added, per cent. w/w	Skimmed milk powder found, per cent. w/w	Standard deviation	Recovery, per cent.
Parsley	5·74	5·65	±0·02	98·4
Onion	4·56	4·54	±0·07	99·6
Savoury white	6·51	6·47	±0·04	99·4
Mushroom	5·19	5·04	±0·03	97·1
Bread	3·50	3·41	±0·03	97·4

for commercial use in the United Kingdom contained different concentrations of lactose.[8] The lactose contents of fourteen different supplies, calculated as the monohydrate, averaged 51·3 per cent. with a standard deviation of ±2·1.[8] By using this average lactose monohydrate content the skimmed milk powder contents of four different brands of soup mixes and two different brands of sauce mixes sold within the London area were determined. The results obtained are given in Tables III and IV.

TABLE III

SKIMMED MILK POWDER CONTENTS IN RETAIL SOUP MIXES

Type of soup mix	Content in brand, per cent. w/w			
	1	2	3	4
Mushroom	25·3	28·2	29·3	23·5
Chicken	26·5	26·4	22·2	12·3
Chicken and leek, Batch 1	27·1	—	23·5	—
Chicken and leek, Batch 2	26·9	—	23·9	—
Thick onion, Batch 1	—	12·5	21·8	—
Thick onion, Batch 2	—	13·0	20·0	—
Asparagus, Batch 1	28·8	4·8	—	—
Asparagus, Batch 2	28·4	3·8	—	—
Minestrone	0·0	0·0	0·0	0·0

TABLE IV

SKIMMED MILK POWDER CONTENTS IN RETAIL SAUCE MIXES

Type of sauce mix	Content in Brand I, per cent. w/w			Content in Brand II, per cent. w/w		
	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3
Parsley	6·1	5·0	5·9	5·9	6·6	6·2
Onion	4·5	4·0	4·1	4·1	3·6	4·5
Savoury white	7·1	5·0	6·6	6·6	6·7	7·3
Mushroom	5·2	5·2	4·3	—	—	—
Bread	3·4	3·4	3·1	—	—	—

CONCLUSIONS

The enzymic method, when used for determinations of skimmed milk powder in soup and sauce mixes, gives excellent recoveries. The determination is free of interference from reducing sugars and other substances present in the mixes, the error due to these substances being eliminated by the use of a sample blank.

The method is very accurate for the determination of skimmed milk powder that has a known lactose content. However, when a sample contains skimmed milk powder that has an unknown lactose content, the accuracy will be affected to an extent that depends on the deviation of the lactose content of the skimmed milk powder from the average value of 51·3 per cent. The coefficient of variation for fourteen different samples analysed by the author[8] was $\pm 4{\cdot}1$.

The method is rapid and simple and the whole determination takes only $1\frac{1}{2}$ hours, of which the actual working time is less than 15 minutes.

The enzymic method is also applicable to canned soups, but the loss of free lactose during storage owing to browning reactions and other causes needs to be investigated. For dried soups and sauce mixes this loss was found to vary from negligible to slightly less than 10 per cent. during storage for 9 months at room temperature.

The author has adopted this method for the determination of milk solids-not-fat in ice cream because of its specificity and reliability over other methods currently in use.[11–13] At present, work is in progress on the determination of milk solids-not-fat in chocolates and bedtime malted drinks by this method.

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A Simple Thin-layer Chromatographic Technique for the Semi-quantitative Determination of Volatile Nitrosamines in Alcoholic Beverages

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A thin-layer chromatographic procedure is described for determining dimethyl-, diethyl- and dipropylnitrosamines in alcoholic drinks. Nitrosamines are first distilled from an alkaline solution of the sample and then extracted into dichloromethane. Ethanol and interfering materials are removed by co-distillation with hexane or benzene and chromatography on a basic alumina column. Finally, the nitrosamines are determined semi-quantitatively by thin-layer chromatography as nitrosamines or, after oxidation, as the corresponding nitramines. The method is sensitive down to 0·025 p.p.m. and applicable to various drinks such as wine, beer, whisky and rum. Percentage recoveries of added nitrosamines varied between 50 and 100 at the 0·1 p.p.m. level and between 20 and 100 at the 0·025 p.p.m. level.

SINCE the publication of reports[1,2] that dimethylnitrosamine or some other nitrosamines had been found in African alcoholic drinks, and because of the possible implications of linking the high incidence of oesophageal cancer in African natives to the drinking of the locally distilled spirits, considerable interest has been aroused in the search for nitrosamines in alcoholic beverages. However, attempts to detect them have been hampered because of the lack of sensitive and specific methods that are suitable for routine use. The polarographic method, which was used by McGlashan and co-workers,[1,2] has some disadvantages. McGlashan, Patterson and Williams[3] pointed out in a recent paper that the technique used is not specific and interference can occur from furfural, which is a natural constituent of many alcoholic beverages. Thin-layer[4–6] and gas - liquid chromatographic[7–9] methods have been published for detecting nitrosamines in various foods, but these methods have been demonstrated to be unsuccessful with alcoholic drinks. Our earlier method[5] was found to be unsuitable because of interference from ethanol and other materials present in the drinks.

EXPERIMENTAL

REAGENTS—

Glycine - hydrochloric acid buffer solution—Dissolve 22 g of glycine in 200 ml of N hydrochloric acid and dilute to 1 litre with water. Adjust the pH of the solution to 2·1 ± 0·1 with N sodium hydroxide or N hydrochloric acid solution.

Griess reagent—Prepare as described previously.[4] (CAUTION—This reagent is a carcinogen and should be handled carefully.)

Ninhydrin solution—Prepare as described previously,[4] but omit the pyridine.

NEDSA reagent—Prepare two solutions in 30 per cent. v/v acetic acid, one containing 1 per cent. w/v of sulphanilic acid and the other 0·1 per cent w/v of *N*-1-naphthylethylenediamine hydrochloride. Store the latter solution at 4 °C. Mix equal volumes just before use.

Nitrosoamine standards—Dimethylnitrosamine, diethylnitrosamine and dipropylnitrosamine. Prepare several dilutions (10, 1 and 0·05 mg ml^{-1}) of working standards in dichloromethane. Store the solutions at −10 °C. (CAUTION—These compounds are highly carcinogenic and appropriate precautions should be taken in handling them.)

Silica gel—MN-Silica gel G-HR for thin-layer chromatography (Macherey, Nagel & Co., Germany).

Basic alumina—Aluminium oxide, Woelm basic, activity grade 1 for chromatography (Alupharm Chemicals).

Hydrogen peroxide, 50 per cent.—Fisher Scientific Co.

Trifluoroacetic acid—Glass distilled.

SOLVENTS—

Dichloromethane, pentane, hexane and benzene, all glass distilled.

APPARATUS—

Thin-layer chromatographic plates—Prepare 0·25 to 0·3-mm thick silica-gel plates, dry them in air for half an hour and then activate at 100 °C for 1 to 2 hours.

Ultraviolet lamp (without filter)—Similar to the General Electric G15T8 germicidal lamp.

Snyder column with 3 sections—Supplied by O. H. Johns Glass Co. Ltd., Toronto.

Micro-Snyder column—As designed in this laboratory (Fig. 1).

Pear-shaped flask, 25-ml capacity—With 10/30 joint and graduated from the bottom to 0·5 ml (Fig. 1).

Alumina column—A glass column 1 to 1·2 cm in diameter and 20 to 25 cm long with a Teflon stopcock is used. Fill the column with pentane, then insert a plug of glass-wool and gently push it to the bottom of the column with a glass rod. Pour about a 25 to 30-mm layer of basic alumina into the column, which should be prepared just before use and always kept filled with solvent.

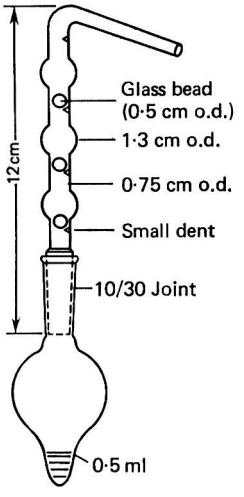

Fig. 1. Micro-distillation apparatus for concentrating volatile nitrosamine solution to a small volume

PRINCIPLE OF THE METHOD

Nitrosamines are distilled and extracted from the distillate with dichloromethane. Interfering amines are removed by washing the dichloromethane extract with glycine - hydrochloric acid buffer. The extract is then concentrated by evaporation and the nitrosamines are determined by thin-layer chromatography with the Griess (or NEDSA) and ninhydrin reagents (method A). Further clean-up is achieved in methods B and C by means of alumina-column chromatography, after removal of ethanol by azeotropic distillation with hexane or benzene. In method C, the nitrosamines are oxidised to the corresponding nitramines, which are then detected by thin-layer chromatography.

METHOD A—

To a 100-ml aliquot of the drink (containing not more than 20 per cent. v/v of ethanol) to be analysed, add 12 g of sodium hydroxide and stir to dissolve. Transfer the solution into a 1-litre distillation flask and add a few carborundum boiling chips. Distil the mixture from an all-glass apparatus and collect about 50 ml of distillate.

Dissolve 5 g of potassium carbonate in the distillate and extract the solution with two 50-ml portions of dichloromethane. Shake the combined extracts with 40 ml of glycine - hydrochloric acid buffer. Discard the aqueous layer. Wash the organic layer by shaking it vigorously with 40 ml of 20 per cent. potassium carbonate solution and dry the final dichloromethane layer over anhydrous sodium sulphate.

Filter the dry extract through a Whatman No. 1 filter-paper and collect the filtrate in a 250-ml distillation flask. Add two or three small boiling chips, fit the Snyder column on to the neck of the flask, and evaporate the solution down to about 5 to 10 ml by heating it in a hot water bath (55 to 60 °C). Transfer the solution into the small pear-shaped distillation flask (without boiling chips) and evaporate the solution down to 0·4 ml, but not less than this volume, after fitting the micro-Snyder column. Remove the apparatus from the water-bath, tilt it and add a few drops of dichloromethane to wash down any nitrosamine trapped in the column. Finally, make the volume up to 0·5 ml with dichloromethane. Stopper the flask, and rinse the sides by a gentle swirling action of the liquid. The solution is now ready for thin-layer chromatographic analysis.

For distilled spirits of high alcoholic content, add 300 ml of water to 100 ml of sample and dissolve 48 g of sodium hydroxide in the mixture. Collect 200 ml of distillate and proceed with the extraction steps as described above by using proportionate amounts of the various reagents. Finally, evaporate the solution down to 0·5 ml as described above.

METHOD B—

Distil the sample and extract the distillate as in method A, then concentrate the extract by evaporating it to about 10 ml. At this stage add 50 ml of hexane through the Snyder column and continue concentrating it. Monitor the temperature of the issuing vapour by inserting a thermometer into the Snyder column. Stop concentrating the extract as soon as the temperature of the vapour rises to between 63 and 65 °C. Do not evaporate the solution down to less than 10 ml at any stage, and add more hexane if necessary.

Carefully transfer the extract to a freshly prepared alumina column and allow the solution to flow through it at a flow-rate of 0·8 to 1 ml min^{-1}. After all of the extract has passed through the column, wash the column successively (flow-rate 2 to 3 ml min^{-1}) with the following mixtures: 2·5 ml of dichloromethane *plus* 47·5 ml of pentane, 5 ml of dichloromethane *plus* 45 ml of pentane, and 10 ml of dichloromethane *plus* 40 ml of pentane. Retain the washings for possible use in the later steps. Elute the nitrosamines from the column by passing through it 75 ml of dichloromethane at the rate of 2 to 3 ml min^{-1}. Evaporate the eluate down to 0·3 ml (by using the Snyder columns) as described under method A and subject the extract to thin-layer chromatographic analysis.

