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Volume 90, No. 1073 August 1965

Summaries of Papers in This Issue

The Determination of Perchlorate in the Presence of Other Halogen Acids by Means of a Coloured Liquid Anion Exchanger

Perchlorate in aqueous solution can be determined absorptiometrically in concentrations down to approximately 5×10^{-5} M in terms of the optical density at 353 mμ of the erdmannate ion, $[Co(NH_3)_2(NO_2)_4]^-$, displaced on equilibration with a solution of tetra-n-hexylammonium erdmannate in a xylene - hexone mixture, which acts as a coloured liquid anion exchanger. Two analytical procedures are described. In the first procedure the optical densities of both equilibrated phases are measured, and the construction of a linear calibration curve demands a knowledge of the relevant molecular extinction coefficients, which are shown to be slightly different in the two solvent media. The second is simpler, in that only the optical density of the aqueous phase is measured to give a linear calibration graph.

Interferences by chlorate, chlorite and hypochlorite ions (as well as nitrite and nitrate ions) can be eliminated by fuming the sample with concentrated hydrochloric acid, since chloride ions (and many others) do not interfere. The procedure lends itself to the determination of perchlorate in admixture with chloride and other species obtained by its electro-oxidation.

H. M. N. H. IRVING and A. D. DAMODARAN
Department of Inorganic and Structural Chemistry, The University, Leeds, 2.

Analyst, **1965**, **90**, 443–452.

A General Method for the Determination of Organophosphorus Pesticide Residues in Foodstuffs

A scheme of analysis is described for the extraction from plant material, separation by paper chromatography and determination of the organophosphorus pesticides used commercially in the United Kingdom. The pesticides, together with important metabolites, are extracted from the crop with acetone, and, after the addition of water, are partitioned into chloroform. The solvent is removed, the extract taken up in acetone and the fats and waxes frozen out at $-80°$ C. Further clean-up is achieved by column chromatography, and the subsequent paper chromatography of the polar pesticides and metabolites is carried out in a different solvent system from that used for the less polar parent organophosphorus compounds. The pesticide and metabolites are characterised by R_F values, and individual compounds are determined from the phosphorus content of the spot on the chromatogram. Results are given for several combinations of crops and pesticides.

J. A. R. BATES
Plant Pathology Laboratory, Hatching Green, Harpenden, Herts.

Analyst, **1965**, **90**, 453–466.

A Simple Concentration-cell Technique for Determining Small Amounts of Halide Ions and Its Use in the Determination of Residues of Organochlorine Pesticides

Quick, simple and sensitive methods for determining small amounts of chloride and bromide ions are described. They are based on the direct measurement of the e.m.f. of a concentration cell with electrodes consisting of thin silver rods that are partially submerged in a suspension, prepared *in situ*, of the corresponding silver halide in dilute acid. These electrodes have the advantage of easy preparation, photostability and comparative immunity from poisoning.

The use of a similar cell for determining iodide ion is discussed.

The application of the method for chloride, after oxygen-flask combustion, to the determination of residues of organochlorine insecticides in extracts of animal and plant tissue is described in an Appendix.

J. ROBURN
Ministry of Technology, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.1.

Analyst, **1965**, **90**, 467–475.

Active Charcoal as an Adsorbent in Clarification of Raw Sugar Solutions

A new approach to the problem of clarifying raw sugar solutions is described.

A procedure is proposed for clarifying raw sugar solutions on the grounds that a decolorisation of the sugar solution may be effected without altering the relative concentration of the component sugars. A departure from the use of traditional defecating agents with their precipitate volume and other rotation effects has been made, and a method that can be used with either visual or photo-electric polarimeters has been evolved.

R. SAWYER

Ministry of Technology, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.1.

Analyst, 1965, **90**, 476–481.

A Concentration-cell Method for the Determination of Trace Amounts of Chloride in Solutions of Lithium Salts

A concentration-cell technique has been applied to the determination of up to 4 μg of chloride per ml in solutions of lithium salts containing 0·025 g of lithium per ml (3·6 M). The method is without bias and has a standard deviation of 0·06 μg per ml at the 1 μg per ml level. This precision remains sensibly constant over the quoted range. A modification of the method, which involves the removal of the chloride from the sample as hydrogen chloride and subsequent absorption in the concentration cell, gives satisfactory results below the 0·25 μg per ml level with a standard deviation of 0·03 μg per ml.

P. EMMOTT

M.G.O. Inspectorates, Chemical Inspectorate, Headquarters Building, Royal Arsenal, Woolwich, London, S.E.18.

Analyst, 1965, **90**, 482–487.

The Radiometric - Spectrophotometric Determination of Microgram Amounts of Niobium in Rocks and Minerals

An improved method for the determination of 0·5 to 150 p.p.m. of niobium in silicate rocks and minerals is described. Niobium-95 is used to determine the yield of the chemical separation. The thiocyanate complex is extracted into ethyl acetate and the concentration is measured spectrophotometrically. The standard deviation of replicate analysis is less than 2 per cent. at the 20 p.p.m. level, but increases at lower concentrations and reaches 20 per cent. at 1 p.p.m.

J. ESSON

Department of Geology, University of Manchester, Manchester, 13.

Analyst, 1965, **90**, 488–491.

The Detection and Estimation of Aflatoxin in Groundnuts and Groundnut Materials

Part IV. Routine Assessment of Toxicity due to Aflatoxin B_1

A sensitive, high-resolution thin-layer chromatographic method is described for determining aflatoxin B_1 in groundnut kernels, groundnut meals and peanut butter by fluorescence. Kieselgel G is used as absorbent and is shown to offer advantages over alumina. Aflatoxin levels are determined by a dilution technique and expressed in terms of categories.

TREVOR J. COOMES, P. C. CROWTHER, B. J. FRANCIS and Miss LINDA STEVENS

Ministry of Overseas Development, Tropical Products Institute, 56–62 Gray's Inn Road, London, W.C.1.

Analyst, 1965, **90**, 492–496.

The Determination of Selenium in Soils and Sediments with 3,3′-Diaminobenzidine

Short Paper

R. E. STANTON and ALISON J. McDONALD
Department of Geology, Imperial College of Science and Technology, London, S.W.7.

Analyst, 1965, **90**, 497–499.

Determination of Chloride in Beer by Radioactivation Analysis

Short Paper

A. G. SOULIOTIS, A. P. GRIMANIS and N. A. TSANOS
Chemistry Department, Nuclear Research Center, "Democritus," Athens, Greece.

Analyst, 1965, **90**, 499–501.

The Absorptiometric Determination of Antimony: Extinction Coefficient of the Tetraiodoantimonate(III) Ion

Short Paper

R. A. WASHINGTON
Atomic Energy of Canada Ltd., P.O. Box 93, Ottawa, Canada.

Analyst, 1965, **90**, 502–503.

The Detection of Thiophosphate Insecticides on Paper Chromatograms with Congo Red

Short Paper

A. IRUDAYASAMY and A. R. NATARAJAN
State Forensic Science Laboratory, Madras, 3, India.

Analyst, 1965, **90**, 503–504.

A Method of End-point Location in Constant-current Coulometry

Short Paper

W. A. ALEXANDER, D. J. BARCLAY and A. McMILLAN
Department of Chemistry, University of Strathclyde, Glasgow.

Analyst, 1965, **90**, 504–506.

The Determination of Sodium Nitroprusside: A Special Application, The Determination of Small Amounts of Sodium Nitroprusside Impregnated on Cellulose Strips

Short Paper

BRIAN A. SEWELL
The Pharmacy Development Laboratory, Miles Laboratory Limited, Bridgend, Glamorgan.

Analyst, 1965, **90**, 507–509.

Determination of Potassium Chromate and Hydrogen Peroxide in the Presence of Each Other

Short Paper

FARHATAZIZ and GHAZANFAR A. MIRZA
Atomic Energy Centre, Ferozepur Road, Lahore, Pakistan.

Analyst, 1965, **90**, 509–510.

AEI SCIENTIFIC APPARATUS
BULLETIN NO. 4

IMPROVED ACCURACY IN THE ANALYSIS OF SOLIDS BY SPARK SOURCE MASS SPECTROMETRY

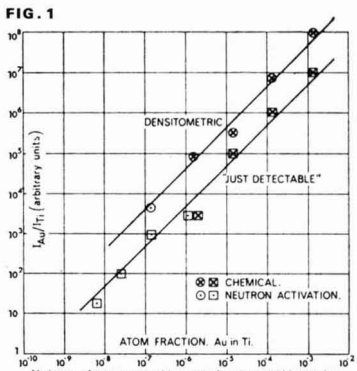
Since the possibility of using spark source ionization for the analysis of solids was first recognised ten years ago, the design of the double focusing mass spectrometer using Mattauch geometry has been considerably improved. And today one instrument—namely the AEI MS7—is capable of detecting impurities at levels as low as 1 part in 10^9. As a result the MS7—which, incidentally, was the first commercially available double focusing instrument to be built expressly for the analysis of solids—has found wide applications particularly where overall coverage of all elements and comparison analysis without standards are valuable.

Now the MS7 technique has been further improved by the careful control of certain parameters, and very good reproducability and accuracy can be guaranteed. This bulletin reviews the parameters affecting analytical accuracy and outlines the methods of control developed by AEI engineers.

It has been shown that the sensitivity of the majority of elements differs from some standard such as iron by no more than a factor of 3. In other words, most relative sensitivity factors lie between 0.3 and 3. In the case of the MS7, the determination of relative sensitivity factors is considerably simplified by the fact that the response of the instrument is linear over a very large range of concentration. Indeed Hannay & Ahearn[1] established linearity over the range 10^4 to 1 using doped silicon samples. More recently, W. A. Wolstenholme (AEI Consultant Lab.) has reported on the investigation of gold doped titanium samples covering a concentration range from 0.5% to .02 ppm by weight; a range of more than 10^5 to 1.

These samples were chosen because of their suitability for neutron activation and wet chemical analysis. Figure 1 shows the relative ion intensity of gold plotted against the concentration determined by chemical or neutron activation techniques. The "just detectable" line is an individual assessment for ion intensity and the "densitometric" line indicates that a microdensitometer was used to scan the spectral lines; the two graphs have been displaced for the sake of clarity. Matrix effects are generally very small, as is illustrated by the relative sensitivities for copper and steel standards reported in tables 1 and 2.

FIG. 1



Variation of ion current with atomic fraction; gold in titanium.

In copper and steel relative sensitivities are very similar for chromium (1·8 and 1·4) and for tin (1·3 and 1·1). Only for some low BP elements is there a marked dependence on the matrix, e.g. (1·4 and 2·6) for lead.

NOTE:

$$\text{Relative Sensitivity} = \frac{\text{uncorrected MS7 value}}{\text{known value}}$$

Homogeneity of standards and samples

Care has to be taken to use homogeneous standards or alternatively to increase the rate of consumption of sample above the usual 5 to 10 milligrams.

As it happens, however, the possibility of inadvertently using an inhomogeneous sample has been materially reduced by the introduction of more reliable methods of sample preparation.

TABLE 1

RELATIVE SENSITIVITIES AND REPRODUCIBILITY OF R.F. SPARK SOURCE ANALYSIS OF COPPER: JOHNSON MATTHEY CA2

30kV r.f.; 19.5kV accel. volts; pulse length and repetition rate varied in the analytical plates.

Impurity	Concentration given	p.p.m wt MS7	Relative sensitivity	Standard deviation % 8 separate analyses	Standard deviation % 15 repeat exposures
Bi	130	110	0.85	31	13
Pb	100	140	1.4	30	11
Sb	120	130	1.1	32	18
Sn	120	160	1.3	25	9.2
Ag	150	210	1.4	23	21
Ga	80	250	3.1	27	13
Cr	60	110	1.8	20	21

FOOTNOTE:

1 Hannay and Ahearn (1954) Anal. Cham 26 1956.

Reproducibility

The most important improvements in reproducibility have been achieved by careful control of certain instrumental parameters. As a result of recent investigations[2] it is now clear that the most important factor is one that is comparatively simple to control; namely, the ion accelerating voltage. When this is always set to the same value, and other source conditions are kept as constant as possible, reproducible analytical results are obtained. The principal reason is the improved constancy of the relative sensitivity factors for different elements. Table 2 shows how close agreement with known values is obtained when such relative sensitivity factors are measured and used to correct observed concentrations.

Variations in Photoplate

The standard deviations on identical exposures on a photoplate using a homogeneous aluminium standard indicate that the best standard deviation obtained for the different elements is about 10%. The standard deviation on isotope ratios, i.e. where the relative sensitivity factor is not involved and where a limited area of plate is used, show better figures of 3 to 4%. This indicates the likely variation due to the plate itself, and represents a limit that would remain even if a calibration spectrum of a standard was placed on the same photoplate as the sample to be analysed.

Reproducibility of Analyses

When different photoplates are used in separate analyses the standard deviation increases.

In repeat analyses different photoplates will be used and also source conditions may vary slightly. To cover the full range of exposures (10^7 to 1) it is sometimes necessary to vary the pulse length and repetition rate of the spark which might also contribute to the variations. An internal standard, although not essential, eliminates the need for such a wide range of exposures.

When the spark pulse-length and repetition rates for the analysis of a copper standard shown in Table 1 were held constant throughout the series of successive analyses of one of the standards, the minimum standard deviation did in fact decrease towards the minimum value previously obtained for repeat exposures (about 10%).

Accuracy of Analysis

The accuracy which can be attained with the MS7 when all parameters are properly controlled is perfectly exemplified by the data set forth in Table 2. In this case, of course, a standard of reasonable homogeneity has been used to establish relative sensitivity factors for the impurities in the given matrix.

It will be noted that only one standard is used, and that the levels of concentration in the standard do not correspond too closely to those in the unknown. A steel matrix has been taken as an example because studies of source conditions indicated that it seemed the most likely (compared with the aluminium and copper standards) to give poor results should the source conditions change. It is clear, however, that the results once corrected for relative sensitivity factors are in excellent agreement with those given by chemical analysis.

FOOTNOTE:

[2] *Halliday, Swift and Wolstenholme; Quantitative Analysis by Spark Source Mass Spectrometry, International Mass Spectrometry Conference, Paris 1964.*

TABLE 2

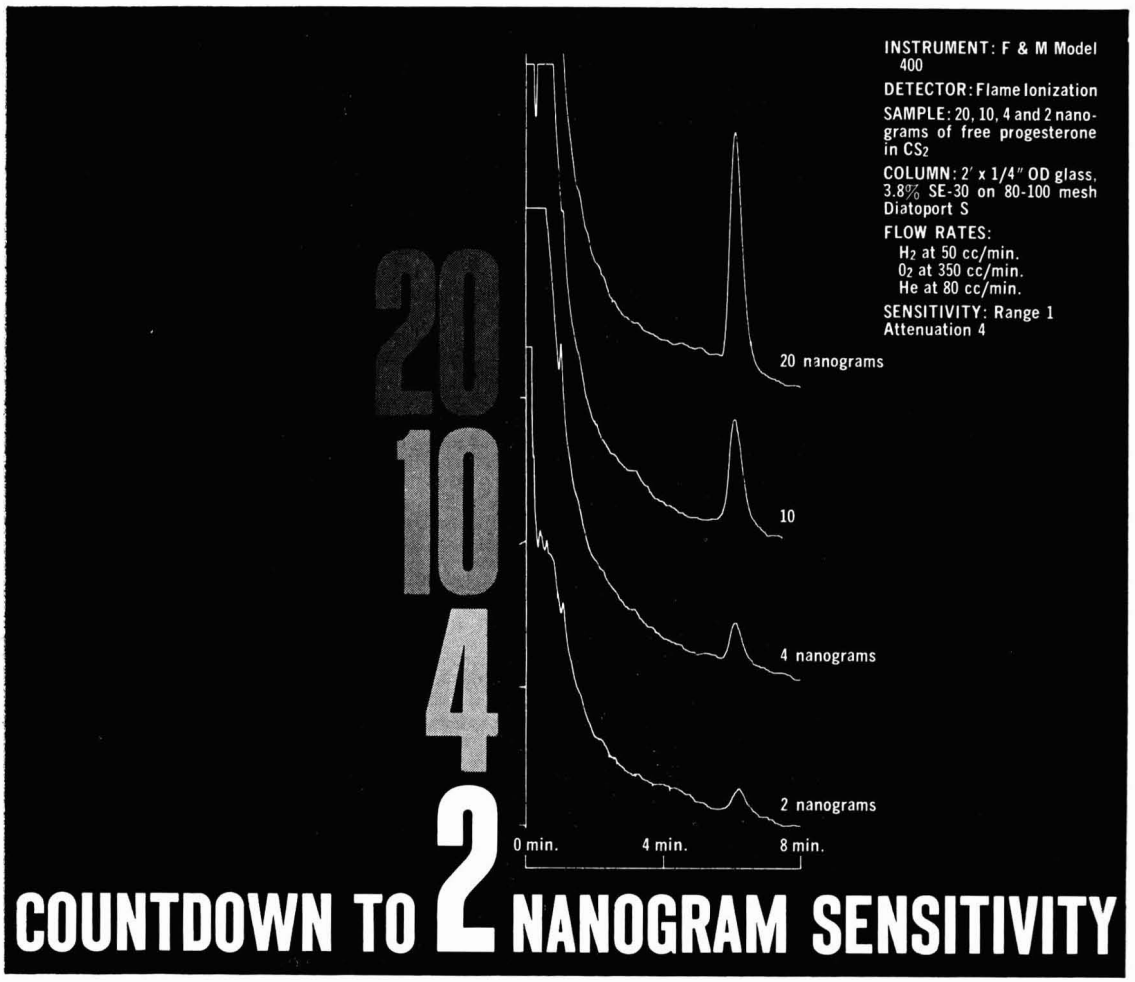
ACCURACY OF ANALYSIS AFTER CALIBRATION: BUREAU OF ANALYSED STANDARDS LTD. MILD STEEL RESIDUAL SERIES SPECTROGRAPHIC STANDARD SS14

Pulse length 100 microsecs; 300 pulses/sec.; 30kV r.f.; 19.5kV accel. volts.

Concentration % wt

Impurity	MS7, average of eight analyses	MS7, corrected using SS12	Given Spectrographic value
Cr	0.26	0.18	0.185
Co	0.19	0.18	0.19
Ni	0.10	0.13	0.13
Cu	0.047	0.029	0.04
Zr	0.017	0.007	(0.005)
Nb	0.072	0.025	(0.05)
Mo	0.053	0.060	0.07
Sn	0.019	0.017	0.02
Pb	0.018	0.007	(0.0075)

( ) not certified—approximate



IN FREE STEROID ANALYSIS

Free steroids are one of the most difficult tests of a gas chromatograph's sensitivity. Normally these compounds are analyzed in the microgram range; even in this day of sophisticated instruments, most chromatographs do well to detect 20 nanograms. ▪ However, as shown in this series of scans of free progesterone, the F & M Model 400 Biomedical Gas Chromatograph easily improves on the 20-nanogram detection level, revealing as little as 2 nanograms . . . *with* practical retention times, *with* substantial freedom from peak tailing and *without* serious background noise. ▪ Why does the Series 400 do so much better than any of its competitors? ▪ *Because* it combines an extremely sensitive flame ionization detector with an instrument environment that is devised specifically for biomedical use. ▪ *Because* its chromatographic system is practically free of dead spaces, thus eliminating the component "loss" phenomenon that stymies other gas chromatographs. ▪ *Because* its many other exclusive features make it the best performing biomedical gas chromatograph, not only for steroid analysis, but for a long list of biological compounds including vitamins, alkaloids, bile acids, fatty acids, amino acids, pesticides, organo-metallics, etc. Want more proof of the Series 400's remarkable performance record? We have it: Write us or call your F & M Sales Representative. F & M Scientific Europa N.V., 40/48 High Street, Acton, London W-3. Tel. ACORN 5221.

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THE ANALYST

The Determination of Perchlorate in the Presence of Other Halogen Acids by Means of a Coloured Liquid Anion Exchanger

BY H. M. N. H. IRVING AND A. D. DAMODARAN
(*Department of Inorganic and Structural Chemistry, The University, Leeds, 2*)

Perchlorate in aqueous solution can be determined absorptiometrically in concentrations down to approximately 5×10^{-5} M in terms of the optical density at 353 mμ of the erdmannate ion, $[Co(NH_3)_2(NO_2)_4]^-$, displaced on equilibration with a solution of tetra-n-hexylammonium erdmannate in a xylene - hexone mixture, which acts as a coloured liquid anion exchanger. Two analytical procedures are described. In the first procedure the optical densities of both equilibrated phases are measured, and the construction of a linear calibration curve demands a knowledge of the relevant molecular extinction coefficients, which are shown to be slightly different in the two solvent media. The second is simpler, in that only the optical density of the aqueous phase is measured to give a linear calibration graph.

Interferences by chlorate, chlorite and hypochlorite ions (as well as nitrite and nitrate ions) can be eliminated by fuming the sample with concentrated hydrochloric acid, since chloride ions (and many others) do not interfere. The procedure lends itself to the determination of perchlorate in admixture with chloride and other species obtained by its electro-oxidation.

QUATERNARY ammonium salts that contain long-chain alkyl groups, R, are almost insoluble in water, although soluble in various organic solvents. If the organic solvent is immiscible with water, such salts can exchange their anions according to the reaction—

$$n\{(R_4N)^+X^-\}_{org.} + Y^{n-} \rightleftharpoons \{(R_4N)_n^+ Y^n\}_{org.} + nX^- \quad .. \quad .. \quad .. \quad (1)$$

where the subscript, org., is used to distinguish the organic from the aqueous phase. In this respect, such quaternary ammonium salts formally resemble anion-exchange resins and they have been used for the analytical separations of a wide variety of metals,[1] after which the separated metals have been determined by conventional methods. A somewhat different approach has been used with cobalt^{II}, which has been extracted as the quaternary ammonium salt of its anionic thiocyanate complex, whose concentration can then be determined absorptiometrically.[2]

Clifford and Irving[3] recently suggested a new method of using liquid anion exchangers. By equilibrating a solution of tetrahexylammonium iodide in a suitable organic solvent with an aqueous solution of Erdmann's salt, $(NH_4)^+[Co(NH_3)_2(NO_2)_4]^-$, they obtained a deeply coloured liquid anion exchanger with a molecular extinction coefficient of approximately 10^4. The coloured erdmannate ion, E^-, could then be replaced by other anions, *e.g.*, perchlorate ions, according to the reaction—

$$\{(NR_4)^+E^-\}_{org.} + ClO_4^- \rightleftharpoons \{(NR_4)^+ClO_4^-\}_{org.} + E^- \quad .. \quad .. \quad (2)$$

so that there was a decrease in the optical density of the organic phase and an increase in that of the aqueous phase. When a mixture of 80 per cent. v/v *m*-xylene and 20 per cent. v/v hexone (methyl isobutyl ketone) was used as the solvent, the equilibrium constant for this reaction was found to be 1·05 at room temperature and substantially independent of the relative concentrations of erdmannate and perchlorate ions. By measuring the concentration of erdmannate ions absorptiometrically in the organic phase before and after equilibration with an aqueous solution of perchlorate, and by assuming a value of $K_{ClO^-} = 1{\cdot}05$ for the

equilibrium constant of reaction (2) it was found possible to calculate the amount of perchlorate present. Furthermore, from the experimental values of $K_{NO_3^-} = 8{\cdot}2 \times 10^{-4}$ and $K_{ClO_3^-} = 2{\cdot}8 \times 10^{-3}$ for the equilibrium constants of reactions corresponding to reaction (2) in which nitrate or chlorate ions are used in place of perchlorate ions, it was possible to determine perchlorate in the presence of chlorate and nitrate, the two ions that most seriously interfere.[3]

Although this procedure is fundamentally sound, there are several practical objections. In the first place, the absorptiometric determinations are carried out on one phase only, so that there is no real check on the mass balance and hence on the confidence that can be attached to individual results. Secondly, attempts to replace calculations involving equilibrium constants by simple calibration curves substantially reduces the precision of the results when only small amounts of perchlorate are involved. A more general criticism, however, is that the procedure outlined by Clifford and Irving[3] does not lend itself to the determination of quite a number of other anions. In some instances the value of the relevant equilibrium constant, K, cannot be obtained with sufficient accuracy. In other instances, the equilibrium processes are more complex than is suggested by reaction (1). For example, the equilibration of a quaternary ammonium erdmannate with a solution containing mercuric cyanide in potassium cyanide simultaneously involves the two replacement reactions—

$$2\{(NR_4)^+E^-\}_{org.} + Hg(CN)_4^{2-} \rightleftharpoons \{(NR_4)_2^+Hg(CN)_4^{2-}\}_{org.} + 2E^- \quad .. \quad (3a)$$

and

$$\{(NR_4)^+E^-\}_{org.} + Hg(CN)_3^- \rightleftharpoons \{(NR_4)^+Hg(CN)_3^-\}_{org.} + E^- \quad .. \quad .. \quad (3b)$$

Admittedly a composite "conditional" equilibrium constant[5] could be derived for specific conditions, but the modified procedures that follow seem preferable.

Theory

Procedure A—

The equilibrium constant of reaction (2) is given by—

$$K_{ClO_4^-} = \frac{[NR_4^+\ ClO_4^-]_{org.}[E^-]}{[NR_4^+E^-]_{org.}[ClO_4^-]} \quad .. \quad .. \quad .. \quad .. \quad (i)$$

$$i.e.,\ K_{ClO_4^-} = \frac{[E^-]^2}{[NR_4^+E^-]_{org}[ClO_4^-]} \quad .. \quad .. \quad .. \quad .. \quad (ii)$$

since $[NR_4^+ClO_4^-]_{org.}$, the concentration of the quaternary ammonium perchlorate in the organic phase, must be equal to the concentration of erdmannate ion, $[E^-]$, liberated into the aqueous phase, provided the volumes of the two phases are the same.

On rearranging equation (*ii*) we obtain—

$$\frac{[E^-]^2}{[NR_4^+E^-]_{org.}} = K_{ClO_4^-}[ClO_4^-]$$

and on taking decadic logarithms—

$$\log \frac{[E^-]^2}{[NR_4^+E^-]_{org.}} = \log K_{ClO_4^-} + \log [ClO_4^-] \quad .. \quad .. \quad .. \quad .. \quad (iii)$$

$$= \log K_{ClO_4^-} + \log ([ClO_4^-]_{tot.} - [ClO_4^-]_{org.})$$

$$= \log K_{ClO_4^-} + \log ([ClO_4^-]_{tot.} - [E^-]) \quad .. \quad .. \quad (iv)$$

where $[ClO_4^-]_{tot.}$ is the total initial concentration of perchlorate ion in the aqueous phase.

It follows from equation (*iv*) that a graph of $\log\left(\frac{[E^-]^2}{[NR_4^+E^-]_{org.}}\right)$ against $\log ([ClO_4^-]_{tot.} - [E^-])$ should be linear with a slope of unity and intercept, $\log K_{ClO_4^-}$. Typical results for equilibrations conducted as described later in the experimental section are summarised in Table II, and are shown plotted in Fig. 1. The points fall on a straight line of unit slope, which confirms the validity of equation (*iv*). The intercept leads to the value $K_{ClO_4^-} = 1{\cdot}7 \pm 0{\cdot}2$ at 18° C, which is somewhat higher than the value previously obtained[3] under different conditions and by a less precise method.

The values of the concentration terms $[E^-]$ and $[NR_4^+E^-]_{org.}$ are, of course, obtained from the measured values of the optical densities of aliquot portions of the aqueous and organic phases, respectively, after equilibration by making use of the appropriate molecular extinction

coefficients (*q.v.*) and the relevant cell lengths. In the analysis of an unknown, the concentration of perchlorate remaining after equilibration in the aqueous phase is read off along the abscissa of the calibration curve (*cf.* equation *iv*). The total initial perchlorate is then obtained from the relationship—

$$[ClO_4^-]_{tot.} = [ClO_4^-] + [E^-] \quad .. \quad .. \quad .. \quad .. \quad (v)$$

This Procedure A is to be preferred to that proposed initially by Clifford and Irving,[3] both from the point of view of convenience and accuracy. There is the additional advantage that there is an immediate check on reliability by calculating the mass-balance of erdmannate, since the sum of $[E^-]$ and $[NR_4^+E^-]_{org.}$ after equilibration should be constant and equal to the concentration of reagent taken initially (see Table II). The relationship formulated by equation (*iv*) has been found to hold over a wide range of concentrations of erdmannate and perchlorate ions when hexone is used as the solvent; it is also substantially independent of temperature and changes in ionic strength.[4]

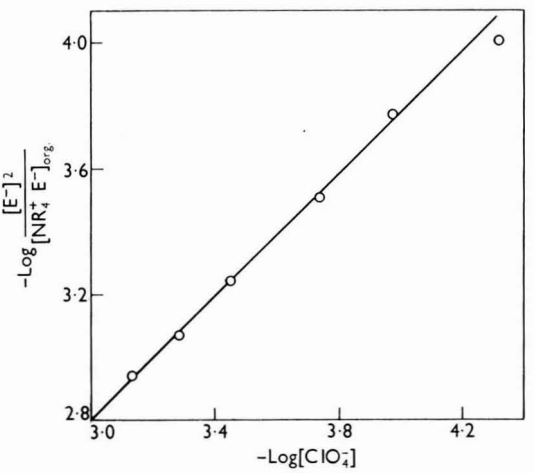

Fig. 1. Graph of equilibration in determination of perchlorate with tetrahexylammonium erdmannate in 20 per cent. hexone — 80 per cent. xylene mixture

PROCEDURE B—

It is obvious from the Law of Mass Action that the position of equilibrium of reaction (2) can be moved to the right for any arbitrary concentration of perchlorate by increasing the concentration of the quaternary erdmannate in the organic phase. Clifford and Irving selected the initial erdmannate concentration such that the equilibrium value was about 10^{-5} M in order to give suitable optical-density readings. However, by using such a concentration a highly curved calibration curve is obtained if a graph of the decrease in the optical density of the organic phase, *i.e.*, initial optical density minus final optical density, $D_0 - D_f$, is plotted against the concentration of perchlorate initially present in the aqueous phase (see Fig. 2, curve A). The curvature can, of course, be reduced by increasing the initial concentration of erdmannate (see Fig. 2, curves B and C), and by taking a 0·01 M solution an almost linear calibration curve can be obtained with initial concentrations of perchlorate up to 0·001 M. However, this method must obviously fail when applied to the determination of small amounts of perchlorate, since the ordinate then represents the measured difference between two large amounts, *viz.*, the initial and final optical densities, both of the order of 10^3. In principle, this difference could be measured by what is essentially an application of differential absorptiometry if a sample of the organic phase after equilibration with pure water (or an appropriate inert salt solution) is placed in the reference cell and a sample of the same organic phase after equilibration with aqueous perchlorate is placed

in the sample cell (both would need to be diluted very precisely to the same extent of approximately 100-fold), and the difference in optical density, $D_0 - D_f$, read directly. Our experiments showed that experimental difficulties prevented the achievement of sufficient reproducibility and accuracy.

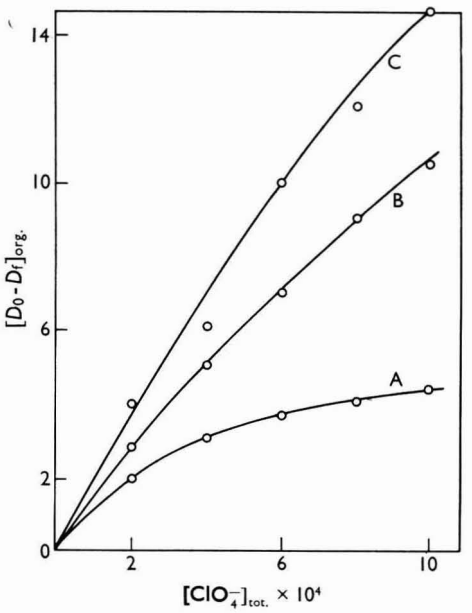

Fig. 2. Graph of decrease in the optical density of the organic phase plotted against concentration of perchlorate in the aqueous phase, curve A. Curves B and C show how the curvature may be reduced by increasing the initial concentration of erdmannate

It is obvious that under these circumstances it is preferable to measure the *increase* in the optical density of the aqueous phase due to the coloured erdmannate displaced by perchloric from the organic phase. If initial concentrations are distinguished by a superscript dash, the initial concentration of erdmannate in the organic phase will be represented by $[NR_4^+E^-]'_{org.}$, and the various terms in reaction (2) after equilibration will be—

$$[NR_4^+E^-]_{org.} = ([NR_4^+E^-]'_{org.} - [E^-])$$

$$[ClO_4^-] = ([ClO_4^-]'_{tot.} - [E^-])$$

$$\text{and } [NR_4^+ClO_4^-]_{org.} = [E^-]$$

so that equation (*ii*) becomes—

$$K = \frac{[E^-]^2}{([NR_4^+E^-]'_{org.} - [E^-])([ClO_4^-]'_{tot.} - [E^-])}$$

whence—

$$[ClO_4^-]'_{tot.} = [E^-] + \frac{[E^-]^2}{K([NR_4^+E^-]'_{org.} - [E^-])} \quad .. \quad .. \quad .. \quad (vi)$$

Provided $[NR_4^+E^-]'_{org.} \gg [E^-]$, the second term on the right hand side will be negligible compared with the first, and the amount of erdmannate displaced into the aqueous phase will be linearly proportional to that of the perchlorate taken initially. In practice, a calibration curve indistinguishable from a straight line will be obtained even when this condition is not exactly fulfilled, for it is easy to show that the calculated value of $[E^-]$ will not be more

than 1 per cent. low provided $[NR_4^+E^-]'_{org.} > 61[E^-]$. Indeed, even with the most concentrated perchlorate solution shown in Fig. 3 in which the initial concentration of quaternary erdmannate is actually only twelve times that of the initial perchlorate, the calculated value of $[E^-]$ is only 5 per cent. low.