METHOD C—

Proceed as in method B up to the elution step of the nitrosamines from the alumina column and then evaporate the eluate down to about 5 ml. Transfer the solution into a 25-ml pear-shaped flask, add 1 ml of water and evaporate off the dichloromethane by heating the flask (with the micro-Snyder column fitted on top) in a water bath. Transfer the aqueous solution into a 50-ml glass-stoppered flask and add 5 ml of trifluoroacetic acid. Use a portion of the trifluoroacetic acid to rinse the flask and the micro-Snyder column. Add 4 ml of 50 per cent. hydrogen peroxide, shaking and cooling the mixture in a cold water-bath, stopper the flask and allow the mixture to stand overnight at room temperature to permit oxidation of the nitrosamines to nitramines.

Pour the reaction mixture on to 20 g of crushed ice and slowly add 50 ml of 20 per cent. potassium carbonate solution, with constant stirring. The pH of the mixture should be between 9·8 and 10·2, otherwise add more potassium carbonate solution. Extract the solution with two equal volumes of dichloromethane, dry the extract over anhydrous sodium sulphate, filter and evaporate it to 0·3 ml as described above. Take 10 μg of nitrosamine standards in 1 ml of water and proceed through the oxidation and extraction steps. Spot an aliquot of the standard extract alongside the sample extract for the quantitative determination and for calculating percentage recoveries.

THIN-LAYER CHROMATOGRAPHIC DETERMINATION—

Mobile phase—Hexane - diethyl ether - dichloromethane (4 + 3 + 2).[4,6]

Method A—Spot 0·1 ml of extract per spot and 1·0, 1·5 and 2·0 μg of each of the standard nitrosamines on both halves of the thin-layer chromatographic plate. After developing the plate in the above solvent system, spray the left half of the plate with the Griess or NEDSA reagent and the right half with 30 per cent. acetic acid. Spray generously until the entire plate appears thoroughly wet and transparent. Irradiate the plate under ultraviolet light for 10 minutes. Nitrosamine spots should appear as red - purple spots on the left-hand side.

Dry the plate in a jet of warm air for 5 to 10 minutes (or allow to air dry in the fume hood for half an hour) and spray the right-hand side with ninhydrin reagent. Heat the plate at 80 to 100 °C for 15 to 45 minutes. Nitrosamines appear as red - purple spots. Determine the amount of nitrosamines in the extract by visual comparison of the relative spot size and intensity of the respective spots.

Methods B and C—Spot all of the 0·3 ml of extract as a single spot (diameter 8 to 10 mm) and three different amounts of each of the standard nitrosamines or nitramines. After developing the plate, spray with the Griess or NEDSA reagent to locate the nitrosamines. If the extract gives a positive spot, prepare a fresh extract and use the ninhydrin spray as described above. For extracts containing too much interfering material, spot one half or one third of the final 0·3 ml of extract.

Results and discussion

Three samples of wine, two of beer, two of whisky and one of rum were analysed by the above methods and the percentage recoveries of nitrosamines added to the drinks are presented in Table I. In addition, two other samples of sherry and two of red table wine were analysed by method A but no recovery studies were carried out with these samples. None of the samples analysed contained detectable amounts of nitrosamines.

Table I

Recovery of dimethylnitrosamine (DMN), diethylnitrosamine (DEN) and dipropylnitrosamine (DPN) added to various alcoholic drinks

Sample	Amount added, p.p.m.			Method	Approximate recovery, per cent.		
	DMN	DEN	DPN		DMN	DEN	DPN
Sherry	0·1	0·1	—	A	100	100	—
Red wine	0·1	0·1	—	A	80	100	—
Beer I	0·1	0·1	0·045	A	50	80	100
Whisky I	0·1	0·1	0·045	A	50	50	100
Whisky II	0·1	0·1	0·045	A	75	75	100
Rum	0·1	0·1	0·045	A	50	50	100
Red wine	0·025	0·025	0·025	B*†	80	Int.	75
Whisky I	0·025	0·025	0·025	B*†	80	Int.	100
Beer II	0·025	0·025	0·025	B*†	80	Int.	50
Red wine	0·025	0·025	0·025	B*	20	40	100
Beer I	0·025	0·025	0·025	B*	50	50	75
Whisky I	0·025	0·025	0·025	B*	50	25	80
Rum	0·025	0·025	0·025	B*	20	Int.	50
Sherry	0·045	0·045	0·045	C	50	80	80
Beer II	0·025	0·025	0·025	C	50	80	80
Whisky I	0·025	0·025	0·025	C	50	80	80
Rum	0·025	0·025	0·025	C	25	70	70

* Resin and polyamide clean-up[5] was used instead of glycine - hydrochloric acid buffer wash.
† Benzene was used for azeotropic distillation of ethanol; hexane was used in all other instances.
Int. denotes interference from a white spot.

Method A is simple and rapid. The entire procedure can be completed within an 8-hour working day. Methods B and C are more time consuming and should be used only if higher sensitivity (down to 0·025 p.p.m.) is required. A white spot interfered with the detection of diethylnitrosamine in method B. However, we were able to detect it by method C because of the difference in the R_F values of the white spot and diethylnitramine.

R_F values of nitrosamines and nitramines obtained with the solvent system hexane - diethyl ether - dichloromethane (40 + 30 + 20) are shown below.

Dimethyl-nitros-amine	Diethyl-nitros-amine	Dipropyl-nitros-amine	*N*-Nitro-dimethyl-amine	*N*-Nitro-diethyl-amine	*N*-Nitro-dipropyl-amine	Interfering white spot
0·28	0·48	0·68	0·38	0·56	0·74	0·48

Yellow pigments sometimes interfered in the analysis by method B, but we were successful in removing these pigments by passing the initial distillate through a resin and polyamide column as described by us previously.[5] In the trifluoroperacetic acid oxidation step of method C, the yellow pigments are oxidised to colourless compounds and, therefore, it is not

necessary to use the resin and polyamide treatment while using this method. The clear and distinct spot of the nitramines obtained from the spiked samples in method C suggested that it would be possible to detect the nitrosamines at the 0·01 p.p.m. level. However, no attempt was made to carry out recovery studies at this level. When 10 μg of furfural were analysed by thin-layer chromatography or 50 mg of furfural were taken through all the steps of method C, no interference was noticed in either instance.

It is important to use efficient Snyder columns for successful operation of the procedures. During the concentration of the extracts care should be taken to ensure that there is always a liquid seal around each floating bubble (or bead) in the column and that there is a constant and steady refluxing action of the condensed solvent. Each Snyder column, especially the micro-column, must be tested for efficiency by using pure reagents. Similarly, the alumina column should be tested to determine percentage recoveries. If alumina is not sufficiently active it can be activated by heating at 300 °C for 4 hours. On the other hand, more than 75 ml of dichloromethane is needed to elute nitrosamine from very active alumina. In such instances, water-saturated dichloromethane can be used for elution. Test runs should be carried out with spiked samples (0·1 p.p.m. level) to determine the elution pattern of various nitrosamines and to determine losses, if any, occurring in various washings from the alumina column.

Ethanol interferes with the alumina clean-up step and is, therefore, removed by azeotropic distillation with hexane or benzene. Extreme care should be taken to stop the azeotropic distillation as soon as the temperature of the issuing vapour reaches 65 °C (for hexane) or 76 °C (for benzene). Excessive concentration or prolonged heating at these temperatures will result in considerable losses of dimethylnitrosamine. Most of the losses of the nitrosamines occur during concentration, especially when the volume is less than 1 ml, and some during azeotropic distillation. Better recoveries might be expected if the extract is evaporated down to 1·0 ml by using method A and then analysed by gas - liquid chromatography (a 50-μl sample is injected) with a Coulson electrolytic conductivity detector.[9]

We have found from experience that the double-spray technique, as indicated previously,[4] is specific for *N*-nitrosamines, and any spot that gives a positive reaction with both of the reagents should be suspected to be a nitrosamine. However, every effort should be made to confirm the identity by other available methods.[5,9–11] The combined gas - liquid chromatographic and mass-spectrometric technique[12] is preferable for this purpose. As there is also a possibility that these compounds will form as artifacts during the analysis, all positive results should be interpreted very carefully. Griess reagent contains a carcinogen (2-naphthylamine) as an impurity and, therefore, it should be used with appropriate precautions. NEDSA reagent should be used instead of Griess reagent whenever possible.

Thus far, no identifiable nitrosamines have been found in alcoholic beverages, but it would be desirable to carry out a thorough search for these compounds. The method described in this paper has been tried only with dimethylnitrosamine, diethylnitrosamine and dipropylnitrosamine, and it is hoped that, with minor modifications, it will be satisfactory for other volatile nitrosamines. There is also a need for sensitive methods for determining non-volatile nitrosamines that could occur in wine, beer and other undistilled alcoholic drinks.

We are grateful to A. Moore for fabricating the micro-distillation apparatus (Fig. 1) used in these experiments.

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Quantitative Esterification of Lipids on Thin-layer Adsorbents

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Lipid classes separated by thin-layer chromatography can be determined by gas chromatography of the methyl esters of the component fatty acids with added internal standard. A simplified procedure is described for the complete analysis of all the lipid classes in a given sample in which transesterification is performed on the thin-layer adsorbent itself. The methyl esters are recovered from the reaction mixture with a less polar solvent than would have been required to elute the original component. As a consequence of the elimination of a step that requires preliminary elution of components from the thin-layer plates, there is less contamination of minor components by trace amounts of impurities in large volumes of solvent, and the results obtained are more reproducible.

It has been shown that the amounts of individual lipid classes eluted from thin-layer chromatographic plates can be quantitatively determined by gas chromatography of the methyl esters of the component fatty acids after addition of an appropriate internal standard.[1] There have recently been several reports on the esterification of lipid samples directly on thin-layer chromatographic plates or on thin-layer adsorbents without prior elution.[2–10] The elimination of this step reduces the risk of loss of sample, particularly of phospholipids, which are strongly adsorbed by silica gel, and of contamination of the samples by trace amounts of impurities in large volumes of solvent. The precision of several methods of esterifying lipids on thin-layer chromatographic adsorbents has been investigated and a simple quantitative modification of the previously described procedure[1] developed.

Experimental

Lipid samples—

The pig-liver lipids used were similar to those described previously.[1] Egg-yolk lipids were obtained from four locally purchased fresh eggs by extraction with chloroform - methanol (2 + 1 v/v).

Determination of major lipid classes—

Triglyceride, diglyceride, monoglyceride, free fatty acid and phospholipid bands, which were separated by thin-layer chromatography in the same manner as before,[1] were scraped into 15-ml test-tubes with ground-glass joints at the top. Dichloromethane (1 ml) was added to the tube containing the triglycerides to effect their dissolution and 1 ml of a standard solution of methyl heptadecanoate in methanol[1] was added to each tube; 1 ml of 2 N sodium methoxide in methanol was then added to all the tubes except that containing the free fatty acids, to which 1 ml of boron trifluoride - methanol reagent* was added instead. The tubes were stoppered, shaken vigorously and then heated at 50 °C for 15 minutes. The sodium methoxide solutions were then acidified with 0·3 ml of glacial acetic acid, 5 ml of water were added to all the tubes and the required esters extracted into diethyl ether, two 5-ml portions of solvent being used. After shaking the solutions with the solvent, the tubes were centrifuged at 2000 r.p.m. for a few minutes to precipitate the silica gel. The solvent layers were removed by Pasteur pipette and washed with 5 ml of 2 per cent. potassium hydrogen carbonate solution before being dried over anhydrous sodium sulphate. The diethyl ether was removed under reduced pressure and the esters were taken up in hexane for gas-chromatographic analysis.

Cholesteryl esters could not be quantitatively esterified in the presence of silica gel so the band containing this component was scraped into a small chromatographic column from

* BDH, Poole, Dorset.

which the cholesteryl esters were eluted with 50 ml of chloroform. The solvent was removed and 1 ml each of dichloromethane, methyl heptadecanoate standard solution and 2N sodium methoxide in methanol were added and the solution was heated at 50 °C for 30 minutes, when the methyl esters were recovered as before.

DETERMINATION OF PHOSPHOLIPID CLASSES—

The phospholipids were separated from the other lipid classes by thin-layer chromatography as described above and the appropriate band was scraped into a small chromatographic column and eluted with chloroform - methanol - acetic acid - water (25 + 15 + 4 + 2). This solvent system was also used to separate about 5 mg of the phospholipids into separate classes on thin-layer chromatographic plates coated with a 0·55-mm layer of Kieselgel G without binder (Camag).[11] Bands were detected by spraying with 2,7-dichlorofluorescein (0·1 per cent. solution in ethanol), identified by comparison with known standards and scraped into test-tubes. Methyl heptadecanoate standard solution (1 ml) was added to each tube and then 1 ml of 2 N sodium methoxide in methanol was added to all the tubes except that containing sphingomyelin. The tubes were then heated at 50 °C for 15 minutes and the methyl esters recovered as before. Methanol - concentrated hydrochloric acid (4 + 1 v/v) (2 ml) was added to the sphingomyelin and the mixture was maintained at 50 °C overnight, when the methyl esters were recovered as described above.

TABLE I

DETERMINATION OF THE MAJOR LIPID CLASSES OF PIG LIVER AND EGG YOLK

Mean values with standard deviations of three determinations

	Total lipids, per cent. w/w				
Method	Cholesteryl esters	Triglycerides	Diglycerides	Free fatty acids	Phospholipids
Liver—					
Esterification after elution:					
Mean	5·5	15·7	—	1·4	77·4
Standard deviation	±0·2	±0·8	—	±0·1	±1·4
Esterification on thin-layer chromatographic adsorbent:					
Mean	4·2	15·4	—	0·9	79·5
Standard deviation	±0·1	±0·4	—	±0·1	±0·2
Egg yolk—					
Esterification after elution:					
Mean	1·0	65·3	1·8	—	31·9
Standard deviation	±0·2	±1·9	±0·2	—	±1·6
Esterification on thin-layer chromatographic adsorbent:					
Mean	1·5	64·8	2·1	—	31·6
Standard deviation	±0·1	±0·6	±0·1	—	±0·3

	Phospholipids, mol per cent.						
	Sphingomyelin	Lysophosphatidylcholine	Phosphatidylcholine	Phosphatidylinositol	Phosphatidylserine	Phosphatidylethanolamine	Phosphatidylglycerol
Liver—							
Esterification after elution:							
Mean	3·2	—	56·0	6·8	1·8	26·5	5·7
Standard deviation	±0·4	—	±2·9	±0·3	±0·2	±1·7	±1·4
Esterification on thin-layer chromatographic adsorbent:							
Mean	6·1	—	54·8	6·5	1·5	26·6	4·5
Standard deviation	±0·9	—	±1·0	±0·5	±0·1	±0·3	±0·3
Egg yolk—							
Esterification after elution:							
Mean	1·0	2·6	77·1	1·6	—	17·7	—
Standard deviation	±0·3	±0·4	±2·1	±0·2	—	±1·6	—
Esterification on thin-layer chromatographic adsorbent:							
Mean	2·3	2·2	76·3	1·8	—	17·4	—
Standard deviation	±0·4	±0·2	±0·9	±0·2	—	±0·6	—

Results and discussion

It was found that quantitative esterification of all lipid classes, with the exception of cholesteryl esters, could be achieved in the presence of silica gel. It was necessary, however, to extract the esters from the reaction medium with a polar solvent such as diethyl ether and to centrifuge the extract to remove solid particles so as to ensure quantitative recovery. This need does not appear to have been appreciated by other workers.

The simplified lipid determination procedure described in the experimental section was applied to the analysis of pig-liver lipids (which had been analysed previously[1]) and of egg-yolk lipids. The amounts of each lipid were calculated by using the factors given earlier[1] and the results compared with those obtained by the full procedure described previously.[1] The results are listed in Table I; they agree closely but the standard deviations of three determinations by the simplified procedure are lower than those obtained by the original procedure. The only important difference was that the sphingomyelin concentration determined by the modified procedure was found to be significantly higher in each sample. Kinetic experiments with pure sphingomyelin confirmed that esterification of this lipid class was not complete under the conditions described earlier[1] and that prolonged heating in methanol - concentrated hydrochloric acid was required for quantitative transesterification. It should be noted that during alkaline transesterification of the total phospholipids, the sphingomyelin will not be affected, so that if the latter is a major component it may be necessary to amend the result for the total phospholipid analysis once the amount of sphingomyelin in the sample has been determined.

There was much less extraneous background material in the gas-chromatographic traces obtained from the methyl esters of minor components that had been esterified directly on thin-layer adsorbents. The determinations of the fatty acid composition of these esters were accordingly more reproducible.

All major lipids, with the exception of cholesteryl esters, can therefore be transesterified without prior elution from thin-layer adsorbents. By using the simplified procedure described above, it is possible to determine the amount, as well as the fatty acid composition, of every lipid class in a given sample in a very short time.

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Studies on the Quantitative Freeze-drying of Aqueous Solutions of some Metabolically Important Aliphatic Acids Prior to Gas - Liquid Chromatographic Analysis

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These studies have shown that the over-all losses observed are due to volatilisation during the freeze-drying process. These losses are related to the variation of the vapour or sublimation pressures of the acids with temperature, and are also related to their latent heats of vaporisation or sublimation.

Reliable data on the latent heats of vaporisation and sublimation of the acids of interest are seldom available and a method for the estimation of the latent heats, based on group contributions, has been developed. The reliability of the method, which is generally applicable to organic compounds, is discussed.

Thermochemical data derived from the use of this method have been used in conjunction with reliable published experimental data to determine the optimum freeze-drying conditions for the complete quantitative recovery of all but the most volatile of the acids studied, with a minimum of preliminary experimental work.

THE study of organic acidurias in human metabolic disorders has been largely neglected although several methods for the detection of the acids in urine (and blood) have been developed. Most of these methods involve the extraction of the acids with solvents or by column chromatography, followed by the separation and determination of volatile derivatives of the acids by gas - liquid chromatography. At some stage in many of these procedures the urine, or an aqueous extract of it, is evaporated to dryness for the preparation of derivatives. Anhydrous conditions must be achieved when trimethylsilyl derivatives are to be prepared because the reagents used react rapidly with all active hydrogen atoms, including those of water.

Most previous workers have concentrated aqueous solutions or organic solvent extracts by rotary evaporation under reduced pressure,[1–4] although freeze-drying (lyophilisation) has sometimes been used.[5–7] Very little rigorously controlled quantitative work has been carried out although the availability of quantitative data would greatly increase the value of studying simultaneously a range of carboxylic acids in relation to human disease. The concentration of aqueous solutions of organic acids by rotary evaporation leads to large losses by volatilisation in the presence of water vapour; this effect is particularly marked for the hydrophilic aliphatic acids, and rotary evaporation should not be used in quantitative studies. Freeze-drying of the extracts, although requiring a longer working time, may allow complete recoveries of all but the most volatile acids if carefully selected conditions are used.

This paper describes a method by which the conditions for the quantitative freeze-drying of organic compounds can be selected, and presents the results of applying the method to some low molecular weight aliphatic acids in aqueous solution and in the aqueous pyridinium acetate eluate from a DEAE-Sephadex ion-exchange column. The compounds studied are of particular importance in the study of certain inborn errors of metabolism.

THEORETICAL

A knowledge of the variation of the vapour or sublimation pressure of the sample with temperature is needed to provide a theoretical basis for the derivation of the most suitable freeze-drying conditions. The greatest losses would be expected to occur when the bulk of the water has been removed and the temperature rises, while the sample remains under a

relatively high vacuum. Therefore, a knowledge of the latent heats of sublimation or vaporisation in the region of the ambient temperature is necessary in order to decide which compounds are most liable to volatilisation losses.

Both of these parameters are related by the Clapeyron - Clausius equation—

$$\frac{d(\ln p)}{dT} = \frac{\Delta H}{RT^2}$$

where p is the pressure, T is the absolute temperature, ΔH is the latent heat of vaporisation (ΔH_v) or of sublimation (ΔH_s) of the substance, and R is the gas constant and is equal to $1{\cdot}98717 \pm 0{\cdot}0029$ cal $°K^{-1}$ mol^{-1} (see Note).[8] When this equation is used it is assumed that ΔH remains constant over the temperature range of the experiments, that the vapour behaves as an ideal gas and that the molar volume of the liquid or solid is negligible. The last two assumptions become less significant at lower saturation vapour or sublimation pressures (in the range 10 to 10^{-2} torr; see Note) and ΔH remains reasonably constant over limited temperature ranges (approximately 50 °K), particularly for solid crystalline materials. The equation therefore provides a reasonably good fit of vapour and sublimation pressure data, even for substances such as the carboxylic acids, which are known to have very imperfect vapours.[8]

NOTE—

The S.I. unit of energy is the Joule (J), but for the sake of clarity the thermochemical calorie is used here in preference; 1 thermochemical calorie = 4·184 J.

Similarly, the S.I. unit of pressure is the newton per square metre (N m^{-2}), but the torr is used here because of the greater understanding it conveys in the present work; 1 torr = 133·32 N m^{-2}.

On integrating and taking common logarithms, the Clapeyron - Clausius equation becomes—

$$\log_{10} p = A - \left(\frac{\Delta H}{2{\cdot}303\ RT}\right)$$

where A is a constant.

The use of this equation requires the knowledge of the latent heat of vaporisation or sublimation of the compound. Extensive reliable data on latent heats for all the acids of interest are not available and for these acids the latent heats have been estimated for the construction of the graphs of pressure against temperature. The method used for the estimation of the latent heats of vaporisation is based on that of Laidler,[9] who related them to the molecular structures of the compounds. This method is considered to give fairly accurate results except for oxygen-containing compounds,[8] and to obtain more reliable estimations of the latent heats of the acids studied here the contributions to the latent heats of vaporisation by particular molecular groups have been re-calculated. It has been assumed, from the work of Laidler,[9] that contributions to ΔH_v arising from a C–C bond, in which both carbon atoms are sp^3 hybridised, is zero. New values for the group contributions to ΔH_v have been calculated from the experimental data of Cox and Pilcher,[8] these being more extensive and reliable than the data that were available to Laidler.[9] The calculations have been made for several series of compounds that contain each group of interest and the average result of these figures was used for the estimation of the latent heats of vaporisation of other compounds. These results, which are given in Table I, do not agree with all of Laidler's values,[9] particularly for oxygen-containing groups, but calculation of the latent heats of vaporisation for a number of compounds for which reliable experimental data are available[8] has shown that the values given here provide much greater accuracy in their estimation. The comparisons between published values and those calculated by this method are given in Table II.

It will be noticed in Table II that there is better agreement between the calculated and experimentally determined values for the latent heats of vaporisation of liquids (ΔH_v) than for the latent heats of sublimation of solids (ΔH_s). This is due to the effect of the crystal lattice geometry of the solid, which is related to the melting-point of the compound. The discrepancy between the determined and estimated values increases with increase in the melting-point, and this is especially apparent with maleic and fumaric acids (*cis*- and *trans*-butenedioic acid, respectively), as shown in Table II. The crystal lattice geometry factor, ΔH_m, is related to the latent heats of vaporisation and sublimation of the compound by the equation[8] $\Delta H_s = \Delta H_v + \Delta H_m$ and can itself be estimated by using the equation given by Bondi,[10] who related it to the melting-point of the compound.

TABLE I

GROUP CONTRIBUTIONS TO THE LATENT HEAT OF VAPORISATION (ΔH_v) OF ORGANIC COMPOUNDS

Values are given in kcal per formula weight in grams at 25 °C (1 kcal = 4·184 kJ)

Group	ΔH_v	Number of experimental values used
C–C	0	15
C=C	1·07 ± 0·70	17
C≡C	3·04 ± 0·65	3
C–H (primary)	0·448 ± 0·02	9
C–H (secondary)	0·601 ± 0·05	8
C–H (tertiary)	0·653 ± 0·14	10
C–COOH	11·00 ± 0·60	9
C–OH	7·42 ± 0·50	15
C–CHO	4·73 ± 0·48	4
C–CO–C	3·99 ± 0·70	9
C–O–C	1·38 ± 0·10	3

The discrepancy caused by ΔH_m is small for substances that have low melting-points (Table II), and for the purposes of the present study ΔH_v has been taken as being essentially representative of ΔH_s when experimental data are not available and the melting-point is low.