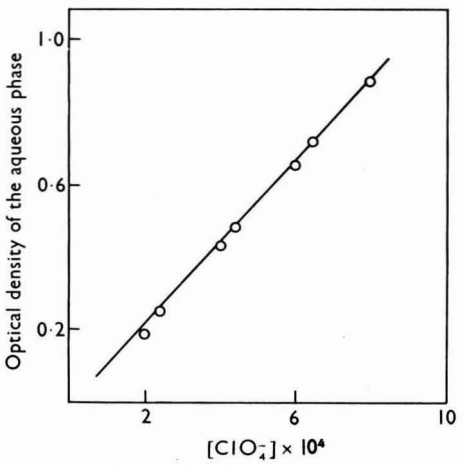

Fig. 3. Graph of optical density of the aqueous phase plotted against the concentration of the perchlorate solution

All the results used in the following discussion were obtained by Procedure B. This was preferred both on the grounds of simplicity (there are fewer measurements of optical density and less calculations needed), but also because it leads directly to an effectively linear calibration curve and does not involve any knowledge of the molecular extinction coefficients of the species involved.

Experimental

Reagents—

All reagents used were of AnalaR quality whenever possible.

Preparation of Erdmann's salt—Erdmann's salt was prepared by Jørgensen's method[6] and recrystallised from water as brownish-red crystals. After decomposition of the complex by fuming with concentrated hydrochloric acid, the cobalt was determined gravimetrically in the form $Co(NH_4)PO_4.H_2O$ with the results—

Cobalt found, per cent.	20·07, 20·18
Cobalt calculated, per cent.	19·98

Tetrahexylammonium iodide—The sample of this salt used initially was supplied by General Mills, Illinois, U.S.A. Later samples were synthesised from n-hexyl iodide and tri-n-hexylamine by heating equimolar amounts of the components under reflux in benzene on a water-bath for 12 hours. The solvent was removed slowly on the water-bath whereupon shining crystals of the quaternary iodide separated, and these were purified by re-crystallisation from xylene.

Solvents—A mixture of 80 per cent. v/v of xylene and 20 per cent. v/v of hexone (methyl isobutyl ketone) was used for reasons previously described in detail.[3]

Standard sodium perchlorate solution—A solution of sodium perchlorate prepared from AnalaR sodium carbonate and AnalaR perchloric acid was standardised by passing a known volume through a cation-exchange resin in the hydrogen ion form and titrating the acid liberated with standard alkali. After standardisation, the solution was diluted to the desired concentrations with de-mineralised water.

Stock solution of quaternary erdmannate—Five millitres of a 0·05 M solution of tetra-n-hexylammonium iodide in hexone were diluted with 20 ml of xylene in a 100-ml separating funnel. Twenty-five millitres of an aqueous solution of Erdmann's salt (approximately 0·01 M) were then added and the mixture shaken for 3 minutes. After the phases had been

allowed to separate the aqueous phase was separated and rejected. The organic phase was then re-equilibrated with a fresh aliquot portion of the erdmannate solution and the whole procedure was then repeated twice more. Finally the separated organic layer was washed with 25 ml of water to remove any adhering erdmannate solution and after rejection of the aqueous phase the organic solution was stored in the dark.

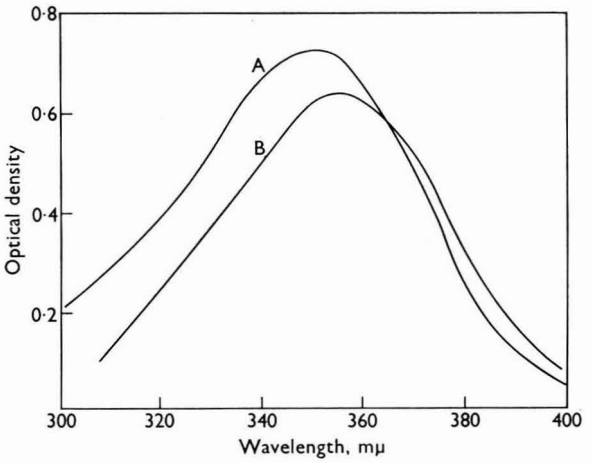

Fig. 4. Spectra of the erdmannate ion in aqueous solution, curve A, and in the xylene - hexone mixture, curve B.

SPECTRA AND MOLECULAR EXTINCTION COEFFICIENTS OF THE ERDMANNATE SOLUTIONS—

The spectra of the erdmannate ion in aqueous and organic media are shown in Fig. 4. In water, curve A, the erdmannate ion gives a single broad band centred at 352 mμ: the spectrum in the xylene - hexone mixture, curve B, is very similar, but there is a small bathochromic shift and the broad band is now centred at 355 mμ. In all subsequent measurements with the Unicam SP500 spectrophotometer a wavelength setting of 353 mμ was used for either media.

A standard solution of Erdmann's salt, 0·009389 M, was prepared by dissolving a known weight of the pure salt in water. The cobalt content, determined absorptiometrically by the thiocyanate method[7] was found to be 0·009365 M, in good agreement. The molecular extinction coefficient, ϵ, calculated from the optical density (average 0·423 in a 1-cm cell) after a 250-fold dilution was 11,300 $\pm$ 100.

A known volume of the stock organic erdmannate solution was then taken and the organic matter destroyed by wet oxidation with a mixture of sulphuric and nitric acids containing a few drops of perchloric acid. The cobalt content was determined absorptiometrically by the thiocyanate method[7] and found to be 0·02441 M. After a 500-fold dilution with the mixed organic xylene - hexone solvent the optical density was found to be 0·693 (average in 1-cm cell), whence the molecular extinction coefficient for the organic phase, $\epsilon_{org.}$, is calculated to be 14,200 $\pm$ 100.

The large difference between the two molecular extinction coefficients for the aqueous and organic solutions was somewhat unexpected, since the absorption spectra are effectively identical in shape in the two solvents (see Fig. 4) and there was no reason to suspect changes in the nature of the species present or any possibilities of decomposition. The observed difference was, however, consistent with the satisfactory mass-balance shown in Table II, for which the respective concentrations were calculated from measured optical densities and the appropriate (different) molecular extinction coefficients given above.

Although similar differences in molecular extinctions have been reported for other salts studied in different media, there remained the doubt whether the results were spurious and due in some way to the different methods used for establishing the concentration of cobalt

in the stock aqueous and stock organic phases, especially since there was no independent check on the latter. The experiment described below was devised to eliminate any such uncertainty.

Five millilitres of a stock solution of the quaternary erdmannate (approximately 10^{-4} M) in the xylene - hexone mixture was shaken with 5 ml of 2·5 M aqueous sodium perchlorate. Calculations with the value $K_{ClO_4^-} = 1{\cdot}7$ (or even with the former value of 1·05) show that this concentration of perchlorate is sufficient to displace more than 99·9 per cent. of erdmannate ion into the aqueous phase. The optical density of the aqueous phase and then that of the organic phase were measured in 0·2-cm cells to confirm that the whole of the erdmannate had been displaced by perchlorate. The results of two such experiments are summarised below—

	Organic phase	Aqueous phase
Optical density before equilibration ..	0·320, 0·320	—
Optical density after equilibration	0·00, 0·01	0·255, 0·250

The ratio of optical densities in the two phases is thus 0·320/0·253 = 1·26, which is almost identical with the ratio calculated from the previously measured molecular extinction coefficients, *viz.*, 1·42/1·13 = 1·27.

Clearly the validity of this experiment rests on the assumption that the absorption spectrum of Erdmann's salt in water is not influenced by high concentrations of sodium perchlorate. To test this, 5 ml of an aqueous erdmannate solution were diluted to 25 ml with (*a*) pure water, and (*b*) 2·5 M sodium perchlorate. The measured optical densities (0·1-cm cell) were, respectively, 0·530 and 0·525, thus establishing the absence of any gross salt effect.

Solutions of the quaternary erdmannate in the organic medium and of the erdmannate ion in the aqueous phase were shown to obey Beer's law, at least up to concentrations of $1{\cdot}3 \times 10^{-4}$ M and 10^{-4} M, respectively: these represent upper limits to the concentrations measured absorptiometrically in Procedures A and B.

Stability of erdmannate solutions—

Although direct sunlight should be avoided,[3] it appears that aqueous solutions of erdmannates can be exposed to good laboratory (fluorescent) lighting for at least 15 minutes without the need for special precautions against decomposition. Some typical results are shown in Table I.

Table I

Stability of approximately 10^{-5} M erdmannate solutions

Optical-density readings were made in 1-cm cells at 353 mμ

Time, minutes	Optical density of— Aqueous phase: In dark	Aqueous phase: In light	Organic phase: In dark	Organic phase: In light
0	0·48	0·48	0·44	0·44
15	0·48	0·48	—	—
25	0·48	0·47	0·44	0·44
45	0·48	0·46	—	—
65	—	—	0·44	0·43
75	0·48	0·44	—	—
100	0·47	0·39	0·44	0·44*

* Unchanged on being kept overnight in the dark.

Solutions of the quaternary erdmannate in the organic solvents are much less sensitive to light.

Results and discussion

Procedure A—

Standard perchlorate solution (5 ml of approximately 10^{-4} M) was vigorously shaken with 5 ml of the stock organic erdmannate solution (approximately 10^{-4} M) in a 20-ml stoppered centrifuge tube for 3 minutes. The mixture was then spun in a centrifuge for 5 minutes to ensure complete phase separation, and appropriate aliquot portions of each phase were then transferred by pipettes into matched spectrophotometer cells of suitable path length and their optical densities measured at 353 mμ. The concentration of erdmannate in each phase was then calculated from a knowledge of the molecular extinction coefficients, *viz.*, $\epsilon_{aq.} = 11{,}300$

and $\epsilon_{org.} = 14{,}200$. The results of some typical measurements are given in the second and third rows of Table II. The fourth and fifth rows give the calculated concentrations of erdmannate in the aqueous and organic phases, respectively: the constancy of the mass-balance given in the sixth row both demonstrate the reliability of the method and the validity

TABLE II

DETERMINATION OF PERCHLORATE BY EQUILIBRATION WITH TETRAHEXYLAMMONIUM ERDMANNATE IN HEXONE - XYLENE MIXTURE ACCORDING TO PROCEDURE A

Initial erdmannate concentration = $4{\cdot}40 \times 10^{-4}$ M

$[ClO_4]_{tot.} \times 10^4$	2·12	4·24	6·36	8·48	10·60
Optical density (0·1-cm cell)					
(*i*) Organic phase.. ..	0·385	0·272	0·210	0·168	0·145
(*ii*) Aqueous phase ..	0·185	0·275	0·328	0·362	0·388
$[E^-] \times 10^4$	1·63	2·43	2·89	3·19	3·42
$10^4[E^-]_{org.} \times 10^4$	2·73	1·93	1·49	1·19	1·03
$([E^-] + [E^-]_{org.}) \times 10^4$ ..	4·36	4·36	4·38	4·38	4·45
$[ClO_4^-]_{org.} \times 10^4$	0·49	1·81	3·47	5·29	7·18
$\log[ClO_4^-]$	−4·31	−3·74	−3·46	−3·29	−3·14
$\log([E^-]/[E^-]_{org.})$	−4·01	−3·51	−3·25	−3·07	−2·94

of using the two different molecular extinction coefficients as explained above. The calibration curve derived from these results is shown in Fig. 1.

PROCEDURE B—

Five millilitres of stock quaternary erdmannate (0·01 M in the xylene - hexone mixture) were equilibrated with a mixture of x ml of sodium perchlorate solution of known concentration (approximately 10^{-3} M) and (5-x) ml of water by being shaken in a 20-ml stoppered centrifuge tube for 3 minutes. After centrifugation for 5 minutes, a portion of the aqueous phase was removed and the optical density measured in a 0·1-cm cell at 353 mμ against a blank solution obtained by carrying out the same determination with water alone ($x = 0$). A calibration curve was then constructed for the range 10^{-3} to 10^{-4} M perchlorate by repeating the determination with different values of x in the range 5 to 0·5 ml. The graph of $[ClO_4]_{tot.}$ against optical density was a straight line as shown in Fig. 3. The determination of an unknown concentration of perchlorate within this range is carried out by the same procedure by using 5 ml of the sample or an appropriately smaller aliquot portion diluted accurately to 5 ml.

If the sample contains free acid, or if this has been introduced by any pretreatment designed to eliminate interfering species (*cf.* p. 450), the pH should be brought within the range 6 to 10 by adding alkali before the dilution to 5 ml.

If it is desired to use still smaller concentrations of perchlorate in the range 10^{-4} to 10^{-5} M, a linear calibration curve (not reproduced) is constructed as before by using various volumes, x ml, of 10^{-4} M sodium perchlorate and measuring the optical densities of the equilibrated aqueous phase in a 1·0-cm cell.

TABLE III

INTERFERENCES IN THE DETERMINATION OF PERCHLORATE IONS BY PROCEDURE B

Initial concentration of quaternary erdmannate was 0·01 M and that of perchlorate approximately $2{\cdot}5 \times 10^{-4}$ M

Added anion, approximately 0·02 M	Optical density of aqueous phase in 0·1-cm cell	Added anion, approximately 0·02 M	Optical density of aqueous phase in 0·1-cm cell
Nil	0·260	Nitrate	0·45
Fluoride	0·260	Chlorate	0·90
Phosphate	0·260	Nitrite	*
Sulphate	0·260	Chlorite	*
Hydroxide	0·265	Hypochlorite	*
Chloride	0·270		

* Rapid decomposition of the erdmannate ion and variable optical densities were obtained.

INTERFERENCES—

Possible interferences were examined by determining perchlorate by Procedure B in the presence of various amounts of other anions, added as their sodium salts. Typical results are given in Table III.

Bromide, iodide and thiocyanate ions gave high results as expected from the increasing tendency $F^- < Cl^- \ll Br^- \ll I^-$ to enter the organic phase. Clearly in analysing a mixture obtained by the electro-oxidation of chloride ion, the most serious interference will derive from chlorate, chlorite and hypochlorite. Chlorate can be reduced to chloride by sulphur dioxide or by ferrous sulphate. The latter reductant is inappropriate here since the ferric ions produced interfere with the subsequent spectrophotometric determination. Moreover, to avoid decomposition of the erdmannate ion, the pH of the aqueous phase should not be less than about 3, although it can be raised as high as 10. However, there is then the risk of precipitating ferric hydroxide or at least of forming a colloidal solution. Reduction by boiling hydrochloric acid was found to be preferable and, furthermore, this would eliminate interference due to chlorite and hypochlorite, and indeed that caused by nitrite and nitrate, if these are present. The procedure is as given below—

The aqueous solution of the sample is heated in a small basin on a water-bath and concentrated hydrochloric acid is added dropwise until the effervescence due to the liberation of "euchlorine" (or chlorine, nitrosyl chloride and nitrous fumes if hypochlorites, nitrates or nitrites are present) ceases. A few more drops of concentrated hydrochloric acid are then added and the solution evaporated to dryness. The residue is taken up in water, made up to a known volume, and aliquot portions used for the determination of perchlorate by Procedure B. Oxyacids of bromine and iodine also interfere, but their reduction by treatment with hot concentrated hydrochloric acid presents no problem. Elimination of iodide, bromide and thiocyanate can be effected by treatment with silver sulphate.[3]

In typical experiments in which a mixture of 4×10^{-4} M sodium perchlorate and 0·4 M potassium chlorate was taken through this procedure the final optical densities of the aqueous phase were 0·470, 0·450, 0·452, 0·452, 0·470 and 0·455 in a 0·1-cm cell (mean, $0{\cdot}46 \pm 0{\cdot}01$) as compared with a value of 0·47 in the absence of added chlorate. Perchlorate can thus be determined at this concentration level in the presence of a 1000-fold excess of chlorate to within about 2 per cent. Similar experiments, not reported in detail, show that the method is equally applicable in the presence of comparable excesses of nitrate and nitrite ions. The effect of chlorite was not studied quantitatively in view of the difficulty of obtaining a sample free from chlorate.

Effect of ionic strength—

Preliminary reports by Clifford and Irving[3] suggested that changes in the ionic strength of the aqueous phase had some appreciable effect on the equilibrium constant of reaction (2). The determination of 8×10^{-4} M perchlorate in solutions consisting of 0·04 M sodium chloride ($\mu = 0{\cdot}04$), 0·04 M sodium sulphate ($\mu = 0{\cdot}12$) and 0·12 M sodium sulphate ($\mu = 0{\cdot}36$), anions that had previously been shown not to be appreciably extracted, gave optical-density readings of 0·485, 0·470 and 0·475 on a 20-fold diluted aqueous phase after equilibration.

Table IV

Interference by high concentrations of chloride ion in determination of perchlorate by Procedure B

Concentration of sodium chloride, M	Optical density (0·1-cm cell) of the aqueous phase at 353 mμ*		
	hexone†	ethylene dichloride†	xylene - hexone mixture†
0·0	0·47	0·46	0·46
0·1	0·47	0·59	0·46
0·2	0·51	0·67	0·46
0·4	0·54	0·73	0·46

* After equilibration with approximately 4×10^{-4} M perchlorate.
† Containing approximately 0·01 M quaternary erdmannate.

This shows the absence of any significant effect, at least over the range of ionic strength $\mu = 0{\cdot}04$ to 0·36. Similar results have been obtained with pure hexone as the solvent.[4] Table II reports some measurements with chloride solutions of even higher concentration. With ethylene dichloride as a solvent for the quaternary erdmannate, there are notable effects of changing the concentration of chloride ion. These may be due in part to a larger tendency to displace erdmannate ions, *i.e.*, a higher value for K_{Cl^-}, and in part to the salting out effect produced by the increase in ionic strength. The effect is smaller in pure hexone and negligible in the mixture of xylene and hexone used routinely in Procedure B.

BLANK VALUES—

Blank values had always to be subtracted, since on shaking 0·01 M quaternary erdmannate solutions with water alone, an appreciable colour appeared in the aqueous phase corresponding to an optical density of approximately 0·5 in a 1-cm cell (and hence to 0·05 for the measurements in 0·1-cm cells used routinely). This blank value is presumably due to the normal partition of the quaternary salt. A similar blank value was obtained when benzene was used as the organic solvent, but the magnitude was reduced to 0·205 and 0·175 (1-cm cell) when ethylene dichloride or hexone, respectively, were used. This is understandable since the ion pair, $(NR_4)^+E^-$, should be more soluble in the more polar solvents, thereby decreasing the proportion in the aqueous phase. However, the effect of chloride ion is greater when pure hexone or ethylenedichloride are used (see Table IV) and similar effects on K_{Cl^-} and $K_{NO_3^-}$ had previously been noted by Clifford and Irving[3] when mixtures of xylene and hexone in various proportions were used for extracting chloride or nitrate. It follows from Table IV that concentrations of chloride as high as 0·4 M can be tolerated when the solvent is xylene - hexone mixture and the blank value is reproducible and almost negligible (0·05 in 0·1-cm cell), if measurements of perchlorate are undertaken in the range 10^{-3} to 10^{-4} M (see Fig. 3). However, when the perchlorate is less concentrated and in the range 10^{-4} to 10^{-5} M and a 1·0 cm cell is used, the blank value may amount to half of the measured optical density when the concentration of perchlorate falls below 5×10^{-5} M, so that, even though the blank value remains constant, the overall precision falls off rapidly. Procedure B should thus be confined to perchlorate concentrations of 5×10^{-5} M and above.

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A General Method for the Determination of Organophosphorus Pesticide Residues in Foodstuffs

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A scheme of analysis is described for the extraction from plant material, separation by paper chromatography and determination of the organophosphorus pesticides used commercially in the United Kingdom. The pesticides, together with important metabolites, are extracted from the crop with acetone, and, after the addition of water, is partitioned into chloroform. The solvent is removed, the extract taken up in acetone and the fats and waxes frozen out at −80° C. Further clean-up is achieved by column chromatography, and the subsequent paper chromatography of the polar pesticides and metabolites is carried out in a different solvent system from that used for the less polar parent organophosphorus compounds. The pesticides and metabolites are characterised by R_F values, and individual compounds are determined from the phosphorus content of the spot on the chromatogram. Results are given for several combinations of crops and pesticides.

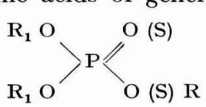
The increasing use of organophosphorus pesticides in agriculture has created many problems for the analyst. One of the most pressing is the need for quick screening methods that can be used for determining whether or not a crop, or food sample, contains pesticide residues, and that can then be used to identify and quantitatively or semi-quantitatively determine the amount present. The determination of organophosphorus pesticide residues in foodstuffs is a complex problem, since in certain instances the original pesticide may be no longer present as such. The problem can only be solved by working out some relatively rapid scheme of qualitative analysis before subsequent quantitative determination.

Such a scheme would ideally involve techniques that were efficient, specific, flexible, simple and inexpensive. Paper chromatography fulfills all these requirements, and Getz[1] has recently reviewed part of the considerable work already carried out involving separations of organophosphorus pesticides and their metabolites by paper chromatography, both in the analysis of residues in foodstuffs and in investigations of the metabolism of organophosphorus compounds in plants and animals. Several paper-chromatographic separations of small groups of pure compounds have been reported, but few attempts have been made to apply such separations to a general procedure for the detection, identification and determination of organophosphorus residues in the presence of plant extracts.[2]

This paper deals with the separation of the organophosphorus pesticides used in the United Kingdom and describes a method by which they can be detected, identified and determined in a range of crops.

General considerations

Most organophosphorus pesticides are esters of phosphoric, phosphorothionic, phosphorothiolic or phosphorothiolothionic acids of general structure—



where R_1 is ethyl or methyl and R is an organic radicle. Recent books by O'Brien[3] and Heath[4] summarise the properties and reactions of these compounds and discuss them in terms of the electronic theory of reactions.

The simplest and most important electronic effect is the inductive effect, which influences the polarity of the molecules. This, in turn, is important in deciding the partitioning behaviour of a compound between immiscible solvents, and in determining partition coefficients and behaviour on chromatograms.

The resultant inductive effect in a molecule is the sum of the effects of individual atoms and groups. The phosphorus atom in organophosphorus esters carries a partial positive charge because it is surrounded by atoms or groups that withdraw electrons from it. The inductive effects of the electrophilic atoms or groups frequently found in organophosphorus pesticides, decrease in the order—

$$=O > =S > -OH$$

$$\text{and} \qquad -OR > -SR$$

Thus $(R_1O)_2 . PO . OR$ is much more polar than $(R_1O)_2 . PS . SR$.

The least polar organophosphorus pesticide is the phosphorothiolothionate, phenkapton,* whereas the phosphorothionate, parathion,* is considerably more polar. A great increase in polarity is achieved by oxidising alkylthioalkyl phosphorothionates to their sulphoxides, for example—

$$(CH_3O)_2 . PO . S . CH_2 . CH_2 . S . C_2H_5 \rightarrow (CH_3O)_2 . PO . S . CH_2 . CH_2 . SO . C_2H_5$$

demeton-*S*-methyl — oxydemeton-methyl

Metabolites usually fall into two well defined classes based on the ionic character of the compounds formed. The first class contains metabolites extracted from aqueous systems by solvents such as chloroform. In this group are oxidation products, *e.g.*, P = O analogues, sulphoxides and sulphones, and for nitrophenol compounds, reduced aminophenol analogues. Parent pesticides are also extracted by chloroform.

The second class contains hydrolysis products, which are anionic at pH 7 and not extracted from aqueous sytems by chloroform. The biochemical activity of an organophosphorus compound that inhibits cholinesterase is destroyed by the hydrolysis of any one bond between the phosphorus atom and any of the ester groups.[4] Thus, in general, the hydrolysis products are of less interest in assessing hazards to consumers of treated crops.

In the separation and identification of solvent-extractable metabolites, the number of compounds to be separated is not large except for alkylthioalkyl phosphorothionates, for which the thio (–S–), sulphoxide and sulphone derivatives of the phosphorothionate and the phosphate give a total of six compounds.

For the hydrolysis products not extractable into chloroform, the problem of adequate resolution is more difficult, since there are often more metabolites (ten for malathion) and some pairs are very difficult to resolve by paper chromatography.

In this paper the emphasis is on the first group, which includes parent organophosphorus pesticides and metabolites extracted from aqueous solutions by chloroform.

Experimental

Extraction—

A general extraction procedure applicable to all crops should be as straightforward and trouble-free as possible. Dichloromethane, recommended by Laws and Webley[5] as an extracting solvent, unfortunately gives emulsions with some crops. The work of Getz[6] suggests that acetonitrile could be used more widely, since, although fats and waxes are not very soluble in it, all organophosphorus pesticides are readily extracted. It might, however, be considered to be too expensive and toxic a solvent for general use. Acetone was chosen as the extracting solvent for the work described in this paper, since it is inexpensive, highly efficient, non-toxic and does not give emulsions. The method was as described by Bates.[7] The extract contains both the non-polar parent organophosphorus pesticides and the more polar pesticides and metabolites.

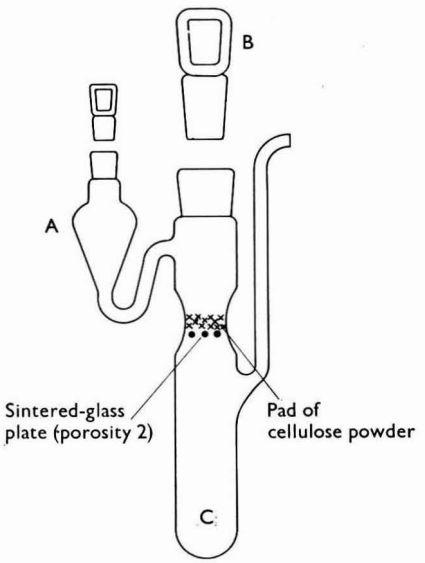
Clean-up—

When pesticide residues are extracted from plant material they are accompanied by a considerable amount of pigments and waxes, and these interfere severely with paper chromatography. A clean-up procedure is needed to remove interfering material, and so several of the alternative clean-up methods described in the literature were examined. The simple and direct technique of freezing fats and waxes out of acetone solution at −70° C,

* The common names used for the pesticides mentioned in this paper are those recommended by British Standards 1831 : 1964.

reported by Anglin and McKinley,[8] is a general procedure applicable to a wide range of crops. Combining the acetone precipitation with Florosil-column chromatography, they obtained better than 97 per cent. removal of co-extractives, and recoveries of DDT and related pesticides were 90 per cent. or better, the whole process comparing very favourably with solvent-partition methods.

This freezing procedure was chosen as the basic clean-up technique. By carrying out the cooling and filtration in the apparatus shown in Fig. 1, transference of the sample during filtration was reduced to a minimum. Two columns are recommended for further clean-up, the choice depending on the solvent system to be used in the paper-chromatographic stage. For the polar pesticides and metabolites a magnesium oxide column for which chloroform is used as the eluting agent is adequate, whereas a cellulose - charcoal - magnesium oxide column for which a mixture of chloroform and benzene is used is more suitable for the less polar organophosphorus compounds. Although the final extracts were occasionally a pale-yellow colour, the amount of pigment left did not interfere with the subsequent paper chromatography.



A = Flask, 10-ml capacity
B = Stopper
C = Tube, about 20-ml capacity, acting as filter flask

Fig. 1. Diagram of apparatus used for freezing procedure

PAPER CHROMATOGRAPHY—

Paper chromatography has been used successfully by many workers both in residue analysis and when following the degradation and metabolism of organo-phosphorus compounds in plants, animals and insects. However, since there is a wide range of polarities amongst the pesticides and their metabolites, chromatographic systems designed to resolve mixtures with a fairly limited range of polarities are not suitable for a general screening method and vice versa.

Choice of paper—The most frequently used papers reported in the literature are Whatman Nos. 1 and 3 MM, the latter being sometimes preferred because of its greater absorptive capacity and faster solvent-flow times. However, Whatman No. 20 has been used for most of the work reported here. It is slower running than No. 1, gives compact spots and provides better resolution. All three grades of paper have been used successfully for most of the separations reported.

Papers usually contain impurities in sufficient amounts to interfere with chromogenic reagents. After development, these impurities are often concentrated near the solvent front,

giving a "curtain" effect. Many authors describe a preliminary washing, usually with water, or the developing solvent, either by dipping several times or washing by upward flow in a chromatographic tank. For satisfactory results is is essential to wash the papers.

Solvent systems—Those reported in the literature for separations of organo-phosphorus esters fall into two groups—

(*a*) Zafforoni-type systems.[9] The paper is impregnated with a polar solvent as stationary phase, then loaded with the sample and developed in a non-polar mobile phase saturated with the stationary phase. Some examples of these systems are given in Table I.

TABLE I

ZAFFORONI-TYPE SOLVENT SYSTEMS

Stationary phase	Mobile phase	Separation achieved
50 per cent. propylene glycol	Skellysolve B - benzene (8 + 2) saturated with propylene glycol	Demeton, phorate,[10,11,12] disulfoton and their metabolites
25 per cent. ethylene glycol	Chloroform - heptane (9 + 1)	Dimethoate and its metabolites[13]
20 per cent. dimethylformamide ..	Iso-octane	11 organophosphorus compounds[14]
10 per cent. formamide		
30 per cent. formamide		

A suitable Zafforoni system can be chosen to resolve most, if not all, mixtures of organophosphorus pesticides and metabolites.

(*b*) Reversed-phase systems. The paper is rendered hydrophobic by impregnation with suitable materials so that the less polar phase is the stationary one and the polar one mobile. These systems are of particular use in separating the weakly polar esters. Table II lists some of the reversed-phase systems reported in the literature on organophosphorus pesticides. The relative R values are for the most part the same in all reversed-phase systems, since they depend primarily on the polarity of the compounds and the systems. There may well be, however, instances in which some of the individual R_F values in certain systems are shifted by the effects of absorption, displacement or hydrogen bonding. It is essential, of course, in reversed-phase systems that all immiscible phases be mutually saturated at the temperature of the experiment.

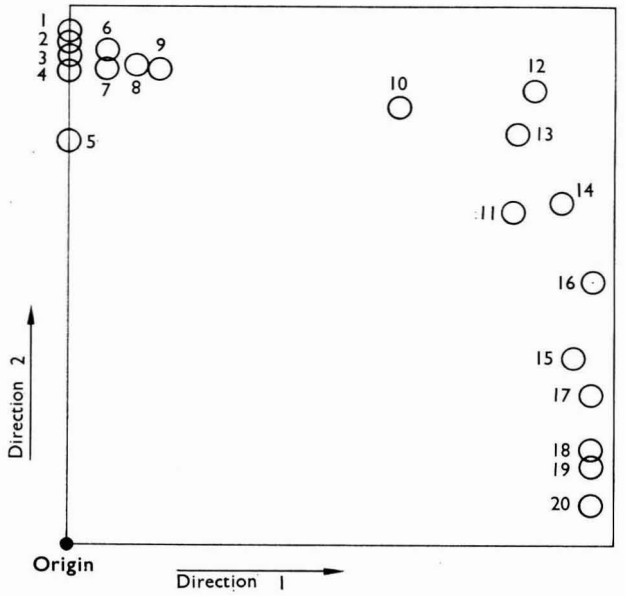
TABLE II

REVERSED-PHASE SYSTEMS

Stationary phase	Mobile phase	Separation achieved
10 per cent. Epotuf E D-1025 or Ucon lubricant	Acetonitrile - water (4 + 6)	Water-soluble metabolites of demeton disulfoton and phorate, especially demeton-*S* sulphoxide and demeton-*S* sulphone[15]
5 per cent. silicone 550	99 per cent. ethanol - chloroform - water (10 + 10 + 6)	Parathion and related esters[16,17]
5 per cent. Carbowax 400	Xylene	Trichlorphon and its metabolites[18]
10 per cent. mineral oil	Dimethylformamide - water (1 + 1)	11 organophosphorus compounds[14]
2 per cent. mineral oil on 100 per cent. acetylated papers	Acetone - water (7 + 3)	19 organophosphorus compounds[19]

Most of the solvent systems listed in Table II were examined. For a general two-directional separation of organophosphorus pesticides with a large range of polarities, the solvent systems chosen were similar to those recommended by Mitchell.[14] The two solvent systems essentially reverse the order of migration of the pesticides. It will be seen from Fig. 2 that identification is most difficult in the group containing the most polar pesticides and metabolites due to poor resolution in this area. To resolve this group it is necessary to run a separate chromatogram with a different Zafforoni-type solvent system. Aliquots of the cleaned-up extract are therefore developed separately in solvent systems (*i*) and (*ii*) by two-dimensional chromatography, and in solvent system (*iii*); see Table III.