The latent heats of a number of other aliphatic acids of importance in metabolic studies have been estimated by using the calculated group contributions (Table III). These latent heats and those experimentally determined values given in papers cited by Cox and Pilcher[8] have been used to calculate the graphs of pressure against temperature by means of the integrated Clapeyron - Clausius equation. The optimum conditions for freeze-drying have been selected by comparing the positions of the lines, shown in Fig. 1, with those of water, pyridine and acetic acid, the last two compounds being derived from the pyridinium acetate present in the buffer used to extract the acids. It is apparent that in the present work a pressure of about 0·5 torr and a temperature of −10 °C would be expected to result in fairly rapid volatilisation of the water with minimum losses of the acids of interest. The time required to dry the specimens has also been considered. The use of the selected conditions and of conditions that differ from them in terms of pressure and sample temperature have confirmed that those chosen result in good recoveries of most of the acids studied.

EXPERIMENTAL

REAGENTS—

Free acids are required for the preparation of trimethylsilyl derivatives and were used in this study. All the acids were of the highest quality available. Oxalic acid dihydrate and citric acid monohydrate were obtained from British Drug Houses Ltd., succinic, fumaric and

TABLE II

CALCULATED AND DETERMINED LATENT HEATS OF VAPORISATION (ΔH_v) AND SUBLIMATION (ΔH_s) OF ORGANIC ACIDS

Values are given in kcal per formula weight in grams at 25 °C (1 kcal = 4·184 kJ)

Acid	Melting-point/ °C	ΔH_v (calculated)	ΔH_v (determined[8])	ΔH_s (determined[8])
Formic (methanoic)	8	11·6$_5$	11·0	—
Acetic (ethanoic)	17	12·3	12·5	—
Propionic (propanoic)	−20	13·5	13·7	—
n-Butyric (butanoic)	−8	14·7	15·2	—
n-Nonanoic	15	20·7	19·7	—
Oxalic (β) (ethanedioic)	101 (dihydrate)	22·0	—	22·3
n-Undecanoic	28	23·3	23·4	29·0
Maleic (*cis*-butenedioic)	130	24·4	—	26·3
Fumaric (*trans*-butenedioic)	286	24·4	—	32·5
Succinic (butanedioic)	184	24·4	—	28·1
Adipic (hexanedioic)	153	26·8	—	30·9
Dodecanedioic	129	34·0	—	36·6

L-malic acids from Sigma (London) Chemical Co., glycollic acid from Fluka A.G. via Fluorochem Ltd., and glyoxylic acid monohydrate from Koch-Light Ltd. Tetracosane for use as an internal standard was obtained from Ralph N. Emmanuel Ltd. and Koch-Light Ltd. Pierce Chemical Co. silylating reagents were purchased from Phase Separations Ltd. All other reagents used were of analytical-reagent grade.

STANDARD SOLUTIONS—

Standard solutions, containing 200 mg l^{-1} of the free acids, were used. They were freshly prepared each fortnight, and stored at 0 to 4 °C when not in use. Aliquots of these solutions (5·0 ml ≡ 1·0 mg of each acid) were used in all the experiments.

RECOVERY OF ACIDS—

The effect of pH on the recovery of the acids in the pH range 3 to 7 was studied. Free acids are required for silylation and recoveries at higher pH values were therefore not investigated.[5] The pyridinium acetate buffer used in the anion-exchange extraction procedure has a pH of about 6·8 and falls within the upper limit of the above pH range. In most of the studies the effect of temperature and pressure on the recoveries was investigated, and the experimental conditions are described below.

TABLE III

CALCULATED LATENT HEATS OF VAPORISATION OR SUBLIMATION (ΔH) OF SOME METABOLICALLY IMPORTANT ALIPHATIC ACIDS

Values are given in kcal per formula weight in grams at 25 °C (1 kcal = 4·184 kJ)

Acid	Melting-point/ °C	ΔH (calculated)
Glyoxylic (oxoethanoic)	98	15·7
Pyruvic (2-oxopropanoic)	13·6	16·3
Glycollic (hydroxyethanoic)	80	19·6
Lactic (2-hydroxypropanoic)	25	20·4
Malonic (propanedioic)	135	23·2
Hydroxypyruvic (2-oxo-3-hydroxypropanoic)	81	23·6
Methylmalonic (2-methylpropanedioic)	132	24·0
Oxaloacetic (keto form) (2-oxobutanedioic)	—	27·2
Glyceric (2,3-dihydroxypropanoic)	—	27·7
2-Ketoglutaric (2-oxopentanedioic)	111	28·4
L-Malic (2-hydroxybutanedioic)	100	31·3
cis-Aconitic (*cis*-propene-1,2,3-tricarboxylic)	130	35·9
Oxalosuccinic (1-oxopropane-1,2,3-tricarboxylic)	—	38·8
Citric (2-hydroxypropane-1,2,3-tricarboxylic)	153	42·8
Isocitric (1-hydroxypropane-1,2,3-tricarboxylic)	125	42·9

FREEZE-DRYING—

The studies on the effect of change in pH were carried out by using a Gallenkamp A.G. freeze-dryer and an Edwards ES 50 single-stage rotary vacuum pump producing an ultimate vacuum having a pressure less than 10^{-2} torr with a swept volume of 53 l min^{-1}. This freeze-dryer has a low capacity and could not be used for evaporating large volumes of the column eluates and in later studies an EF1 freeze-dryer (Edwards High Vacuum Ltd.), fitted with individual closures for each of the eight ports, was used. The condensers of both freeze-dryers were cooled with solid carbon dioxide - methanol at −70 °C. In the studies at low vacuum (pressure above 0·5 torr) an Edwards two-stage 2SC30 rotary vacuum pump producing an ultimate vacuum having a pressure of 10^{-3} torr with a displacement of 30 l min^{-1} was used, together with a Vacustat for measuring the pressure produced. Studies at higher vacuum (pressure below 0·5 torr) were carried out by using Edwards single-stage ES100 or two-stage ED100 rotary vacuum pumps. These produce ultimate vacua having pressures of less than 5×10^{-3} torr and less than 2×10^{-4} torr, respectively, and both have displacements of 100 l min^{-1}. A Pirani gauge was used to measure pressures below 0·5 torr.

When necessary, the samples were cooled externally by using a eutectic mixture of ice and sodium chloride (−20 °C), ice and potassium chloride (−10·6 °C) or ice and anhydrous sodium sulphate (−3·6 °C) contained in an open-topped Dewar flask. These eutectic mixtures remain at constant temperature for more than 24 hours under the above conditions.

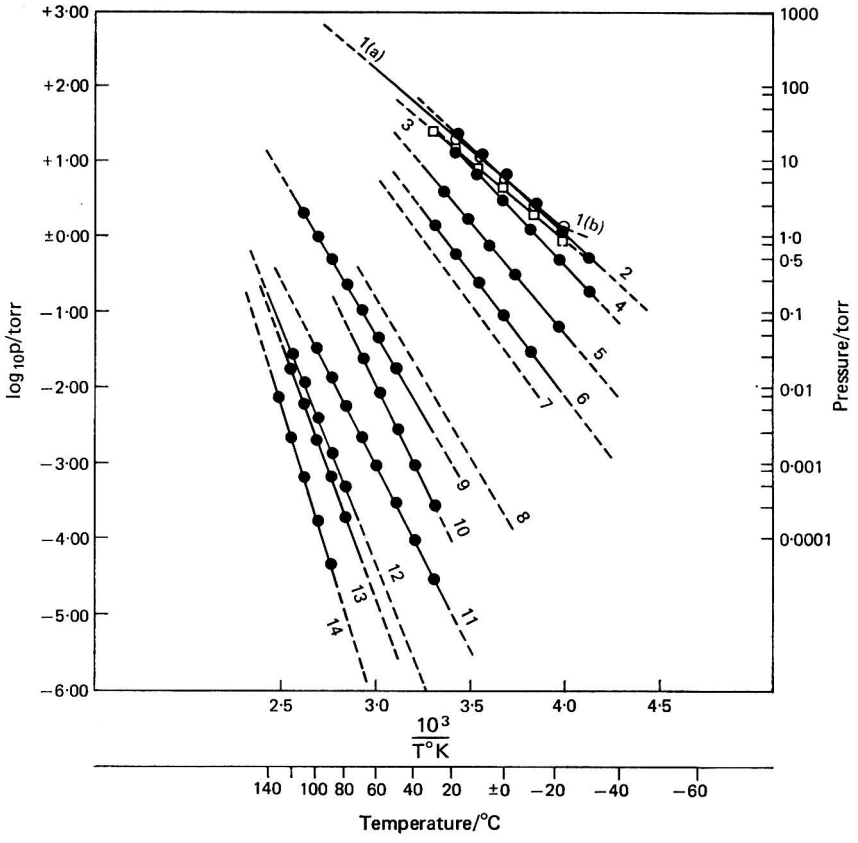

Fig. 1. Pressure - temperature diagrams for aliphatic carboxylic acids, water and pyridine. Lines are: 1(*a*), water; 1(*b*), ice; 2, formic acid; 3, pyridine; 4, acetic acid; 5, propionic acid; 6, n-butyric acid; 7, glyoxylic acid (estimated); 8, glycollic acid (estimated); 9, n-nonanoic acid; 10, n-undecanoic acid; 11, β-oxalic acid (anhydrous); 12, succinic acid; 13, adipic acid; 14, dodecanedioic acid

GAS - LIQUID CHROMATOGRAPHY—

A Hewlett-Packard F & M 402 gas chromatograph fitted with dual U-shaped columns and dual flame-ionisation detectors was used.

Carboxylic acids were converted into their trimethylsilyl esters and hydroxy acids into their trimethylsilyl ethers - trimethylsilyl esters and were separated by chromatography on 6-foot columns packed with 10 per cent. OV-101 on HP Chromosorb W (AW-DMCS), temperature-programmed from 110 to 280 °C at 5 °C min^{-1}. Peak areas were measured by multiplying the peak height by the width at half-height and were expressed quantitatively relative to the peak area of tetracosane as the internal standard.

Full details of this gas - liquid chromatographic analysis will be published elsewhere.

RESULTS

The results of the freeze-drying studies are given in Tables IV to VI. Duplicate or quadruplicate analyses were made in all cases.

Table IV shows the recoveries obtained at different pH values. No consideration was given at this stage to the effect of temperature or pressure on the sample. The samples remained frozen throughout the freeze-drying process, and drying was carried out only during the day, samples being removed as soon as they were dry. The ES50 pump used does not produce a vacuum having a pressure less than 0·3 torr under the load conditions used in the experiment.

TABLE IV

EFFECT OF pH AT THE START OF FREEZE DRYING ON THE RECOVERIES OF SOME ORGANIC ACIDS

Number of determinations	pH	Acid recovery, per cent w/w*						
		Glycollic	Oxalic	Glyoxylic	Succinic	Fumaric	L-Malic	Citric
2	3·5	70·6 (67·4; 73·8)	91·3 (87·3; 95·2)	38·4 (37·5; 39·3)	104·5 (101·4; 107·6)	102·3 (98·9; 105·7)	74·5 (72·2; 76·7)	94·8 (93·3; 96·2)
2	4·5	70·4 (69·0; 71·8)	95·9 (95·6; 96·1)	25·3 (24·8; 25·7)	107·3 (105·5; 109·0)	104·0 (102·5; 105·6)	74·9 (74·2; 75·5)	96·1 (95·5; 96·6)
4	7·0	91·7 (82·6— 106·4)	89·1 (81·0— 102·1)	25·1 (22·4— 26·7)	96·0 (94·5— 101·4)	99·3 (97·4— 101·8)	76·1 (74·5— 77·5)	93·9 (93·1— 95·0)

*The range of values upon which the mean result is based is shown in parentheses.

NOTE—

The Edwards ES 50 rotary vacuum pump used does not produce a vacuum having a pressure less than 0·3 torr under the load conditions used. The required pH was obtained by using acetic acid or 1·5 M pyridinium acetate buffer solution.

Tables V and VI give the recoveries obtained under various conditions of temperature, pressure and time. The term "no cooling" in Table VI indicates that no external cooling was applied, although the sample remained frozen (at below −5 °C) for the greater part of the drying process, and warmed to ambient temperature only after most of the water had been removed.

DISCUSSION

The only previous quantitative study of the freeze-drying of organic acids is that of Rumsey and Noller,[5] who studied the effect of change in pH on the recovery of some aliphatic acids. They did not consider the effects of temperature and pressure on the samples and the present study has shown that the effects of these parameters are of greater importance in achieving quantitative recoveries than are those effects produced by changes in pH (Table IV). The pH range used in the present study was chosen so that the acids remained in their free acid forms in the final dry residue, this being essential for successful trimethylsilylation, and

TABLE V

EFFECT OF THE LENGTH OF TIME FOR WHICH THE MATERIAL REMAINED UNDER VACUUM ON THE RECOVERIES OF SOME ORGANIC ACIDS

Number of determinations	Time/ hours	Acid recovery, per cent. w/w*						
		Glycollic	Oxalic	Glyoxylic	Succinic	Fumaric	L-Malic	Citric
4	7 (just dry)	79·6 (73·0— 88·0)	80·3 (71·8— 87·8)	41·9 (36·1— 45·4)	109·5 (106·0— 112·9)	99·7 (93·2— 106·8)	73·3 (69·2— 75·7)	77·5 (72·4— 81·0)
2	15	57·7 (54·8; 60·5)	43·3 (42·0; 44·7)	42·1 (41·7; 42·5)	111·1 (110·2; 112·0)	100·7 (95·0; 106·4)	72·1 (72·0; 72·1)	82·7 (82·0; 83·4)
2	19	54·7 (54·5; 54·8)	44·3 (41·4; 47·2)	45·6 (45·1; 46·2)	106·1 (103·3; 109·0)	98·7 (98·2; 99·1)	78·2 (77·8; 78·5)	81·0 (78·9; 83·0)
2	31	39·1 (38·2; 40·1)	34·4 (29·6; 39·1)	39·7 (39·1; 40·3)	103·3 (102·4; 104·2)	96·1 (93·5; 99·8)	68·5 (63·4; 74·5)	74·9 (73·5; 74·9)

* The range of values upon which the mean result is based is shown in parentheses.

NOTE—

All determinations were made at pH 3·5 (obtained by use of acetic acid) and by using an Edwards ED 100 rotary vacuum pump.

TABLE VI

THE EFFECT OF TEMPERATURE, PRESSURE AND THE LENGTH OF TIME FOR WHICH THE MATERIAL REMAINED UNDER VACUUM ON THE RECOVERIES OF SOME ORGANIC ACIDS

Number of determinations	Conditions used: Pump (Edwards code)	Time/hours	Pressure/torr	Temperature/°C	Acid recovery, per cent. w/w* Glycollic	Oxalic	Glyoxylic	Succinic	Fumaric	L-Malic	Citric
2	ED 100	10	0·15	−20	75·7 (73·0; 78·4)	90·3 (89·1; 91·5)	59·6 (58·0; 61·2)	101·2 (99·7; 102·7)	99·0 (98·9; 99·1)	68·4 (66·1; 70·7)	84·2 (80·1; 88·3)
2	ED 100	3·5	0·15	no cooling	88·7 (87·0; 90·4)	96·8 (92·0; 101·6)	67·4 (65·3; 69·5)	99·6 (99·4; 99·8)	101·6 (100·1; 103·1)	70·5 (70·3; 70·7)	81·1 (77·9; 84·3)
2	ED 100	6	0·5	no cooling	73·7 (72·2; 75·3)	96·9 (86·6; 107·2)	52·9 (46·1; 59·7)	100·0 (100·0; 100·0)	99·4 (97·7; 101·2)	70·0 (68·8; 71·1)	97·1 (93·1; 101·2)
3	2SC30	20	2·5 ± 1·0	no cooling	72·5 (70·0— 77·0)	119·1 (112·7— 123·0)	33·5 (31·8— 37·4)	114·9 (110·0— 117·8)	113·1 (108·1— 115·6)	76·2 (72·1— 78·8)	96·8 (95·0— 98·2)
3	2SC30	9	3·0 ± 0·1	no cooling	90·2 (86·0— 93·5)	124·2 (123·9— 124·6)	47·3 (45·6— 49·8)	107·8 (104·4— 111·0)	100·6 (99·1— 101·8)	79·2 (74·1— 82·1)	97·7 (97·3— 98·0)
2	2SC30	41 ± 1	0·9 ± 0·3	−6 ± 3	103·2 (103·1; 103·3)	114·6 (113·6; 115·7)	46·8 (44·4; 49·2)	113·6 (113·1; 114·1)	100·0 (99·2; 102·0)	85·9 (85·7; 86·2)	99·1 (97·2; 101·0)
4	2SC30	17	1·5 ± 0·1	−2 ± 1	86·4 (84·0— 90·1)	113·0 (105·5— 118·0)	31·6 (27·2— 35·0)	103·4 (101·9— 104·1)	100·2 (99·2— 102·0)	74·4 (72·3— 76·6)	94·0 (87·6— 96·5)
4	2SC30	42	2·5 ± 0·1	−5 ± 2	99·0 (94·9— 101·2)	102·9 (94·4— 108·9)	40·0 (19·8— 52·8)	108·5 (106·9— 110·0)	99·0 (95·5— 101·8)	77·7 (72·2— 80·5)	90·3 (88·7— 92·6)
4	ES 100	17	0·5	−10	95·6 (89·2— 100·2)	110·9 (108·4— 112·5)	30·1 (27·1— 34·5)	113·1 (111·6— 115·0)	101·0 (95·3— 105·5)	79·3 (79·2— 79·8)	92·8 (86·7— 97·0)
4	ES 100	7	0·5	−10	96·8 (94·1— 99·1)	108·7 (105·0— 111·2)	35·7 (29·9— 38·5)	111·1 (104·2— 113·5)	102·2 (100·6— 104·5)	81·1 (79·8— 82·4)	96·1 (93·6— 97·3)

* The range of values upon which the mean result is based is shown in parentheses.

NOTE—
All determinations were made at pH 7 in the presence of 1·5 M pyridinium acetate buffer solution.

the results obtained are sufficiently consistent to show that variations within this pH range have little effect. The buffer used in the elution from the DEAE-Sephadex column contains pyridinium acetate, and on freeze-drying the acids tend to remain in the form of their pyridinium salts. However, these salts are only weakly ionic and tend to dissociate into their separate components under conditions in which the pyridine is being withdrawn from the system by volatilisation, leading to increased losses of the free acid during prolonged evacuation.

Studies of the effect of the time the sample was under vacuum were made initially without control of the sample temperature or pressure. The ED100 pump used in these studies produces vacua having pressures less than 0·1 torr under load conditions and Table V shows that long periods of evacuation (*i.e.*, overnight) led to greater losses, especially of the low molecular weight acids. It is apparent that the time spent under vacuum after the bulk of the water has been removed is most critical. The sample warms to the ambient temperature at this stage and consideration of the graphs of pressure against temperature (Fig. 1) shows that losses would increase sharply under these conditions as the new temperature - pressure coordinates intersect and pass the lines followed by the more volatile acids.

Consideration of the Clapeyron - Clausius equation showed that if the latent heat of vaporisation or of sublimation of the compound is known, a good estimate can be made of the effect of freeze-drying and the most suitable freeze-drying conditions can be selected. When good experimental data are not available, the present method for estimating latent heats, which is based on that of Laidler[9] but which utilises new group contribution figures derived from reliable experimental data,[8] gives reasonably accurate results. The use of the method in the present study on organic acids has shown that practical conditions can be pre-selected to give the optimum results without extensive experimentation, and the method should be generally applicable to organic compounds. Aromatic compounds have not been considered here because they are relatively involatile compared with the aliphatic compounds studied; however, the theoretical estimation of latent heats could easily be extended to aromatic systems, if required, by use of the extensive experimental data that are now available.[8] It would, however, probably be necessary to use freeze-concentration[11] rather than freeze-drying for very volatile compounds, such as short-chain fatty acids.

The present application showed that water and pyridine and acetic acid derived from pyridinium acetate could be removed at 0·5 torr and −10 °C with satisfactory recovery of all the compounds studied except glyoxylic acid. Higher pressures or lower temperatures would prolong the drying time excessively and higher temperatures would cause greater losses of the acids. The bottom two rows in Table VI show the effect of the use of these pre-selected conditions on the recoveries of the acids, both for an overnight run and for samples that were taken just to dryness. The effect of the time for which the dry sample remains under vacuum is apparent and the recoveries for the 7-hour freeze-drying period are mainly in the range 100 ± 10 per cent.

Glyoxylic acid has a very low latent heat of vaporisation in the free aldehyde form (estimated $\Delta H_v = 15{\cdot}7$ kcal per formula weight in grams), and is theoretically more volatile than pyruvic acid. It is said to form a hydrate analogous to chloral hydrate in both the solid state and in solution, and this has an estimated ΔH_v of 26·5 kcal per formula weight in grams, *i.e.*, it is less volatile than succinic acid. The losses of glyoxylic acid that were observed in the present study indicate that it is present in the free aldehyde form under the conditions used or that the hydrate is relatively unstable, the water being easily removed. Studies are being undertaken to improve the recoveries of glyoxylic acid, which is of particular interest in the study of some metabolic disorders.

Conclusions

The use of thermochemical data derived from both experimental and theoretical work has enabled the optimum conditions for the freeze-drying of aliphatic acids to be developed with the minimum amount of preliminary experimental investigation. The method is generally applicable to organic compounds, and has been used to establish the best conditions for the quantitative freeze-drying of some aliphatic acids that are of interest in the study of certain inborn errors of metabolism. The results obtained indicate that complete recovery of all but the most volatile of the acids studied can be achieved.

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The Microdetermination of Calcium by the Use of Amberlite IRC-50 Resin and Glyoxal Bis(2-hydroxyanil)

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Conditions for the use of Amberlite IRC-50 resin and glyoxal bis(2-hydroxyanil) for the microdetermination of calcium in solutions containing phosphate are described.

Despite the attractive properties of glyoxal bis(2-hydroxyanil) as an indicator for calcium (see King and Pruden[1] for a recent summary of the literature), as it is sensitive to less than 0·1 μg of calcium in a volume of about 4 ml, has high specificity for the calcium ion in biological material and is, perhaps, the only known colourless calcium indicator, it has not been widely used. This is, no doubt, largely because of the inhibition by phosphate and carbonate of colour production. As it may be desirable to know how much phosphate (or silicate) is present in the solutions to be percolated, and, as they appear quantitatively in the eluates, simple methods for their determination are described at the end of the paper.

Colorimetric measurement

Reagents—

De-ionised distilled water.

Hydrochloric acid—B.D.H. Aristar grade.

Sodium hydroxide solution—B.D.H. 40 per cent. w/v solution, free from carbon dioxide and containing about 1 μg g^{-1} of calcium.

Glyoxal bis(2-hydroxyanil) (*GBHA*)—Available commercially as a white crystalline powder. Pigmented products should not be accepted. A solution of 50 mg in 10 ml of methanol, protected from light, will remain usable for 1 to 2 days.

Calcium stock solution—A solution containing 1 mg ml^{-1} of calcium, with 0·001 per cent. of thiomersal, is made up in slightly acidic solution.[2]

Methanol—B.D.H., conforming to the AnalaR specification.

Equipment—

An Evans Electroselenium Limited colorimeter was used, with 8-ml tubes and an Ilford filter (green, 480 nm).