Various sources of contamination will interfere with the paper chromatography. The acetone, benzene and hexane must be redistilled before use and care must be taken to avoid using compressed air contaminated with oil for evaporating solvents from samples.[2]



Key

1. Vamidothion sulphoxide
2. Dimethoate
3. Vamidothion
4. Oxydemeton-methyl
5. Menazon
6. Formothion
7. Phosphamidon
8. Mevinphos
9. Azinphos-methyl
10. Azinphos-ethyl
11. Parathion
12. Malathion
13. Mecarbam
14. Fenthion
15. Phorate
16. Diazinon
17. Disulfoton
18. Ethion
19. Trithion
20. Phenkapton

Fig. 2. Diagram of a two-way chromatogram showing the resolution of twenty organophosphorus compounds in solvent systems (*i*) and (*ii*). The origin corresponds to point A in Fig. 3

TABLE III

R_F VALUES FOR ORGANOPHOSPHORUS COMPOUNDS

Solvent system	Stationary phase	Mobile phase
(*i*)	Dimethylformamide, 30 per cent. v/v in acetone	Hexane
(*ii*)	Liquid paraffin, 5 per cent. v/v in diethyl ether	Dimethylformamide - water (1 + 1)
(*iii*)	Formamide, 20 per cent. v/v in acetone	Benzene - chloroform (9 + 1)

Spot number*	Chemical	R_F values calculated from leading edge of spots with solvent systems— (*i*)	(*ii*)	(*iii*)	Colour with 2,6-dibromo-*N*-chloro-*p*-quinonimine
1	Vamidothion sulphoxide ..	0·0	0·97	0·06	Yellow
2	Dimethoate	0·0	0·95	0·78	Orange-brown
3	Vamidothion	0·0	0·95	0·5	Yellow
4	Oxydemeton-methyl ..	0·0	0·90	0·18	Yellow
5	Menazon	0·0	0·78	0·08	Red
6	Formothion	0·09	0·96	—	Red-brown
7	Phosphamidon	0·10	0·92	—	No colour
8	Mevinphos	0·15	0·92	—	No colour
9	Azinphos-methyl	0·18	0·92	—	Orange-brown
10	Azinphos-ethyl	0·63	0·85	—	Orange-brown
11	Parathion	0·82	0·65	—	Red-brown
12	Malathion	0·85	0·85	—	Orange-brown
13	Mecarbam	0·85	0·79	—	Red-brown
14	Fenthion	0·91	0·65	—	Red
15	Phorate	0·96	0·36	—	Orange-brown
16	Diazinon	0·98	0·51	—	Red-brown
17	Disulfoton	0·98	0·29	—	Orange-brown
18	Ethion	0·98	0·20	—	Orange-brown
19	"Trithion"	0·98	0·18	—	Orange-brown
20	Phenkapton	0·98	0·10	—	Yellow-brown

* See Fig. 2.

Amount of sample—The amount of sample used in paper chromatography depends on the purpose of the chromatography, the solubility of the substance in the solvent system used and the ease of detection of the material on the chromatogram. For the chromatography of pure samples of pesticides and metabolites, 5 to 10 μg of material was the most satisfactory range. Small amounts of material permit better separations, but in order to detect minor constituents, as in the chromatography of cleaned-up crop extracts, samples of the extract containing larger amounts of the pesticide are usually necessary. Detection methods for organophorphorus compounds on paper will generally detect 1 μg or less of the compound, so detection is not a limiting factor. Since about 5 μg is the least that can be satisfactorily determined by the wet-digestion method, the crop sample at the 0·5 p.p.m. level can be as small as 10 g. However, it is better to have at least 10 μg of pesticide for the final total-phosphate determination, and a suitable equivalent crop sample at the 0·5 p.p.m. level is 25 g.

Detection—Several spray reagents suitable for detecting organophosphorus esters on chromatograms have been described in the literature. The specificity and sensitivity of five methods of detection were investigated.—

(1) By ultraviolet light. Most of the compounds tested could be located on a chromatogram at the 1-μg level by the dark, quenched areas visible in ultraviolet light. Dimethoate, oxydemeton-methyl and disulfoton could not be detected below 5 μg, and mevinphos, ethion, mecarbam, malathion, formothion and vamidothion required 3 to 5 μg for detection.

(2) 2,6-Dibromo-*N*-chloro-*p*-quinonimine. The colours obtained range from yellow to to red and about 1 μg of most organophosphorus compounds containing sulphur can be detected. In a few instances the colour first produced is quite characteristic; for example, an almost brick-red colour with menazon and a yellow-brown colour with phenkapton. Mevinphos and phosphamidon give no reaction (see Table III).

(3) Blue tetrazolium. Only phosphamidon, vamidothion and its sulphoxide, oxydemeton-methyl, phorate and phenkapton give blue spots in amounts less than 5 μg. Parathion gives a yellow spot due to alkaline hydrolysis to sodium *p*-nitrophenate.

(4) Bromine - 4-methylumbelliferone. Compounds on the chromatogram are detected by first exposing the paper to bromine fumes for 30 seconds and then by spraying with the reagent and observing the paper under ultraviolet light. About 1 μg of most compounds can be detected.

(5) Silver nitrate - bromophenol blue. Only sulphur-containing esters react to give a blue or purple colour and the intensity of the spot varies with the position and number of sulphur atoms in the molecule. Limits of detection are 1 μg for phosphorothiolothionates and 3 μg for phosphorothiolates and phosphorothionates.

In this work, chromatograms of treated crops were always first examined under ultraviolet light, then sprayed with a suitable reagent. Of the reagents described, the blue tetrazolium was generally least useful, but was the best for detecting phosphamidon.

Chromatography of pure compounds—Chromatograms were developed for each compound in three solvent systems, and the R_F values obtained are given in Table III. The results obtained for standards were used as a guide in the analyses of crop extracts, but not for positive identification. Variations in temperature, the nature and concentration of the components from the crop extract and other factors influence the mobilities on paper, and it is essential to include suitable standards on each chromatogram. Within certain limits, which will be obvious on studying Fig. 2, good separations of up to 12 organophosphorus pesticides in mixtures can be achieved with two-dimensional chromatography.

DETERMINATION OF PHOSPHORUS—

Quantitative determination of organophosphorus compounds on paper chromatograms was achieved by cutting out the spot after detection, wet digesting it to orthophosphoric acid, which was then determined spectrophotometrically as the molybdenum-blue complex. The wet-digestion technique used was that described in the Report of the Joint Demeton-methyl Panel[20] with the final colorimetric procedure as modified by Caverly and Hall.[21] Oxygen-flask combustion of the spot from the chromatogram was also used successfully to convert the pesticide to orthophosphate before the colorimetric determination.

METHOD

APPARATUS—

Macerator—A top-drive macerator.

Cooling flask—Thermos flask, wide-mouthed, 2-pint capacity. Part fill this flask with a mixture of solid carbon dioxide and acetone to maintain a bath temperature of −80° C.

Freezing apparatus—See Fig. 1.

Chromatography equipment—Aimer "Universal" outfit based on 12 × 12 × 12-inch tank taking papers 10 inches square. Whatman papers for chromatography, grades 1, 3 MM and 20. Wash the paper in continuously changing warm solvent, (*a*) for 6 hours with hexane then, (*b*) for 6 hours with a 50 + 50 mixture of acetone and water. A liquid - liquid downwards-displacement extractor was used for this purpose.

Absorption spectrophotometer—Unicam SP500, or similar instrument, with 1-cm matched glass cells.

Ultraviolet lamp—Hanovia Chromatolite, or equivalent.

Rotary film evaporator

Liquid - liquid extraction apparatus—A downwards-displacement type.

GENERAL REAGENTS—

Acetone—Analytical-reagent grade. Redistil the solvent through a short column of glass helices before use.

Benzene—Analytical-reagent grade. Redistil the solvent through a short column of glass helices before use.

Chloroform—Analytical-reagent grade.

Hexane—Standard hexane, boiling-range, 66·5° to 70·5° C (obtainable from Carless, Capel and Leonard Ltd., Hope Chemical Works, Hackney Wick, London, E.9). Redistil the hexane through a short column of glass helices before use.

Carbon—Nuchar carbon (obtainable from Eastman Kodak).

Magnesium oxide—Chromatographic grade (obtainable from the British Drug Houses Ltd.).

Powdered cellulose—Whatman, standard grade.

Sodium sulphate, anhydrous—Analytical-reagent grade.

Other reagents required for the determination of phosphorus by the spectrophotometric measurement of the molybdenum-blue complex are as described by Laws and Webley[5] and Caverly and Hall.[21]

DEVELOPING SOLVENTS—

For the two-way development of non-polar compounds, the developing solvents required are—

Zafforoni system, stationary phase—Dimethylformamide, 30 per cent. v/v solution of laboratory-reagent grade material in acetone.

Zafforoni system, mobile phase—Hexane.

Reversed-phase system, stationary phase—Liquid paraffin, 5 per cent. v/v solution of B.P. grade material in diethyl ether.

Reversed-phase system, mobile phase—Dimethylformamide - water (1 + 1) mixture.

The Zafforoni system for developing polar compounds requires—

Stationary phase—Formamide, 20 per cent. v/v solution of laboratory-reagent grade material in acetone.

Mobile phase—Benzene - chloroform (9 + 1) mixture.

SPRAY SOLUTIONS—

2,6-*Dibromo*-N-*chloro*-p-*quinonimine*—Prepare a 0·5 per cent. w/v solution of the compound in cyclohexane. Renew the solution at weekly intervals.

Blue tetrazolium—Prepare a 0·1 per cent. w/v solution of blue tetrazolium [3,3′-dianisole-bis-4,4′-(2,5-diphenyl)tetrazolium chloride] in water. Mix 1 volume of this with 9 volumes of 2 N sodium hydroxide just before spraying.

4-*Methylumbelliferone*—Dissolve 0·15 g of 4-methylumbelliferone in 100 ml of ethanol and dilute to 200 ml with water. Add 10 ml of 0·1 N ammonia solution.

Silver nitrate - bromophenol blue mixture—Prepare a 2 per cent. w/v solution of silver nitrate in water. Mix 90 ml of this with 10 ml of a 0·4 per cent. w/v solution of bromophenol blue in acetone just before spraying.

Citric acid—Prepare a 0·01 per cent. solution of citric acid in water.

STANDARD SOLUTIONS—

Prepare solutions in acetone containing 100 μg per ml from the available samples of the pesticides and metabolites (these are generally more than 90 per cent. pure). Take aliquots of these as required.

EXTRACTION PROCEDURE—

Macerate a 100-g sub-sample of the crop with 200 ml of acetone for 5 minutes (for recovery experiments, aliquots of standard solutions of pesticides are added to the crop sample before solvent addition and mixed for 1 minute). Set the mixture aside for 15 minutes. Filter the mixture by suction through nylon cloth on a Buchner funnel or through a No. 541 Whatman filter-paper on a Hartley-pattern three-piece Buchner funnel. If the first portion is turbid, filter again.

Rinse the macerator jar with three 50-ml portions of acetone and use the rinsings to wash the solid on the filter-pad. Transfer the filtrate to a 1-litre separating funnel and add 300 ml of water.

Extract the filtrate with five successive portions of chloroform (100 ml for the first three and 50 ml for each subsequent extraction). Shake the funnel for approximately 30 seconds for each extraction, but only gently during the first extraction so as to avoid the formation of stable emulsions. Wash the combined chloroform extracts with 200 ml of water. Separate the water, wash it with two 25-ml portions of fresh chloroform, and add the washings to the main chloroform extract.

Dry the chloroform extract over anhydrous sodium sulphate, filter and distil it to a small volume in a rotary film evaporator. Transfer the residual solution to a beaker and remove the last traces of solvent on a steam-bath in a current of air.

FREEZING PROCEDURE—

Prepare a thin layer of cellulose powder on the sintered-glass filter-disc of the apparatus in Fig. 1 by using a slurry of the powder in acetone. Transfer the residue from the extract to the flask A with 2 ml of acetone. Wash the beaker twice with 1 ml of acetone and add the washings to the extract in flask A. Place the apparatus in the cooling-bath at −80° C. After 30 minutes apply a vacuum to the side-arm with the stopper B in place. The extract is then filtered into C. Remove the vacuum tube, stopper B and wash the flask with 1 ml of acetone. When the solvent has cooled to the bath temperature wash the precipitate on the filter-pad by replacing stopper B and again applying vacuum. Repeat the washing procedure once more. Remove the apparatus from the cold bath, and transfer the filtrate carefully to a small beaker, wash out the flask C twice with acetone, add the washings to the beaker and evaporate the solution almost to dryness using a stream of air. Dissolve the residue in 3 ml of benzene - chloroform (1 + 1) mixture, and transfer the solution to a 10-ml stoppered measuring cylinder. Wash the beaker several times with 1 ml of the same solvent mixture and dilute the solution to 10 ml.

COLUMN CLEAN-UP PROCEDURE—

Non-polar compounds—Prepare a column, 1·5 cm in diameter, from a slurry of 0·5 g of Nuchar carbon, 2·0 g of magnesium oxide and 1·5 g of powdered cellulose in benzene - chloroform (1 + 1) mixture. Wash the column with 50 ml of the same solvent and discard the washings. Add 5 ml of the extract to the top of the column, and wash the column with the same solvent until 150 ml of eluate are collected. Evaporate the eluate nearly to dryness on a water-bath; use a stream of air to assist evaporation. Make up to 10 ml with chloroform in a stoppered measuring cylinder and take aliquots for paper chromatography.

Polar compounds—Prepare a column, 1·5 cm in diameter, from a slurry of 3 g of magnesium oxide in chloroform. Wash the column with 50 ml of chloroform and discard the washings. Add 5 ml of the extract to the top of the column, and wash the column with chloroform until 150 ml of eluate are collected. Evaporate the eluate nearly to dryness as for non-polar compounds. Make up to 10 ml with chloroform in a stoppered measuring

cylinder and take aliquots for paper chromatography. After chloroform elution, wash the column with 50 ml of acetone. Discard the acetone elutate. Wash the column with methanol until 100 ml are collected. Evaporate nearly to dryness as before, and transfer the residue to a 10-ml measuring cylinder with small portions of chloroform. Take aliquots for paper chromatography. Run both the chloroform and methanol eluates in solvent system (*iii*) as described below.

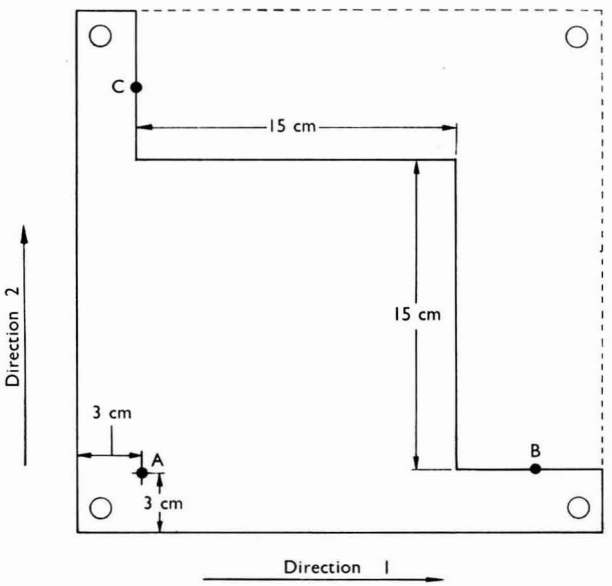


Fig. 3. Diagram of template for two-dimensional chromatogram on 10 × 10-inch paper with solvent systems (*i*) and (*ii*)

PAPER-CHROMATOGRAPHIC PROCEDURE—

Non-polar compounds—Evaporate a 5-ml aliquot of the cleaned extract to a small volume and transfer it to the point A of a Whatman 10 × 10-inch paper, prepared according to the template shown in Fig. 3, by using a disposable glass capillary. The spot should not exceed 5 mm in diameter. Include suitable marker spots at points B and C. When the spots are dry, dip the paper in a 30 per cent. v/v solution of dimethylformamide in acetone. Allow the acetone to evaporate, then develop the paper in direction 1 with hexane as the mobile solvent until the solvent front on the paper has risen to the boundary line 15 cm above the base-line. Remove the paper and dry it for 30 minutes at room temperature. Dip the paper in a 5 per cent. v/v solution of liquid paraffin in ethyl ether and allow the ether to evaporate. Develop the paper in water - dimethylformamide (1 + 1) mixture in direction 2, at 90° to the first direction, until the solvent front has risen to the other boundary. Dry the paper in an oven at 50° C. Run at least two papers with aliquots of the extract so that more than one spray reagent can be used if necessary. If more than two papers are required then smaller aliquots can be taken.