Procedure—

Place a neutral or very slightly acidic solution containing less than 5 μg of calcium into a colorimeter tube and make the volume up to 1·4 ml by the addition of water, if necessary. Add 2 ml of methanol, then 0·2 ml of GBHA solution. To the resulting mixed solution add 0·33 ml of 0·10 N sodium hydroxide solution and mix at once. A clear red colour will rapidly develop, which should be read after 3 minutes; after 10 to 15 minutes, depending on room temperature, the colour will become brownish. There is linearity of readings up to 5 μg of calcium.

In instances when the test solution is markedly acidic, titrate a separate small portion, *e.g.*, 0·25 ml, with 0·10 N sodium hydroxide solution. The additional amount of sodium hydroxide solution required by the test portion must then be added to the 0·33 ml required by neutral solutions, although keeping the total aqueous volume constant.

This method of adjusting the acidity is preferable to buffering, as used by Kerr,[3] as it permits a wider range of reaction of test solutions and avoids the possibility of introducing interfering substances in the buffers.

Readings are recorded against a blank of 1·4 ml of water. The minimum reading obtainable is from 1 to 2 spaces on the Evans Electroselenium Limited colorimeter scale, the limiting factors being contaminants in the sodium hydroxide used and the age of the GBHA. The presence of 55 per cent. of methanol does not increase the blank, but it should be noted that a corresponding proportion of ethanol produces a yellow colour.

Under the conditions described, 1 μg of calcium gives a reading between 10 and 12 on the colorimeter scale, corresponding to an optical density of 0·10 to 0·12.

Effect of various ions on the reaction—

Phosphate and carbonate—It has been observed by various workers that the presence of phosphate, and of carbonate, reduces the amount of colour produced in the reaction of calcium with GBHA. The presence of 0·4 μg of phosphorus as orthophosphate results in a 50 per cent. reduction in the colour produced by 1 μg of calcium, and the presence of 100 μg of carbon dioxide, as carbonate, depresses colour production by about 20 per cent.

Carbon dioxide is an insidious interfering factor. If reasonable precautions are taken in the preparation of 0·10 N sodium hydroxide solution from the best analytical material, it is unlikely that more than 10 μg of carbon dioxide could be present in the reaction mixture, at which level interference is negligible. From a 1 N solution, protected from carbon dioxide, 10 ml of an accurate 0·10 N solution can be made for immediate use, although with repeated withdrawals from and re-entries of pipettes into such solutions a concentration of carbonate can soon be built up that could interfere with full colour development. By checking the linearity of colour readings with various amounts of calcium, a solution of sodium hydroxide of accurately known concentration can be maintained.

Other ions—The presence of up to at least 50 mg of sodium chloride does not interfere with colour development and the presence of 50 to 100 μg of strontium or barium yields a negligible colour.

No attempt has been made to observe the effects of metals unlikely to be present in significant concentrations in biological materials, but it should be mentioned that trace amounts of copper, zinc or nickel, as noted by Bayer,[4] give intense colours with GBHA. Lanthanum, which has been suggested as a possible means of removing phosphate, yields a pink colour.

Calcium-complexing agents such as EDTA and citric acid inhibit colour production.

Use of ion-exchange resin

Removal of phosphate—

From the observations of Vedsø and Rud[5] and Winters and Kunin[6] it seemed that Amberlite IRC-50 resin might be used quantitatively in the micro range with GBHA as indicator. As there are few published reports of the quantitative use of resins the observations recorded here may be of some use in laboratories where classical chemical methods are still in use. Also, because of the many problems encountered during this investigation, it is necessary to describe in some detail the conditions required for the successful use of the resin in the determination of trace amounts of calcium in the presence of phosphate.

Equipment—

A set of six uniform hard-glass tubes (length 20 cm, outside diameter 9 mm, bore 6·5 mm, tapered to about a 2-mm tip to take a silicone tube of outside diameter 3·5 mm and bore 1 mm) is used, the flow through each being controlled by a small Perspex screw-clamp. Resin is retained in the tube by a plug of glass-wool. Into each tube is placed 500 mg of Amberlite IRC-50(H) resin, prepared as described below. The tubes are held in a row above graduated 10-ml centrifuge tubes with clips, such that the centrifuge tubes will catch the eluates from the columns.

Remarks on equipment—

Silicone tubing—This is the only material that was found to be suitable for the tips; it compresses repeatedly without cracking, releases without sticking and adds no interfering substances to eluates.

Amberlite IRC-50(H) resin—The best commercial grades have little colour, but must be washed repeatedly with hot hydrochloric acid (1 to 2 N) to remove calcium and other materials.

The resin must then be washed and fully expanded with sodium hydroxide solution, re-converted to the H-form, re-expanded with methanol and washed with acid and water. After filtering and drying in air, it must then be sieved through a plastics mesh of about 1 mm and finally through a mesh of 355 μm. The intermediate fraction, which is added to the tubes, then has a particle size between 0·355 and 1·0 mm.

Colour-inhibiting substance—When the moist resin is allowed to stand overnight, or for a longer period, it accumulates a colour-inhibiting substance. One millilitre of the eluate from 500 mg of resin that has stood for several days may be more than enough to suppress completely the colour normally given by 1 to 2 μg of calcium when using this method. Amberlite IRC-50 resin is a polymerised form of methacrylic acid, which will itself, of course, complex calcium. It would therefore seem that the resin is slightly soluble or that a slow breakdown of the polymer occurs, rather than that unpolymerised methacrylic acid has not been washed out of the resin, because repeated washings over long periods fail to remove this factor. As the production of this inhibiting substance is very slow, the difficulty is overcome by washing the resin in the tubes with 5 to 10 ml of water before use.

METHOD—

With a syringe attached to the tip of each tube, water is injected slowly upwards to remove air bubbles. The water is then drained to the level of the top of each resin column, 1 ml of 1 N hydrochloric acid is added and drained at the rate of 1 drop per 10 to 15 s, and finally 2·5 ml of water are added. Any further water passed through the columns should give no blank reading on the colorimeter with the test described above.

Control run—The resins are loaded with 2·5 ml of 1 N sodium hydroxide solution. This is more than enough to expand 500 mg of resin at the rate of flow mentioned above. The excess of base is removed with 2·5 ml of water, and the total base in each eluate is titrated with 0·1 N hydrochloric acid.

The precise amount of sodium hydroxide held by the resin after loading and washing is calculated, and an equivalent amount of 4 N hydrochloric acid, *plus* a 0·05-ml excess to ensure that the eluate is acidic, is now added to the resin. Elution proceeds into clean, graduated tubes. The acid is followed by 2 ml of water, yielding approximately 3-ml volumes of eluate. The reactions of the eluates should be definitely acidic, but as the degree of acidity will vary, 0·25 ml is titrated with 0·10 N sodium hydroxide solution. If the volume required exceeds 0·10 ml, the acidity should be reduced by the addition of a calculated amount of 1 N sodium hydroxide solution. Test portions of the eluate are now taken for colorimetric measurement as already described.

The above operation serves as a control of the uniformity and quality of the resins and indicates the magnitude of the blank that arises mostly from the sodium hydroxide used for loading. With the reagents and equipment described, 1 ml of eluate has been found to give a reading of from 3 to 5 units on the colorimeter scale when measured against a water control.

REMARKS ON METHOD—

No significant amount of the H-form of the resin should be left after loading, as this will produce hydrochloric acid in the eluate when sodium chloride is present in the percolating solutions. The presence of this acid may lower the pH to a level at which calcium is lost from the resin.

The test portions of the eluates used for colorimetry should not be alkaline as this condition initiates changes in the indicator before the final addition of base as described in the method.

The 4 N acid is used for elution to minimise the final volume of eluate.

The presence of 1 μg of calcium in the 3-ml volume of eluate is clearly recognisable and roughly measurable.

APPLICATIONS AND RESULTS

The method can be used, for example, for the determination of calcium in de-ionised distilled water, and in the distilled water used in this hospital. Results are given in Table I.

The resin columns, A to F, are loaded with 2·5 ml of approximately 1 N sodium hydroxide solution, equivalent in this instance to 0·675 ml of approximately 4 N hydrochloric acid. Volumes of final eluate are about 3 ml.

Then, the total colorimeter reading on a 3-ml volume is: 48 − 4 = 44; 44 × 3 = 132.
From standard, 1 μg of calcium ≡ 12 colorimeter units.
Therefore, 100 ml of distilled water contained 11 μg of calcium.
100 ml of de-ionised distilled water contained no calcium.

TABLE I

DETERMINATION OF CALCIUM IN DISTILLED WATER

Column	A	B	C	D	E	F
Excess of base (0·1 N)/ml	1·2	1·0	0·7	0·8	0·9	0·4
Volume percolated/ml	5*	5*	100*	100*	100†	100†
4 N Hydrochloric acid added/ml	0·650	0·655	0·660	0·660	0·660	0·670
Titration of 0·25 ml/ml of 0·1 N HCl	0·08	0·21	0·14	0·01	0·17	0·03
1 N Sodium hydroxide added/ml	0	0·14	0·07	0	0·10	0
Final titration of 0·25 ml/ml of 0·1 N NaOH	0·08	0·06	0·07	0·01	0·07	0·03
Colorimeter reading on 1 ml of eluate	4·5	3·5	4·8	3·0	50·0	46·0
Mean colorimeter readings	4·0		3·9		48·0	

* De-ionised distilled water.
† Distilled water used in this hospital.

REMOVAL OF PHOSPHATE—

The following three solutions were made up: (*i*) 0·1 M sodium chloride, (*ii*) as (*i*) but containing 1 μg ml^{-1} of calcium, and (*iii*) as (*ii*) *plus* 0·5 μg ml^{-1} of phosphorus (as phosphate). The colorimeter readings on 1 ml of each of the three solutions were 5·0, 10·0 and 1·5, respectively.

Six resin tubes were used, as indicated in Table I, and 5 ml of each solution were percolated through three pairs. The mean colorimeter readings on 0·5 ml of the 3·2 ml of final eluate were 3·0, 10·8 and 10·9, respectively. That is approximately 11 − 3 = 8; 8 × 2 × 3·2 = 51·2, which represents approximately 5 μg of calcium, or complete recovery from both solutions (*ii*) and (*iii*).

SODIUM CHLORIDE IN PERCOLATING FLUIDS AND IN ELUATES—

A resin loaded with 2·5 ml of 1 N sodium hydroxide solution will yield about 150 mg of sodium chloride to the final eluate of about 3 ml. The presence of 120 mg of recrystallised sodium chloride gave a colorimeter reading of about 2 units. The colour, however, was brownish, indicating the presence of some impurity, although the magnitude of the interference is not regarded as significant. This impurity does not affect the rate of colour development but adds slightly to its magnitude.

Calcium is extracted by the resins from solutions of sodium chloride up to at least 4 M.

From a 4 M solution of AnalaR sodium chloride were prepared 1, 2 and 3 M solutions. Volumes corresponding to equivalent amounts of salt, namely 20, 10, 6·7 and 5 ml, respectively, were percolated through the resins and the usual procedure was followed. The four values found lay in the range from 3·1 to 3·7 p.p.m. of calcium in this commercially available product.

CALCIUM IN SODIUM HYDROXIDE SOLUTIONS—

With the reagents now available it has become practicable to determine calcium in sodium hydroxide solution, which is the least certain reagent used in the method.

Sodium hydroxide is dissolved in water to a concentration of 10 to 12 N and the solution is neutralised with the Aristar acid (about 13 N, see Reagents), yielding a solution of sodium chloride of concentration 5 to 6 M, as determined by titrations and volume measurements. Such solutions are diluted to 4 M or less and carefully neutralised to sodium alizarin sulphonate.

The percolation of 20 ml of a 4 M solution is equivalent to 3·2 g of sodium hydroxide and at a level of 1 p.p.m. of calcium, with a final eluate volume of 3 ml and a test portion of 1 ml, it should give a net reading of 10 to 12 on the colorimeter scale. There is little advantage in having net readings much greater than 12 to 15 colorimeter units.

The best commercial grades of sodium hydroxide from two sources were found, by this method, to contain 3·4 and 2·0 p.p.m. of calcium. B.D.H. 40 per cent. w/v sodium hydroxide

solution, claimed to be free from carbonate and to contain about 1 p.p.m. of calcium, was found to contain about 1·5 p.p.m. of calcium. It is convenient and satisfactory for use.

Besides calcium the best available sodium hydroxide contains trace amounts of other materials, which are partly responsible for the blank values.

Determination of phosphate and silicate

In the course of the work described above, phosphate was determined as follows.

Stock tin(II) chloride solution—This solution, which will remain stable for a period of years, was made by dissolving 4 g of hydrated tin(II) chloride in 10 ml of concentrated hydrochloric acid.

Working solution of tin(II) chloride—This solution, which remained stable for a few hours, was made by adding 0·02 ml of the stock solution to 4 ml of 20 N sulphuric acid.

Ammonium molybdate solution—This solution contained 5 g of ammonium molybdate dissolved in and made up to 100 ml with 4 N sulphuric acid.

Method

Equipment—

The E.E.L. colorimeter and tubes were as used in the calcium method (p. 233), but with the use of an Ilford 205 filter (red, 640 nm).

To 4 ml of an approximately neutral test solution, or eluate from the resin retaining the calcium, add 1 drop of ammonium molybdate solution followed by 1 drop of tin(II) chloride solution. The blue colour produced immediately in the presence of phosphate is directly proportional to the amount of phosphorus up to 3 μg. Blank values at normal room temperatures are close to zero. The presence of 1 μg of phosphorus gives a reading of about 30 colorimeter units.

Silicates—

When testing the method at temperatures of about 50 °C with certain solutions, the colour produced was much greater than could be attributed to the presence of phosphate. This additional colour was traced to the presence of silicate.

A cursory study showed that in the presence of phosphate alone heating did not affect the net colour production, although it increased the blank reading. The colour produced by 1 μg of silicon, as silicate, gives a colorimeter reading of about 50, indicating that this is a very sensitive test for silicate.

It would seem that, under present conditions of the test, some heating is necessary for the formation of molybdosilicate, a pre-condition for molybdenum-blue formation on reduction.

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Accepted *October 15th*, 1971

Book Reviews

SPECTRAL DATA OF NATURAL PRODUCTS. Volume 1. By K. YAMAGUCHI. Pp. xii + 765. Amsterdam, London and New York: Elsevier Publishing Co. 1970. Price £21.

When, from 1946 onwards, commercial photoelectric spectrophotometers became generally available, ultraviolet spectra of organic compounds were more and more widely measured by organic chemists and biochemists. As data accumulated, the emphasis was on spectroscopy as a tool rather than a topic for specialist study. Improved instrumentation had a similar effect on infrared spectroscopy a little later. Chromatography in its various forms made easier the separation and isolation of previously unrecognised naturally occurring compounds.

During the past twelve years the revolution in methods has been carried further and classical approaches to structure, once obligatory, are now being by-passed. Nuclear magnetic resonance has proved a very powerful weapon and high-resolution mass spectrometry has given a new precision to the determination of molecular weights and partial structures. X-ray analysis of crystals, optical rotatory dispersion, circular dichroism and other physical measurements have become increasingly valuable in solving many special problems.

Dr. Yamaguchi has made a large collection in which natural products have been classified according to structural type and the spectroscopic information used in determining structures is concisely presented. The work is to consist of two volumes and this is the first. It deals with compounds the structures of which were elucidated before 1963. The second volume is to cover compounds studied in 1964 and 1965 with an Appendix on compounds the structures of which were established in 1966–1968. The author says that "if this kind of collection is needed in the future . . . it can and should be done only by the co-operation of several specialists, using an electronic computer."

This is a large book with 726 pages of closely packed text and two good indexes. There are 2297 references. The classification is logical and information is not repeated unnecessarily; thus ultraviolet spectra are often noted by referring to Sadtler Card numbers. There are over 400 diagrams showing various types of spectra and there is a vast array of structural formulae and tabulated data. As might be expected from the period covered, the book is particularly rich in respect of steroids, terpenoids, quinones, carotenoids and alkaloids (these occupy pages 473 to 725).

This is a costly reference book, which is likely to be a good library purchase wherever the structures of natural products are under investigation by current methods. Users may find minor mistakes but the work is beautifully produced and a prodigious amount of labour has been put into it.

R. A. MORTON

ANALYTICAL METHODS FOR ORGANIC CYANO GROUPS. By M. R. F. ASHWORTH. Pp. x + 142. Oxford, New York, Toronto, Sydney and Braunschweig: Pergamon Press. 1971. Price £3·75; $10.

This is the sixth monograph in the series entitled "Organic Functional Group Analysis," and deals with the qualitative and quantitative analysis of organic cyano groups. The author has classified the data into those obtained from chemical methods and those obtained from physical methods. While this seems at first to be a somewhat simple classification, it does mean that the data for any particular individual compound that is currently under investigation are distributed throughout the various chapters.

The nature of the cyano group and its electronic effect on other functional groups within the molecule serve as the basis for the classification of the various chapters and this tends to give at least one chapter of the book a dual purpose, not only for analytical chemistry, but also for studying the chemistry of activation by the cyano group. One particular chapter, entitled "Reactions of groups activated by the cyano group," certainly is very useful for teaching purposes. The mechanisms of the activation processes are not given, but the practical examples will be useful to all organic chemistry teachers.

The five chapters dealing with physical methods are fairly short but are quite useful as indications of the methods that are available; this applies especially to the chapter on chromatographic methods. Overall, the book should prove to be a useful addition to most laboratories dealing with the analysis of organic materials.

L. S. BARK

TOPICS IN LIPID CHEMISTRY. Volume 2. Edited by F. D. GUNSTONE. Pp. x + 313. London: Logos Press Ltd. 1971. Price £8.

This volume is a worthy successor to Volume 1 in the series. There are five main chapters, written by recognised authorities in lipid chemistry, appendices containing a list of books and review articles on lipids that have appeared recently, and a comprehensive catalogue of references to nuclear magnetic resonance spectra of fatty acids and their derivatives.

The chapter by Polgar on "Natural alkyl-branched long-chain acids" will probably have most general interest and is a concise but readable account of these little known but very interesting lipids. Chapters by Pryde and Cowan ("Ozonolysis"), Naudet and Ucciani ("Allylic halogenation and oxidation of unsaturated esters") and Maerker ("Nitrogen and sulphur analogues of epoxy- and hydroxy-acids") cover comprehensively subjects in which there is a growing interest, particularly in industrial laboratories. The last two of these subjects have not been reviewed before. C. W. Bird's chapter on "Olefin reactions catalysed by transition-metal compounds" represents a novel experiment in that it covers a branch of chemistry completely neglected by lipid chemists. There are obvious applications of the reactions described to lipids, however, and this chapter will be stimulating reading for those interested in the chemical reactions of fatty acids and in industrial uses of fatty acids and their derivatives. Finally, the appendices contain a wealth of valuable material on nuclear magnetic resonance spectroscopy, compiled in a readily accessible form.

The volume is well produced and has seen rapid publication as references cover the literature up to 1970. It will be of less interest than Volume 1 to lipid biochemists, but chemists, particularly those in industrial laboratories, will find it very useful. W. W. CHRISTIE

ORGANIC FLUORINE CHEMISTRY. By MILOS HUDLICKÝ. Pp. xii + 198. New York and London: Plenum Press. 1971. Price $16.50.

This book is based on a graduate lecture course given at Virginia Polytechnic Institute in 1969. After a short factual introductory chapter, fluorinating agents and nomenclature are briefly discussed. Methods of introducing fluorine into organic compounds are then described. Subsequent chapters outline analytical methods, physical and biological properties, and practical applications. The final third of the text is devoted to reactions of organic fluorine compounds. The statement on the jacket that "A major chapter is devoted to a systematic review of analytical methods applicable to organic fluorine compounds" makes one wonder, rather wistfully, about the validity of the Trades Descriptions Act as applied to blurb-writers. The competent analyst will be surprised by the superficial treatment given; the less knowledgeable may be misled.

As an introductory survey for undergraduate students, the text is probably adequate, although misleading in places. However, its price is excessive for a mere 153 pages of text, 618 references and 27 pages of index. The book fails to convey any notion of the sheer "cussedness" of fluorine compounds, which pervades so many of the review articles from which it has been culled. It is unlikely to inspire any further study of fluorine chemistry and intending specialists will require something far more authoritative. A. M. G. MACDONALD

pH METERS. By A. WILSON. Pp. 119. London: Kogan Page; New York: Barnes and Noble. 1970. Price £2.

It is difficult to assess to whom this book is directed. The first 84 pages offer an excellent description of underlying theory and practice of pH measurement that is probably most applicable to the student of analytical chemistry and possibly the practising analyst. The last 35 pages consist of a comparison of commercial pH meters. In all, specifications for ninety-four instruments from twenty-three manufacturers are compared, and I can only surmise that this section would be of little use to anyone not contemplating the purchase of such an instrument. In fact, this portion of the book appears to be rather a pointless exercise, in that throughout only six parameters are compared. However, if the author and publisher considered this a worthwhile feature of the book, then the exercise would have been better presented as an expandable pull-out supplement, allowing for a reduction in the over-all price.

The academic portion of the book deals with a discussion of theoretical principles of pH, use of buffers, measurement of pH in chemical laboratory and plant situations, and in biological and medical control. A discussion of indicator and reference electrodes is also included, together

with a brief description of ion-selective electrodes, misleadingly referred to as "ion-specific." The text is well written, is illustrated with diagrams and electrical circuits that are refreshingly simple and easy to understand, and the publisher should be congratulated on the quality of the paper and the style of printing adopted.

I consider the major portion of this book to be of value, in that the author has attempted to join together both physical principles and practical details of pH measurement, all too frequently found under the separate headings of "physical" and "analytical" chemistry, and for this reason the book should find a place on many college and industrial library shelves. Finally, if the publishers could be persuaded to issue a paper-backed version at a considerably reduced price, eliminating the latter section, then the text could form a suitable monograph on pH for purchase by students of analytical chemistry.

B. W. WOODGET

IONIZING SOLVENTS. By J. JANDER and CH. LAFRENZ. Edited by WILHELM FOERST and HELMUT GRÜNEWALD. Pp. xii + 202. London: John Wiley & Sons Ltd.; Weinheim, Germany: Verlag Chemie. 1971. Price £3.

Ionising solvents, other than water and glacial acetic acid, have never found favour with analytical chemists, mainly because of the great experimental complications involved in their use and their obnoxious properties. Nor indeed do there appear to be any protagonists to indicate any great advantages that might outweigh those detractions in particular circumstances. Perhaps this is partly the fault of the literature on non-aqueous solvents. In general, books and review articles tend to concentrate on the physico-chemical aspects of the subject, utilising suitable examples just for illustration of the operation of the principles involved. The present text is rather different. It devotes one chapter each to a range of ionising solvents (ammonia, hydrogen halides, sulphuric and fluorosulphuric acids, hydrogen sulphide, hydrogen cyanide, sulphur dioxide, halogens and a few other liquids) from the point of view of the reactions that occur therein. The numerous examples that are given include those which can be classed as dissolution, acid - base, solvolysis, precipitation, redox and complex-forming reactions, involving inorganic and organic compounds. Not that basic concepts are left out—a brief discussion of acid - base theories is given, and physico-chemical properties of each solvent are described.

This is a useful, easily understood student text, although after a brief perusal the book had become detached from its cover! It provides a lot of information in one of the neglected areas of chemistry, and has an extensive index (30 pages). I fear its value to the analytical chemist, however, is merely to confirm his suspicions that such solvent systems are not for him.

A. TOWNSHEND

LECTURES AND COURSES

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Head of Department R. Stock B.Sc. Ph.D. F.R.I.C.

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Wednesday June 21st 1972

or

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The Continuous Determination of Sodium in High Purity Water by Using a Sodium Monitor Incorporating a Sodium-responsive Glass Electrode

An Electronic Instruments Limited sodium monitor, Model 89C, incorporating a sodium-responsive glass electrode, has been shown to be basically suitable for use in power stations to determine continuously the sodium contents of steam and boiler feed and make-up waters, except when filming amines (*e.g.*, octadecylamine) are present.