Polar compounds—Prepare the paper as described for the non-polar compounds. Allow the spots to dry at room temperature and then dip the paper in a 20 per cent. v/v solution of formamide in acetone. Allow the acetone to evaporate at room temperature and develop the paper with hexane. Remove the paper, allow the hexane to evaporate and repeat the development with hexane once more. After the hexane has evaporated turn the paper and develop at 90° to the first direction in a benzene - chloroform (9 + 1) mixture. Dry the paper in an oven at 50° C. Run at least two papers as before.

SPRAYING PROCEDURE—

First examine the paper in ultraviolet light and mark lightly in pencil any dark quenched areas. Then spray with one of the reagents given below—

2,6-*Dibromo*-N-*chloro*-p-*quinonimine*—Spray the paper lightly with a 15 per cent. v/v solution of formamide in acetone, then spray both sides of the paper with the reagent. Heat the paper in an oven at 120° C for 10 minutes.

Blue tetrazolium—Spray the paper lightly and dry it in an oven at 50° C for 5 minutes.

Bromine - 4-methylumbelliferone—Expose the paper to bromine fumes for 30 seconds, spray with the reagent and observe under ultraviolet light.

Silver nitrate - bromophenol blue—Spray the paper with the reagent and heat in an oven at 50° C for 10 to 15 minutes. Immerse the paper in 0·01 per cent. citric acid solution, wash it once in water and dry.

DETERMINATION OF PHOSPHORUS—

Wet digestion—Cut out an area of the chromatogram that encloses the coloured spot indicating the presence of an organophosphorus compound and place this in a 100-ml conical flask. Add 10 ml of water, 4 ml of N perchloric acid, 2 ml of concentrated nitric acid and 0·5 ml of concentrated hydrochloric acid. Heat very carefully until the paper has completely disintegrated and then heat until dense white fumes are evolved. The remainder of the procedure is described elsewhere.[20,21]

Oxygen-flask combustion—Mark a square of approximately 1 square inch in area on the sprayed chromatogram to enclose the coloured spot indicating the presence of an organophosphorus compound. Cut this area out of the chromatogram, allowing a suitable narrow length of paper to act as a fuse. Fold the paper in half and, with the fuse protruding, fix in the dry platinum holder of the combustion apparatus. Charge the separator with 10 ml of N sulphuric acid as absorbing solution and flush with a fast flow of oxygen for 30 seconds. Ignite the fuse in a flame and immediately insert the stopper into the separator. Simultaneously invert the separator so that the absorbing solution forms a seal around the stopper. Hold the stopper and flask firmly together during the combustion, which lasts a few seconds.

Set the separator aside for 2 minutes, then shake it vigorously for 1 minute to ensure complete absorption. Raise the stopper and rinse the joint and platinum holder with water. Drain off the absorbing solution and washings into a 100-ml conical flask. Wash the separator twice with small amounts of water and run the washings into the conical flask.

Evaporate the absorbing solution washings on a hot plate until only about 3 ml remain, add 3 ml of concentrated ammonia solution and boil the solution to remove any excess of ammonia. Allow the solution to cool, then determine phosphorus as described by Caverly and Hall.[21]

RESULTS

CLEAN-UP—

In a general technique the extraction procedure must be exhaustive, since it is required to extract compounds with widely different solubility characteristics. Unfortunately, such a procedure will also yield a large amount of plant extractives and this places a bigger load on the clean-up. The efficiency of the over-all clean-up procedure was checked by weighing the extractives before and after clean-up. Table IV gives the percentage removal of extractives for several crops.

TABLE IV

PERCENTAGE REMOVAL OF EXTRACTIVES IN CLEAN-UP PROCEDURE

Crop	Weight of extractives, mg, from 100 g of crop,	Removal of extractives, per cent.
Apple	62, 70	97, 98
Bean, runner	31, 32	94, 95
Bean, French	45	87
Brussels sprout tops	68	94
Cabbage (outer leaves)	80	94
Lettuce	40	90
Potato	27, 28	96, 95
Tomato	37	88

Since the weight and nature of extractives from each crop will vary to some extent with the variety of the crop, the maturity and the sampling technique, the figures in Table IV can only be taken as a general indication of the extractives obtained and the clean-up achieved.

As stated earlier, a suitable equivalent crop sample for the final phosphate determination is 25 g if the residue is at the 0·5 p.p.m. level. From Table IV it can be calculated that interfering material remaining in a 25-g sample of cabbage after clean-up can be as much as 1·2 mg. This is equivalent to a ratio of pesticide to interfering material of 1 to 100, if the crop originally contained 0·5 p.p.m.

RECOVERIES AND BLANK VALUES—

There are few published figures on the recoveries obtained with the wet-digestion method of Laws and Webley as modified by Caverly and Hall. Recovery experiments were carried out to check the accuracy of the techniques used at various stages. The mean reagent blank value for 42 determinations was 0·31 μg of phosphorus with a standard deviation of 0·17 μg and a range of 0·04 to 0·66 μg. Batches of reagents tested for the analytical procedure and giving a blank value higher than 0·5 μg phosphorus were unacceptable. The mean reagent blank value for six determinations by the oxygen-flask combustion method was 0·21 μg of phosphorus with a standard deviation of 0·08 μg and a range of 0·10 to 0·31 μg.

Recovery of phosphate—The mean recovery of aliquots of phosphate (1 μg to 10 μg of phosphorus as sodium dihydrogen orthophosphate) added before the wet-digestion stage was 95·5 per cent. with a standard deviation 1·93 from 20 determinations and a range of 91·5 to 98·5 per cent.

Recoveries of pure pesticides—Mean recoveries of pure dimethoate and menazon, in the range 10 μg to 80 μg, added before the wet-digestion stage are given in Table V, pesticides 1 and 2.

Recoveries of pure pesticides after paper chromatography—Aliquots of standard pesticides solutions, in the range 10 μg to 80 μg of pesticide, were spotted on to Whatman No. 20 paper and developed in the solvent systems—

Dimethoate and menazon .. .. Solvent system (*ii*)
Azinphos-methyl and parathion .. .. .. Solvent system (*i*)

The R_F values are given in Table III. After the spots had been sprayed, they were cut out and wet digested as described; results are given in Table V, pesticides 3 to 6 inclusive, and are corrected for paper blank values, which include all reagents.

TABLE V

RECOVERIES OF PURE PESTICIDES

	Pesticide	Mean recovery, per cent.	Number of determinations	Standard deviation	Range, per cent.
1	Dimethoate*	93	5	1·1	92 to 94
2	Menazon*	87	15	6·2	77 to 98
3	Azinphos-methyl†	82	7	3·3	79 to 88
4	Dimethoate†	91	8	4·6	84 to 99
5	Menazon†	81	8	6·4	71 to 91
6	Parathion†	97	6	2·0	91 to 101
7	Parathion‡	91	7	6·0	83 to 102

* Pure pesticides added to the digestion stage.
† After paper chromatography and by wet digestion of the pesticide spot on paper.
‡ Applied to chromatographic paper immediately before oxygen-flask combustion.

For paper blank values, 1-inch square pieces of paper were taken from various parts of a Whatman No. 20 chromatography paper, run in the above solvents and sprayed with 2,6-dibromo-*N*-chloro-*p*-quinonimine. The mean blank value of ten determinations by wet digestion was 0·49 μg of phosphorus with a standard deviation of 0·1 μg. The mean paper blank value of six determinations by oxygen-flask combustion was 0·35 μg of phosphorus with a standard deviation of 0·05 μg and a range of 0·30 to 0·43 μg. These include reagent blank values. The over-all mean recovery for the 56 determinations was 89 per cent. with a standard deviation of the means of 5·7 and a range of 71 to 102 per cent.

Recoveries of pesticides added to crops—Aliquots of standard solutions of pesticides were added to crop samples so that the concentration of pesticide in the crop was either 0·5 p.p.m. or 1·0 p.p.m. This range was considered to be the important one to the pharmacologist in assessing the significance of residues of most of these compounds in foodstuffs. The crop samples were processed and the final spots on the chromatogram were cut out and phosphorus determinations carried out as described earlier. The blank-value determinations were carried out on corresponding areas of papers run after an untreated cleaned-up crop extract had been spotted on to the paper. Owing to difference in R_F values of the pesticides and the variation in interference from crop extractives in various parts of the chromatogram, the blank values may be different for all combinations of crop and pesticide. These crop blank values were usually slightly higher than the ordinary paper blank values.

The recoveries of some pesticides added to various crops are given in Table VI. The over-all average recovery is approximately 70 per cent. and it is suggested that this figure be used as the correction factor for all determinations made by this general method.

TABLE VI

PERCENTAGE RECOVERIES OF PESTICIDES ADDED TO CROPS

Crop	Pesticide	Recovery, per cent.
Apples	Azinphos-methyl	70, 68
	Parathion	74
	Dimethoate	64
	Malathion	50
	Vamidothion*	90
Tomato	Dimethoate	80, 64
	Parathion	66, 74
	Azinphos-methyl	89
Potato	Menazon†	60, 65, 57
	Dimethoate	60, 61
	Disulfoton*	72
	Vamidothion	64, 73, 74
Cabbage	Disulfoton*	70, 65
Spring greens	Vamidothion	60
Lettuce	Dimethoate	67, 69
Peas	Parathion	88
Cucumber	Azinphos-methyl	68, 71
	Dimethoate	68, 69

* Total recoveries representing the parent pesticide *plus* metabolites formed during the procedure.

† Chloroform eluate only.

DISCUSSION

The object of this paper is to provide a robust method for detecting and determining organophosphorus compounds in harvested crops following the application of organophosphorus pesticides. In selecting the recommended techniques, attention has been paid to the need for simple, inexpensive apparatus and readily available chemicals of the required analytical standards. The general method can also be readily made specific for any of the compounds mentioned. Recoveries of pesticides, added to macerated crop samples were not always as high as might be required by the research analyst, but the average recovery of approximately 70 per cent. is adequate for a screening method.

In assessing the hazard of residues to the consumer of treated crops it is not considered necessary to differentiate accurately between residues of, say, 0·1 and 0·2 p.p.m. of an organophosphorus compound. Exaggerated mathematical accuracy is not in keeping with the aims of residue analysis, and it should always be borne in mind that residue analysis is carried out against a very variable biological background. However, it is desirable to be able to detect these compounds in a concentration as low as 0·1 p.p.m. and to differentiate with confidence between, say, 0·1, 0·5 and 1·0 p.p.m.

Of the 26 organophosphorus pesticides that are commercially available in this country, mevinphos, dichlorvos and trichlorphon are not included in the scheme of analysis. This is because residues resulting from their use rarely persist more than 48 hours and so these compounds are not likely to offer a consumer hazard. Demeton, dimefox, schradan, sulfotep

and TEPP have also been left out because their use has declined to such an extent that they are not greatly employed. In applying this scheme of analysis to a crop, it is a great advantage to be familiar with the relevant spraying practice. For example, in the United Kingdom phenkapton is used on apples only. Phorate and disulfoton, which are only available as granular formulations, are not used on either soft or top fruit. Knowledge of such limitations can obviously be of use in the interpretation of any doubtful identifications of compounds with similar R_F values on chromatograms.

Demeton-methyl, disulfoton, phorate and thiometon form a group of systemic organophosphorus pesticides that follow a substantially similar pattern of metabolism in the plant.[4,5] The parent non-polar compounds are fairly stable outside the plant and can be characterised by two-dimensional paper chromatography of the extract. All the toxic metabolites, however, are polar, and their resolution is difficult. In solvent system (*iii*), three metabolites of disulfoton run very close to the solvent front and can be determined together. If separation is required, then a less polar mobile phase such as hexane - benzene (6 + 4) mixture with the same stationary phase will resolve them. Since reference standards were not available for most of these metabolites, positive identification was not possible. Total recoveries of metabolites were made by adding the recoveries of phosphorus in the several spots usually obtained on the paper chromatograms. In a general screening method, however, it will probably be impossible from the chromatogram alone to determine with any degree of certainty which of this group of pesticides was originally used. In particular, since the final residue from demeton-methyl, oxydemeton-methyl and thiometon will be demeton-*S*-methyl sulphone, there is no way of identifying the original pesticide. The polar vamidothion also forms a sulphoxide and this can be separated by solvent system (*iii*).

Another organophosphorus insecticide unlikely to be detected in crops is formothion, since it is rapidly metabolised in the plant to dimethoate. A detected residue of dimethoate does not indicate therefore whether formothion or dimethoate was the pesticide applied to the crop. The *O*-analogue of dimethoate is not usually found in amounts greater than 10 per cent. of the total residue in crops following the application of formothion or dimethoate, and is not determined separately by the procedure described here.

Chloroform elutes most of the organophosphorus compounds quantitatively from the magnesium oxide column. Menazon is only partly recovered, the remainder being in the acetone wash, which generally contains sufficient plant materials to interfere with paper chromatography. The recovery of very polar metabolites such as oxydemeton-methyl are sometimes difficult. With a more active magnesium oxide these cannot be recovered by chloroform elution, but can be eluted with methanol.[22] In instances of doubt, a paper chromatogram of the methanol eluate should also be run in solvent system (*iii*).

The recoveries in Tables V and VI to a certain extent reflect the variation in the purity of the samples of "pure" pesticides available for this work. Only parathion was synthesised and purified to 99 per cent. in this Laboratory. The others, collected from various sources, were not purified further. It was known that some of the samples used would deteriorate even when stored in a refrigerator. Thus, a sample of vamidothion sulphoxide, when examined chromatographically on Whatman No. 7 paper with formamide (20 per cent. v/v in acetone) as stationary phase and benzene - chloroform (6 + 4) mixture as mobile phase, showed five minor components, one of which was the parent vamidothion, in addition to the major component. It was assumed that workers, who might find a use for the screening technique described in this paper, would also use the same quality of reference materials, usually 90 per cent. or more and would not wish to purify them further.

Lower limit of determination—

The lower limit of determination of a method of analysis can be defined satisfactorily in terms of the standard deviation of the blank value and the probability of detecting amounts of pesticide residue.[23] This limit can be set as $2s/\sqrt{n}$, where s is the standard deviation of the blank value and n is the number of replicate analyses carried out on the treated sample. If $n = 2$, as is often so, then the lower limit of determination becomes $\sqrt{2}\,s$ or approximately $1{\cdot}5s$. By using the wet digestion the mean paper blank value was 0·44 μg of phosphorus with a standard deviation of 0·1 μg. The lower limit of determination can be calculated, therefore, as 0·15 μg of phosphorus, or approximately 1·5 μg of an organophosphorus pesticide (the percentage of phosphorus in organophosphorus pesticides varies from 8·2 per cent. for phenkapton to 13·5 per cent. for dimethoate).

The standard deviation of the blank value found by oxygen-flask combustion is 0·05 μg of phosphorus, and the lower limit of determination can be calculated as 0·075 μg of phosphorus, equivalent to approximately 0·75 μg of an organophosphorus pesticide.

Most of the methods used for detecting organophosphorus compounds on paper chromatograms will detect 1 μg of the pure compound. However, detection of residues in a crop extract is more difficult and up to 5 μg is necessary for some pesticides. This is considerably more than the lower limit of determination calculated as above and the method is considered to be capable of determining organophosphorus pesticides at 0·05 p.p.m. in the crops tested.

The time taken from start to finish on a single analysis, including all the steps described in this paper, would be about 20 hours. However, the techniques are such that, provided the equipment and staff are available, 20 analyses could equally well be carried out in the time taken to carry out a single analysis.

I thank Miss P. A. Sanders for careful skilled experimentation in obtaining the results given in this paper.

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A Simple Concentration-cell Technique for Determining Small Amounts of Halide Ions and Its Use in the Determination of Residues of Organochlorine Pesticides

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Quick, simple and sensitive methods for determining small amounts of chloride and bromide ions are described. They are based on the direct measurement of the e.m.f. of a concentration cell with electrodes consisting of thin silver rods that are partially submerged in a suspension, prepared *in situ*, of the corresponding silver halide in dilute acid. These electrodes have the advantages of easy preparation, photostability and comparative immunity from poisoning.

The use of a similar cell for determining iodide ion is discussed.

The application of the method for chloride, after oxygen-flask combustion, to the determination of residues of organochlorine insecticides in extracts of animal and plant tissue is described in an Appendix.

The direct potentiometric determination of chloride[1,2,3,4] and bromide[5] has been described by a number of authors. This paper describes simple methods for determining chloride and bromide ions in the approximate concentration range 10^{-8} to 10^{-4} g per ml. The high sensitivity and improved accuracy at low concentrations are achieved by an empirical approach and by the use of an unusual type of electrode.

In all direct potentiometric methods, the indicator is a silver halide electrode, with either a silver - silver halide (in dilute hydrochloric acid), a mercury - mercurous sulphate or a calomel electrode used as a reference. The use of mercury reference electrodes introduces several complications in the determination of halides at very low concentrations, owing to a significant junction potential and appreciable measurement or temperature errors that can occur due to the high e.m.f. of the cell. These errors can be minimised by using a silver - silver halide electrode in a solution containing very little halide as the reference electrode, by temperature control and by the addition of a constant excess of a suitable electrolyte to all solutions. Careful calibration is necessary, since the response, in terms of the e.m.f. of the cell, is no longer logarithmic, due to the effect of dissolved silver halide and traces of halide ion in reagents becoming increasingly important.

Conventional silver - silver halide electrodes obtained by electro-deposition are not easy to prepare and have a limited life. They are photosensitive and easily poisoned by oxidising or reducing agents and by ions forming complex or sparingly soluble silver salts.[6] It was thought likely that the electrode potential of the silver - silver halide electrode is governed by the concentration of the silver cation in the layer of solution in immediate contact with the surface of the metal. This layer, having percolated through a finely divided deposit of silver halide, is saturated with that salt. This being so, the saturation of the whole solution with a silver halide should be an alternative to coating silver metal with that halide. Addition of a prepared silver chloride suspension has, in fact, been reported by Naumann,[7] whose results show that silver wire stored in dilute hydrochloric acid behaved as a silver - silver chloride electrode in the presence of sufficient amounts of chloride ion, but below approximately 35 p.p.m. the presence of a silver chloride suspension was necessary. In the work described in this paper, the suspension was prepared *in situ* to ensure instantaneous saturation. This procedure was found to have several other advantages. Equilibrium is quickly established in the presence of other ions that form very sparingly soluble silver salts and apparently also in the presence of some oxidising agents, so that halides can be determined in the presence of very small, known amounts of such interfering ions. The "suspension" type of electrode is photostable and is less easily poisoned than the conventional type. When poisoned, it can be easily restored to normal condition. A disadvantage of this type of electrode is the effect of "ageing" of the suspension on the electrode potential. This effect is largely compensated for by the experimental procedure as described below.

METHOD

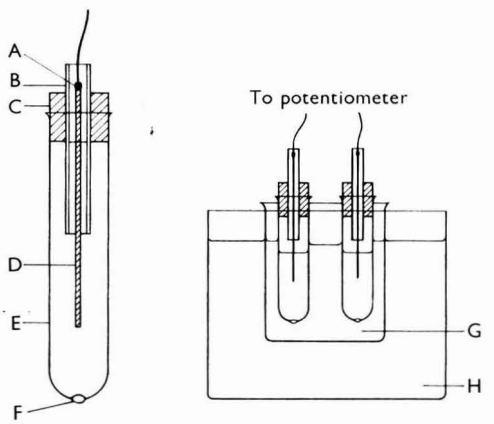
APPARATUS—

Potentiometer—A high-input-impedance instrument with the most sensitive range giving full-scale deflection at potentials of 100 mV or less, and capable of being read to the nearest 0·2 mV. The direct-reading E.I.L. (Electronic Instruments Ltd.) Vibron Electrometer model 33B is suitable.

Thermostatic bath—Water-bath with a working range of 20° to 30° C, controlled to ±0·5° C.

Electrode compartments—Seal a soft-glass bead into the base of a small Pyrex-glass test-tube[8] (see Fig. 1*a*) and calibrate the compartment to hold 5 ml of solution. Test it by filling with water. Reject it if there is no leak or if the leak exceeds one small drop per minute.

Electrodes—Solder a 2-inch length of a pure silver wire or rod, 1 to 2 mm in diameter, to an insulated copper lead. Fix the electrode as shown in Fig. 1(*a*), so that approximately 15 mm is below the 5-ml mark when inserted into the electrode compartment; use short pieces of thin insulated sleeving as wedges to hold the silver rod firmly in the glass tube.



(*a*) (*b*)

A=Soldered joint
B=Glass tube
C=Cork
D=Silver rod
E=Pyrex-glass tube
F=Soft glass bead
G=Beaker containing dilute sulphuric acid
H=Water-bath at 25° C

Fig. 1. Diagram showing (*a*) electrode compartment assembly and (*b*) electrodes in position

New electrodes and electrodes that have not been used for a long time, or have acquired an excessive asymmetry potential should be cleaned with a fine abrasive powder, washed well with water and placed in a suspension of the silver halide in 0·25 M sulphuric acid for 20 minutes.

Between determinations store the electrodes in a suspension of the silver halide in 0·25 M sulphuric acid.

Switches—One or more single-throw switches, according to the number of electrode pairs used.

Syringe—A 50-μl syringe for dispensing ammoniacal silver bromide.

Electrode-compartment holder—Two Terry clips fixed 1½ inches apart to a frame that can be adjusted vertically in a clamp.

REAGENTS—

In order to eliminate variations in halide content, use the same batch of distilled or de-mineralised water and sulphuric acid for preparing all standard solutions and for dissolving or diluting samples.

Sulphuric acid, 0·5 M and 0·25 M.

Standard solutions—A range of solutions containing suitable amounts of sodium chloride or potassium bromide in 0·25 M sulphuric acid.

Silver chloride solution—Dissolve 0·56 g of freshly prepared, well washed silver chloride in 4 ml of ammonia solution, sp.gr. 0·88, and make up to 50 ml with water. Store the solution in the dark. Dilute 1 ml with 3 ml of water before use.

Silver bromide solution—Dissolve 0·077 g of well washed silver bromide in 25 ml of ammonia solution, sp.gr. 0·88. Store the solution in the dark. Dilute 1 ml with 2 ml of water before use.

TRANSFER OF ELECTRODES AND SOLUTIONS—

When transferring electrodes from one suspension to another, first remove surplus liquid by touching the tip of the electrode with a small piece of filter-paper. Dip the electrode several times in a suspension of the silver halide in the reference solution (in a test-tube fixed in the water-bath and set aside for the purpose), removing the surplus drop of liquid each time. Leave the electrode in this suspension while refilling the compartment. Return the electrode to the compartment in a similar way, and stir the new suspension with the electrode for a few seconds before pushing the cork into position.

When replacing solutions in electrode compartments, dry the outside of the compartment with a clean cloth, pour out the contents, drain for a few seconds and remove the liquid remaining on inside walls with a clean strip of filter-paper. If the next solution is likely to contain much less halide, use it for washing the compartment twice with small portions, removing surplus liquid each time. Add fresh solution to the 5-ml mark and inject 50 μl of silver halide solution. Shake the tube for a few seconds, replace in the electrode-compartment holder and lower into the bridging beaker.

CALIBRATION—

Place the standard solution and the bridging beaker containing approximately 0·25 M sulphuric acid, in the water-bath at 25° C. Wash and drain both electrode compartments. Put 5 ml of the reference solution in each compartment and then add either 1 drop of the silver chloride solution when chloride is to be determined, or exactly 50 μl of the silver bromide solution when bromide is to be determined. Place electrodes and compartments in position and allow to equilibrate for 30 minutes. Replace the solution in the indicator compartment in turn by the reference solution and a range of suitable standard solutions, adding silver halide each time, and measure the e.m.f. of the cell 1 minute after the electrodes have been inserted when chloride is to be determined or 3 minutes when bromide is to be determined. Subtract the reading obtained with fresh reference solution from each of the other readings, and plot a graph of the difference against the concentration of added halide. For chloride contents above 5 p.p.m. or bromide contents above 1 p.p.m., when high accuracy is not required, draw a straight line between two suitably spaced points plotted with the deflection on a linear axis and concentration on a logarithmic axis. Above approximately 20 p.p.m. of chloride or 4 p.p.m. of bromide, use only the latter procedure.

One reference solution can be used for obtaining a calibration curve for the whole range. This solution should contain no added halide if some samples are likely to contain very little of the halide ion. Otherwise it is better to use a reference solution containing some added halide.

For more accurate results, different calibration curves should be obtained for different ranges of halide concentration and the respective reference solutions used in the determination. Thus, to calibrate for 0 to 2 p.p.m. of chloride and 0 to 0·4 p.p.m. of bromide, use a reference solution containing no added halide; for between approximately 2 and 20 p.p.m. of chloride or 0·4 and 4 p.p.m. of bromide, use solutions containing 5 p.p.m. of chloride or 1 p.p.m. of bromide respectively; above these limits, use solutions containing 50 p.p.m. of chloride or 10 p.p.m. of bromide. If the range of concentration of halide in samples is wide, two or more reference compartments, each containing an electrode in a different solution, can be inserted in the same bridging beaker, and readings taken consecutively. In this instance, separate calibration curves should be obtained allowing appropriate time intervals between the insertion of the indicator electrode and the measurement of the e.m.f.

DETERMINATION OF HALIDE—

Dissolve the sample in 0·25 M sulphuric acid or, if it is an aqueous solution, add an exactly equal volume of 0·5 M acid. Dilute the solution with 0·25 M acid as necessary. Place the diluted samples and reference solution in the water-bath at 25° C some time before the determination.

Allow the electrodes to come to equilibrium as for calibration. Replace the solution in the indicator compartment by a fresh reference solution, and measure the asymmetry potential of the electrodes after 1 or 3 minutes. Replace the solution in the indicator compartment by sample solutions, measuring the deflection 1 minute after the electrode had been inserted when chloride is to be determined and 3 minutes when bromide is to be determined. Read the halide concentration from the calibration curve after subtracting the asymmetry potential. (If many determinations are being carried out, check periodically for a slow change in asymmetry potential by using fresh reference solution in the indicator compartment.)

Several substances interfere with the determination of halides. They include organic solvents, soluble silver salts, ions forming sparingly soluble silver salts, and oxidising, reducing and complexing agents. These substances should be removed, if possible, by boiling or a simple chemical treatment. When the interfering substance does not produce a drift in the e.m.f. of the cell exceeding 1 mV per minute, and its amount is known, a special calibration procedure can be used. This is, however, extremely time consuming and not practicable, unless the amount of the interfering substance is constant for many samples.

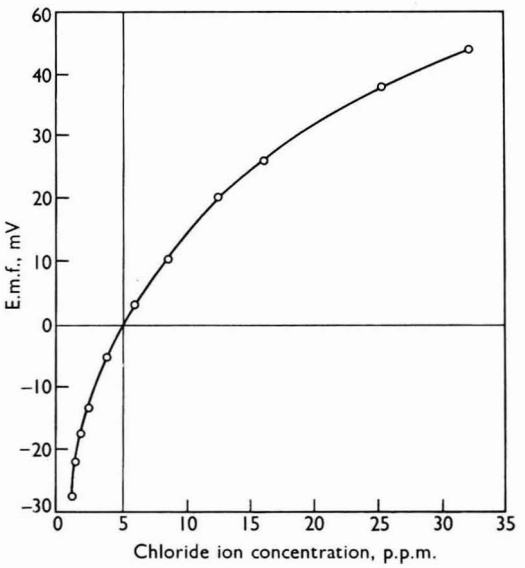


Fig. 2. Calibration curve for chloride obtained by using a reference solution containing 5 p.p.m. of added chloride ion. The e.m.f. of the cell is expressed as the potential of the reference electrode measured against that of the indicator electrode

Experimental

Calibration curves for chloride and bromide were obtained by the procedures described above. Fig. 2 shows the calibration curve for chloride obtained with a reference solution containing 5 p.p.m. of added chloride ion, and Fig. 3 the curve for bromide with a reference solution containing 1 p.p.m. of bromide ion.

Effect of ionic strength—

The effect of ionic strength on electrode potential was tested by measuring the e.m.f. of cells containing equal amounts of chloride, in M sulphuric acid in one compartment and in 0·25 M sulphuric acid in the other compartment. The e.m.f. for 0·5, 2·0 and 5·0 p.p.m. of chloride was 40, 46 and 49 mV, respectively.

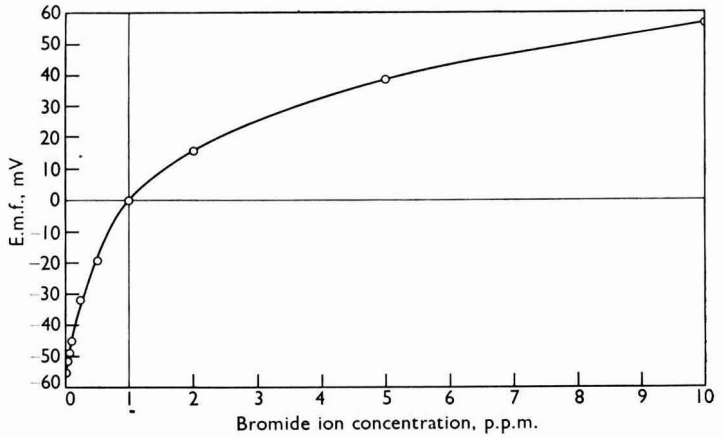


Fig. 3. Calibration curve for bromide obtained by using a reference solution containing 1 p.p.m. of added bromide ion. The e.m.f. of the cell is expressed as the potential of the reference electrode measured against that of the indicator electrode

EFFECT OF TEMPERATURE—

Fig. 4 gives calibration curves for chloride obtained at 21·5° C and 28·5° C. They show that the lower the amount of chloride, the greater the errors caused by small differences between the temperature of the experiment and that at which the calibration was carried out. At higher chloride concentrations, *i.e.*, above 10 p.p.m., this effect was negligible. The slope of response curve and, therefore, the sensitivity at extremely low concentrations was higher at lower working temperature.

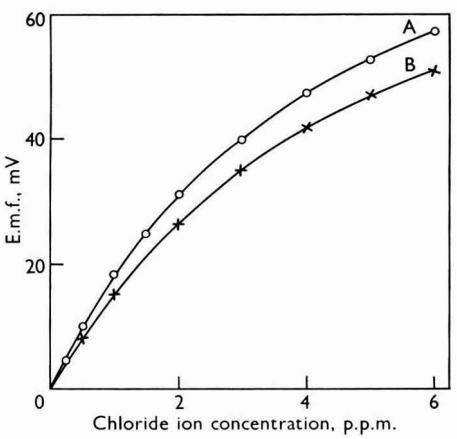


Fig. 4. Calibration curve for chloride at 21·5°C, curve A, and at 28·5°C, curve B, obtained by using a reference solution containing no added chloride. The e.m.f. of the cell is expressed as the potential of the reference electrode measured against the indicator electrode

The effect on the e.m.f. of difference in temperature of two solutions, which were otherwise identical, was found to be approximately 0·5 mV per °C.

A similar pattern of response to temperature differences was observed in the determination of bromide ion, as for chloride ion.

INTERFERENCE WITH DETERMINATION OF CHLORIDE—

Solutions containing small amounts of inorganic salts in 0·25 M sulphuric acid were analysed by using the method for chloride. Table I gives the e.m.f. values, obtained against

TABLE I

EFFECT OF OTHER IONS ON CHLORIDE DETERMINATION

Salt	E.m.f., mV	Salt	E.m.f., mV
Potassium nitrate	0·0	Sodium nitrite*	12
Sodium carbonate	−0·4	Potassium dichromate	79
Sodium phosphate	0·0	Potassium permanganate	91
Sodium sulphite	0·0		
Potassium chlorate	−3·0	Sodium thiosulphate	−56
Potassium bromate	3·0	Ferrous sulphate	0·0
Potassium iodate	2·2	Ammonium ferric sulphate	8·5
Potassium thiocyanate*	−115	Potassium bromide	−17·6
Potassium ferricyanide	−101	Potassium iodide	−9·9

* Continuous drift occurred and readings were taken after 2 minutes.

a reference solution containing no added chloride, with solutions containing 19 μg of the salt or, for potassium bromide and iodide only, 2 μg per ml. Gaseous oxygen and carbon dioxide were also tested for interference.

Substances that were examined can be classified, according to their behaviour, into four groups—

(*a*) Inert. Nitrate, carbonate, phosphate, sulphite, ferrous iron and gaseous oxygen and carbon dioxide did not interfere.

(*b*) Ions forming sparingly soluble silver salts in acid solution. Bromide and iodide were precipitated almost quantitatively as the silver salts, liberating an equivalent amount of chloride. Steady readings were not obtained unless sufficient silver chloride was present to complete the precipitation. Ferricyanide and thiocyanate were partially precipitated as the silver salts, liberating chloride. Soluble sulphide was partially lost as hydrogen sulphide gas and partially precipitated as silver sulphide, the latter liberating an equivalent amount of chloride.

(*c*) Oxidising agents. These can be divided into weakly (iodate and bromate), moderately (ferric iron and nitric oxide) and strongly (dichromate and permanganate) interfering.

(*d*) Reducing and complexing agents represented by sodium thiosulphate. Both groups could be expected to release chloride ion from un-ionised silver chloride.