Details are given of the tests required to show the essentially Nernstian response of the electrode to changes in sodium concentrations; a regular standardisation procedure is also described.

A. A. DIGGENS, K. PARKER

Electronic Instruments Limited, Chertsey, Surrey

and H. M. WEBBER

Central Electricity Research Laboratories, Kelvin Avenue, Leatherhead, Surrey.

Analyst, 1972, **97**, 198–203.

Electrochemical Studies of Copper Lactate and Glycollate Complexes at the Mercury Cathode

Polarographic studies of the copper complexes formed with lactic and glycollic acids have been undertaken using water and mixtures of water and organic solvents. The stability constants have been determined. The effects of pH, concentration of ligand, non-aqueous solvents and surface-active substances have also been investigated. The values of ΔH, ΔF and ΔS have been calculated.

O. N. SHRIVASTAVA and C. M. GUPTA

Department of Chemistry, University of Rajasthan, Jaipur, India.

Analyst, 1972, **97**, 204–208.

A Semi-automated Method for the Determination of the Available Carbohydrate Content of Poultry Feeds

A rapid method is described for the determination of the sugar content of takadiastase digests of poultry feeds. The reproducibility of the method has been assessed and the results of recovery tests on pure carbohydrates are presented. The method gives results in reasonable agreement with those obtained by a previously published titrimetric method involving Fehling's reaction.

D. W. F. SHANNON

Agricultural Research Council's Poultry Research Centre, King's Buildings, West Mains Road, Edinburgh 9, Scotland.

Analyst, 1972, **97**, 209–212.

An Enzymic Method for the Determination of Skimmed Milk Powder in Soup and Sauce Mixes

An enzymic method for the determination of skimmed milk powder in soup and sauce mixes is described. The method is based on the determination of free lactose by its hydrolysis with β-galactosidase to galactose and glucose, the latter being determined by the hexokinase method. The determination is free of interference from reducing sugars and other substances present in soup and sauce mixes. The method is more rapid, accurate and reliable than other methods in current use.

R. K. BAHL

J. Sainsbury Ltd., Stamford House, Stamford Street, London, S.E.1.

Analyst, 1972, **97**, 213–215.

A Simple Thin-layer Chromatographic Technique for the Semi-quantitative Determination of Volatile Nitrosamines in Alcoholic Beverages

A thin-layer chromatographic procedure is described for determining dimethyl-, diethyl- and dipropylnitrosamines in alcoholic drinks. Nitrosamines are first distilled from an alkaline solution of the sample and then extracted into dichloromethane. Ethanol and interfering materials are removed by co-distillation with hexane or benzene and chromatography on a basic alumina column. Finally, the nitrosamines are determined semi-quantitatively by thin-layer chromatography as nitrosamines or, after oxidation, as the corresponding nitramines. The method is sensitive down to 0·025 p.p.m. and applicable to various drinks such as wine, beer, whisky and rum. Percentage recoveries of added nitrosamines varied between 50 and 100 at the 0·1 p.p.m. level and between 20 and 100 at the 0·025 p.p.m. level.

N. P. SEN and CLAUDETTE DALPE
Food and Drug Research Laboratories, Tunney's Pasture, Ottawa 3, Canada.

Analyst, 1972, **97**, 216–220.

Quantitative Esterification of Lipids on Thin-layer Adsorbents

Lipid classes separated by thin-layer chromatography can be determined by gas chromatography of the methyl esters of the component fatty acids with added internal standard. A simplified procedure is described for the complete analysis of all the lipid classes in a given sample in which transesterification is performed on the thin-layer adsorbent itself. The methyl esters are recovered from the reaction mixture with a less polar solvent than would have been required to elute the original component. As a consequence of the elimination of a step that requires preliminary elution of components from the thin-layer plates, there is less contamination of minor components by trace amounts of impurities in large volumes of solvent, and the results obtained are more reproducible.

WILLIAM W. CHRISTIE
The Hannah Research Institute, Ayr, Scotland.

Analyst, 1972, **97**, 221–223.

Studies on the Quantitative Freeze-drying of Aqueous Solutions of some Metabolically Important Aliphatic Acids Prior to Gas - Liquid Chromatographic Analysis

These studies have shown that the over-all losses observed are due to volatilisation during the freeze-drying process. These losses are related to the variation of the vapour or sublimation pressures of the acids with temperature, and are also related to their latent heats of vaporisation or sublimation.

Reliable data on the latent heats of vaporisation and sublimation of the acids of interest are seldom available and a method for the estimation of the latent heats, based on group contributions, has been developed. The reliability of the method, which is generally applicable to organic compounds, is discussed.

Thermochemical data derived from the use of this method have been used in conjunction with reliable published experimental data to determine the optimum freeze-drying conditions for the complete quantitative recovery of all but the most volatile of the acids studied, with a minimum of preliminary experimental work.

RONALD A. CHALMERS and R. W. E. WATTS
Division of Metabolism, Medical Research Council, Clinical Research Centre, Watford Road, Harrow, Middlesex, HA1 3UJ.

Analyst, 1972, **97**, 224-232.

The Microdetermination of Calcium by the Use of Amberlite IRC-50 Resin and Glyoxal Bis(2-hydroxyanil)

Conditions for the use of Amberlite IRC-50 resin and glyoxal bis(2-hydroxyanil) for the microdetermination of calcium in solutions containing phosphate are described.

G. HUNTER
Medical Research Council, Churchill Hospital, Oxford.

Analyst, 1972, **97**, 233–237.

Preliminary Announcement

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REPORTS OF THE ANALYTICAL METHODS COMMITTEE

The Reports of the Analytical Methods Committee listed below may be obtained direct from The Society for Analytical Chemistry, Book Department, 9/10 Savile Row, London, W1X 1AF (not through Trade Agents), at the price of 10p. to members of the Society and 15p. to non-members. Remittances must accompany orders and be made payable to "Society for Analytical Chemistry."

Certain Reports published before 1946 have been omitted from this list, but are still available.

Additives in Animal Feeding Stuffs Sub-Committee:

Report of the Antibiotics Panel: The Determination of Penicillin, Chlortetracycline and Oxytetracycline in Diet Supplements and Compound Feeding Stuffs.
Report of the Hormones Panel: The Determination of Stilboestrol and Hexoestrol in Compound Feeding Stuffs.
Report of the Prophylactics Panel: The Determination of Nitrofurazone in Compound Feeding Stuffs.
Report of the Vitamins (Water-soluble) Panel: The Determination of Water-soluble Vitamins in Compound Feeding Stuffs.
Report of the Vitamins (Fat-soluble) Panel: The Determination of Fat-soluble Vitamins in Diet Supplements and Compound Feeding Stuffs.
Report of the Prophylactics in Animal Feeds Sub-Committee: The Determination of Amprolium in Animal Feeding Stuffs.
Report of the Prophylactics in Animal Feeds Sub-Committee: The Determination of Sulphaquinoxaline.
Report of the Prophylactics in Animal Feeds Sub-Committee: The Determination of Acinitrazole.
Report of the Prophylactics in Animal Feeds Sub-Committee: The Determination of Ethopabate in Feeds.
Report of the Prophylactics in Animal Feeds Sub-Committee: The Determination of Furazolidone in Feeds.
Report of the Prophylactics in Animal Feeds Sub-Committee: The Determination of Dimetridazole in Animal Feeds: Revised Method.
Report of the Prophylactics in Animal Feeds Sub-Committee: The Determination of Dinitolmide (Zoalene) in Animal Feeds.
Report of the Prophylactics in Animal Feeds Sub-Committee: The Determination of Amprolium, Sulphaquinoxaline and Ethopabate when Present Together in Animal Feeds.
Report of the Prophylactics in Animal Feeds Sub-Committee: The Determination of Carbarsone in Animal Feeds.

Analytical Standards Sub-Committee:

Sodium Carbonate as a Primary Standard in Acid - Base Titrimetry.
Sulphamic Acid as a Primary Standard in Acid - Base Titrimetry.

Essential Oils Sub-Committee:

Report No. 15. Determination of Linalol in Essential Oils.
Application of Gas - Liquid Chromatography to Essential-oil Analysis: Interim Report on the Determination of Citronellol in Admixture with Geraniol.
Spectral Characteristics of Eugenol.
Application of Gas - Liquid Chromatography to the Analysis of Essential Oils. Part 1: Determination of Cedrol.

Fish Products Sub-Committee:

Nitrogen Factor for Cod Flesh.
Nitrogen Factor for Coal Fish.

Meat Products Sub-Committee (formerly Meat Extract Sub-Committee):

Analysis of Meat Extract.
Determination of Gelatin in Meat Extract and Meat Stocks: Interim Report.
Nitrogen Factors for Pork *and* Nitrogen Content of Rusk Filler (as one reprint).
Nitrogen Factors for Beef.
Nitrogen Factors for Chicken.
Nitrogen Factors for Liver.
Nitrogen Factor for Veal.
Nitrogen Factors for Turkey.
Nitrogen Content of Rusk Filler.
Nitrogen Factor for Kidney.
Nitrogen Factor for Tongue.
Nitrogen Factor for Barley.
Nitrogen Factor for Blood.

Metallic Impurities in Organic Matter Sub-Committee:

Methods for the Destruction of Organic Matter.
Notes on Perchloric Acid and its Handling in Analytical Work.
The Determination of Lead.
The Determination of Small Amounts of Arsenic in Organic Matter.
The Determination of Small Amounts of Copper in Organic Matter.
The Determination of Small Amounts of Mercury in Organic Matter.
The Determination of Small Amounts of Tin in Organic Matter. Part 1.Amo unts of Tin up to 30 μg.
The Determination of Small Amounts of Zinc in Organic Matter.
The Use of 50 per cent. Hydrogen Peroxide for the Destruction of Organic Matter.
The Determination of Small Amounts of Tin in Organic Matter. Part 2. Amounts of Tin from 30 to 150 μg.
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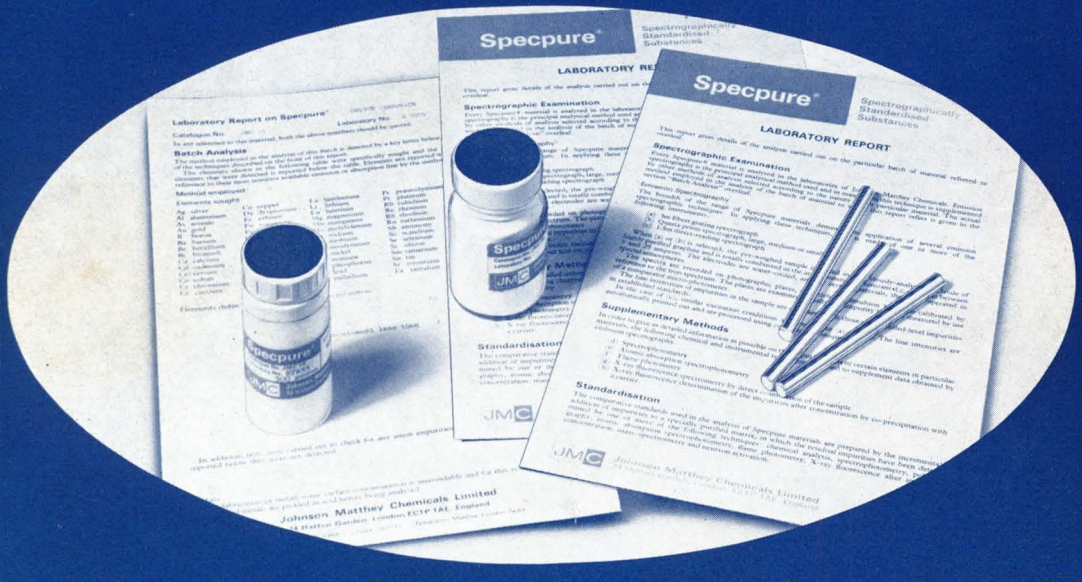

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THE ANALYST

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