INTERFERENCE WITH DETERMINATION OF BROMIDE—

The effect of chloride ion on the determination of bromide was examined. There was interference shown as an apparent bromide content, corresponding to a small fraction of the chloride present. The deflections due to chloride in the presence of 0·1 p.p.m. of bromide are shown below—

Concentration of chloride ions, p.p.m.	1	4	12	32	64
E.m.f., mV	−5·1	−15·5	−28·1	−39·1	−49·2

DETERMINATION OF IODIDE—

An attempt was made at using a suspension type of electrode for determining iodide ion. Silver iodide is not sufficiently soluble in ammonia solution to give a precipitate on addition of the solution to dilute acid. Instead a solution in aqueous potassium cyanide was used.

The method was similar to those used for the other halides, the main difference being in the use of M sulphuric acid as the electrolyte. Suspension was obtained by adding 20 μl of a 0·2 per cent. w/v solution of silver iodide in 0·5 per cent. w/v aqueous potassium cyanide to 5 ml of each solution. Table II gives e.m.f. values obtained at 24° C against a reference suspension containing no added iodide ion, and the effect of the presence of bromide ion on the determination of iodide is indicated below—

Concentration of bromide ions, p.p.m.	0·25	0·5	1·0	2·5	5·0
E.m.f., mV	−32	−37	−49	−72	−88

Discussion

The methods as described are suitable for the determination of extremely small amounts of chloride and bromide ions, when interfering subtances are not present in significant amounts and a high degree of accuracy is not required. In such instances, they provide a simple and sensitive means of determining these two ions, with an accuracy of approximately ± 5 per cent. of the amount found. The limit of detection of chloride is approximately 0·03 p.p.m., corresponding to an e.m.f. of 0·5 mV, and the sensitivity of the method for bromide is about eight times higher.

Table II

Results for the determination of iodide

Added iodide, p.p.m.	Time*	E.m.f., mV, after— 1 minute	3 minutes	5 minutes
0	0	6	5	5·5
0·0125	14	− 8·2	− 10	− 9·8
0·025	27	− 27	− 26	− 28
0·05	36	− 76	− 70	− 69
0·10	46	−181	−177	−177
0·15	55	−218	−218	−220
0·20	63	−237	−237	−235
0·25	74	−252	−251	−250
0·5	85	−282	−281	−280
1·0	92	−299	−298	−298
2·0	100	−326	−326	−326
0·0	117	− 26·4	− 24·4	− 24
0·0	against a fresh reference suspension	2	3·5	4·8

* Minutes after preparation of the reference electrode.

The methods were not used for determining chloride and bromide in concentrations above 100 and 10 p.p.m., respectively, but there is no obvious reason why they should not work above these limits. Since less stringent precautions are necessary as the concentration of halide increases, the procedures would be greatly simplified.

Interference with chloride determination caused by substances that react with silver or silver chloride is dealt with in the "Experimental" section. To these groups of compounds must be added soluble silver salts that precipitate some of the chloride present in the sample, and organic solvents when present in sufficient amounts to lower the ionisation constant of silver chloride.

Bromide cannot be determined in the presence of a large variable excess of chloride, owing to interference by the latter. This effect could probably be expressed as a function of chloride and bromide concentrations, but insufficient results were obtained for working out such a formula. No other substances were tested for interference with the determination of bromide; the general trend can be deduced from their effect on chloride determination, provided that the respective solubilities of the two silver salts are remembered.

The "suspension" type of electrode showed promise as a possible means of determining iodide. Its sensitivity to the iodide ion was extremely high, but a considerable drift in the electrode potential of the reference electrode occurred with time. A possible explanation of this drift is the alteration of solubility of silver iodide, owing either to the initial formation of a colloidal suspension or to a slow change of the crystalline form of the precipitate or to the loss of hydrogen cyanide. Presence of a large excess of bromide interfered with the determination of iodide in a manner suggesting a similar mechanism to the interference with the determination of bromide caused by chloride.

The type of electrode described in this paper, *i.e.*, pure metal in contact with a suspension of its salt prepared *in situ*, may have other practical applications, particularly when a coated electrode of the "second type" is difficult to prepare or unstable.

Appendix

The determination of residues of organochlorine pesticides by a total-chlorine method

Oxygen-flask combustion with subsequent use of the concentration-cell technique for determining chloride ion was used for determining residues of organochlorine pesticides. Animal tissue, eggs and butter were extracted and cleaned-up by using procedures described recently,[9] and plant tissue was extracted with n-hexane with no subsequent clean-up. Suitable portions of the extracts were transferred to small pieces of specially purified and stored filter-paper, and the organic matter destroyed by oxygen-flask combustion (500-ml flasks were used, and the dry weight of the extracts was not allowed to exceed 60 mg, so as to ensure complete combustion). The products of combustion were absorbed in 10 ml of 0·25 M sulphuric acid, and 0·5 ml of 4 per cent. ammonium sulphamate solution was added to destroy nitric oxide, which interferes with the electrometric stage. (Nitric oxide is formed by fixation of the atmospheric nitrogen trapped in the flask and probably also from nitrogenous components of sample extracts.) The solution was then examined for chloride by the concentration-cell method. The reliability of the oxygen-flask procedure used was checked by carrying out recovery tests with pure pesticide solutions, and with pesticides added to butter extracts. The results of tests with pure pesticides are given in Table III. This table includes

Table III

Recovery of pure pesticides

Pesticide	Method for determining chloride	Amount added, μg	Recovery, per cent.
γ-BHC	Colorimetric	50, 50, 62·5	95, 95, 93
γ-BHC	Potentiometric	76, 76	90, 89
Aldrin	Potentiometric	25, 25	99, 96
Heptachlor	Colorimetric	50, 50, 50	98, 96
Dieldrin	Colorimetric	12, 25, 37, 50, 62	97, 90, 88, 90, 82
Dieldrin	Potentiometric	105, 105, 105, 105	95, 93, 94,* 96*
DDT	Colorimetric	100, 100, 100, 60	90, 87, 84, 98
DDT	Potentiometric	113, 113	95, 95

* Combustion products were absorbed in 5 ml of water, and 5 ml of 0·5 M sulphuric acid was added afterwards.

for comparison, results obtained by the colorimetric mercuric thiocyanate method,[10] which is approximately ten times less sensitive than the concentration-cell method (nitric oxide also interferes with this test, a fact that might explain the difficulty some workers had when applying it after oxygen-flask combustion). Recoveries from butter extracts of γ-BHC, DDT and dieldrin in amounts equivalent to 5 to 20 p.p.m., were 91 to 96 per cent.

Table IV

Organochlorine-compound content of wild-life samples

Description of sample	Chlorine content, p.p.m.— found by total-chlorine method	calculated from results obtained by gas-chromatographic method
Kestrel muscle	12·1	14·2
Kestrel liver	32·2	32·7
Kestrel brain	44·3	50
Kestrel liver	10·0	10·4
Peregrine falcon muscle	21·6	15·4*
Peregrine falcon liver	66	43*
Peregrine falcon brain	41	35*
Buzzard egg	5·3	2·0*
Montagu harrier egg	8·6	9·4
Grebe egg	4·2	1·9*

* These chromatograms showed several large peaks due to unidentified substances.

Several samples of tissues and eggs of wild birds that had been found, by a gas-chromatographic method,[11,12] to contain high residues of organochlorine pesticides, were analysed by the total-chlorine method. Results are given in Table IV, and they show that whenever the gas-chromatographic examination revealed the presence of significant amounts of several unknown electron-capturing compounds, the total-chlorine results were considerably higher than the amounts calculated from the levels of known chlorinated hydrocarbons found in a sample. The fact that these unidentified compounds were not found in samples containing extremely small amounts of known chlorinated hydrocarbons supports the hypothesis that they are the metabolic intermediates of one or more organochlorine pesticides.

Three samples of butter were also analysed. Their total-chlorine content was 0·24, 0·0 and 0·06 p.p.m. compared with 0·20, 0·05 and 0·05 p.p.m. calculated from the gas-chromatographic results.

Analysis of water-washed hexane extracts of surfaces of leaves and grass by the total-chlorine method gave satisfactory results only when high residues were present. Untreated plant material appeared to contain variable amounts of organic chlorine so that some clean-up is necessary before low residues of pesticides are determined.

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Active Charcoal as an Adsorbent in Clarification of Raw Sugar Solutions

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A new approach to the problem of clarifying raw sugar solutions is described.

A procedure is proposed for clarifying raw sugar solutions on the grounds that a decolorisation of the sugar solution may be effected without altering the relative concentration of the component sugars. A departure from the use of traditional defecating agents with their precipitate volume and other rotation effects has been made, and a method that can be used with either visual or photo-electric polarimeters has been evolved.

The commercial value of sugars and the duty to be charged on them is assessed by their "polarisation," *i.e.*, the optical rotation of a standard solution (26·00 per cent. w/v) under defined conditions, the rotation being measured on the international Sugar Scale[1] with a visual polarimeter. A necessary stage in the preparation of solutions for visual polarimetry is the clarification by mineral-salt defecants, and this is essential even though solutions may be only slightly coloured.

Solutions of lead salts have been accepted, with reservations,[2] as suitable for clarification purposes, although acknowledged side effects do occur in their use.[3] The magnitude of these side effects has long been the subject of controversy and of extensive research.[4,5]

Filtration through bone charcoal is a standard process for decolorising sugar solutions during refining, and it was thought that a similar process might be adapted to the decolorising of solutions for polarisation. The adsorption of sugars on activated (vegetable) charcoal has been studied in this Laboratory,[6] and it has been shown that if a solution of mixed sugars is passed through a column of a suitable grade of charcoal, the concentration of each sugar in the effluent builds up in turn until the composition of the mixture leaving the column is the same as that entering it. If the colouring matter of raw sugar were adsorbed more strongly than any of the sugars, then it should be possible to obtain a fraction of the effluent that contains all the sugars in the same relative proportions as they occur in the original solution and that contains none of the colouring matter.

Experimental

Clarification efficiency—

Various mixtures of activated charcoal, Hyflo Supercel and Celite 560 were examined for flow-rate and decolorising power.

The most efficient decolorising mixture consistent with a flow-rate of 3 to 4 ml per minute and an advantageous ratio of bed volume to cleared solution proved to be a mixture of activated charcoal powder (British Drug Houses Ltd.) and Celite 560 (Koch-Light Laboratories Ltd.) in the ratio 1 to 3. The decolorising capacity of a 16-mm internal diameter column containing 2·5 g of such a mixture was sufficient to reduce the optical density ($E^{26\ \%}_{1\ cm}$ at 540 mμ) of 60 ml of a dark, raw sugar solution from 0·50 to less than 0·03. The optical density of a solution, clarified with lead acetate, of the same original sugar was 0·04. A progressive increase in the colour of the effluent solution became obvious visually, after approximately 60 ml of solution had passed through the column. The flow-rate was chosen to be comparable with the average filtration rate of solutions of raw sugar clarified with lead acetate.

Optical-rotation measurements—

The optical rotation of effluent from a column can be measured continuously by the Bendix Ericsson ETL/NPL Automatic Polarimeter, Type 143A, coupled with a pen recorder that gives a full-scale deflection over any prescribed interval of 5° Sugar scale; and fitted with a 1-cm flow-through cell (see Fig. 1). Such measurements (see Table I) showed that the rotation increased to a constant, maximum value after approximately 15 ml of solution had passed through the column, and also that the constant rotation was maintained for at

least a further 25 ml of effluent. This is a sufficient amount for use with a standard polarimeter tube for visual reading. The final constant rotation observed was found to be independent of column flow-rates between 3 and 8 ml per minute.

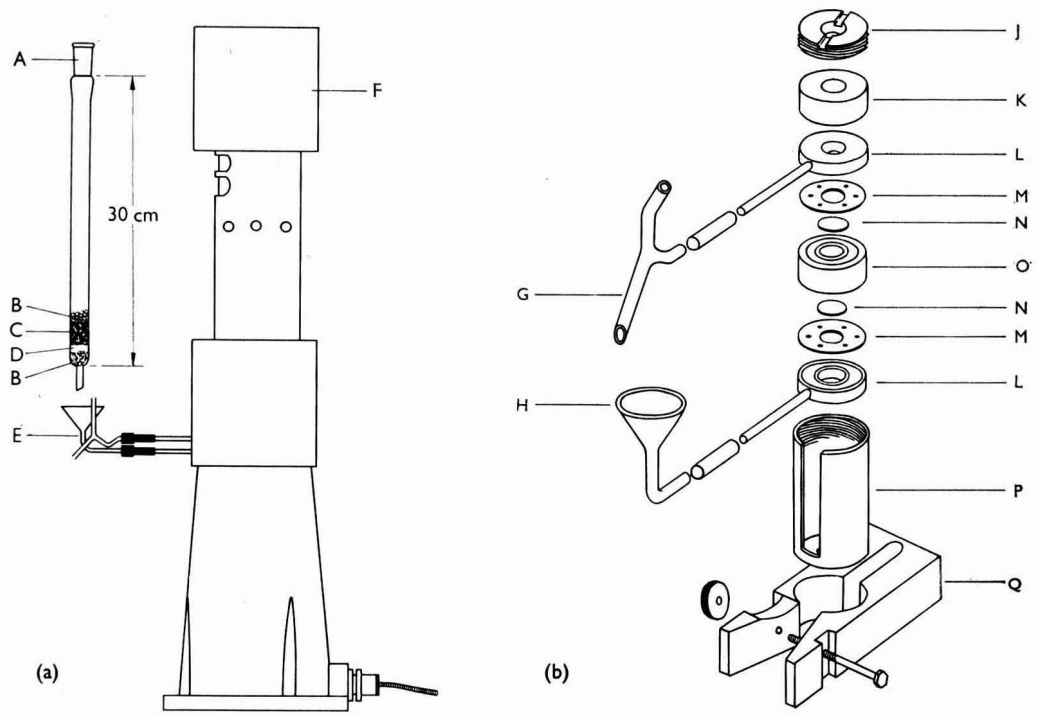

Fig. 1. Diagram of photo-electric instrument: (*a*) set up for continuous flow; (*b*) exploded diagram of flow-cell

TABLE I

ROTATION AND OPTICAL DENSITY OF EFFLUENT FROM CARBON - CELITE MIXTURE COLUMNS

Effluent, ml	Cane sugar: Rotation, °S	Cane sugar: Optical density measured in a 1-cm cell	Beet sugar: Rotation, °S	Beet sugar: Optical density measured in a 1-cm cell
5	95·37	0·00	95·40	0·00
10	97·37	0·00	96·37	0·00
15	97·50	0·00	96·75	0·00
20	97·53	0·00	96·81	0·00
25	97·53	0·00	96·82	0·00
30	97·53	0·00	96·81	0·01
40	97·53	0·00	96·81	0·01
50	97·51	0·01	96·81	0·02
60	97·50	0·02	96·80	0·02
Original unclarified solution	*97·30*	*0·35*	*96·37*	*0·19*

In order to test whether other sugars found with sucrose were removed, artificial mixtures of sugars were made and passed through the charcoal column. Table II gives a summary of results obtained for the various mixture, and shows that only insignificant changes in rotation are caused by the treatment.

TABLE II

COMPARISON OF ROTATIONS OF SUGAR SOLUTIONS BEFORE AND AFTER TREATMENT ON CARBON - CELITE MIXTURE COLUMNS AT AN APPROXIMATE CONCENTRATION OF 26 g PER 100 ml

Mixture composition		Polarisation, °S, found by— photo-electric observation		visual observation		Theoretical effect of removing the non-sucrose sugars, °S		
Sugar	Percentage	before treatment	after treatment	before treatment	after treatment	Glucose	Fructose	Raffinose
Sucrose	100	100·02	100·02	100·01	100·02	—	—	—
Sucrose	99·75	99·68	99·68	99·66	99·67	−0·10	+0·16	—
Glucose	0·12							
Fructose	0·12							
Sucrose	99·5	99·27	99·28	99·27	99·27	−0·20	+0·33	—
Glucose	0·25							
Fructose	0·25							
Sucrose	99·0	98·66	98·67	98·68	98·69	−0·40	+0·66	—
Glucose	0·5							
Fructose	0·5							
Sucrose	98·0	97·45	97·44	97·46	97·47	−0·79	+1·30	—
Glucose	1·0							
Fructose	1·0							
Sucrose	97·9	97·37	97·36	—	—	−0·79	+1·43	—
Glucose	1·0							
Fructose	1·1							
Sucrose	96·2	94·96	94·93	94·97	94·95	−1·51	+2·47	—
Glucose	1·9							
Fructose	1·9							
Sucrose	95·8	94·64	94·63	—	—	−1·67	+2·74	—
Glucose	2·1							
Fructose	2·1							
Sucrose	97·5	97·47	97·47	97·47	97·47	—	—	—
Sucrose	98·0	97·63	97·62	97·62	97·62	−0·79	+1·17	−0·16
Glucose	1·0							
Fructose	0·9							
Raffinose	0·1							
Sucrose	95·4	95·50	95·50	—	—	−1·67	+2·74	−0·64
Glucose	2·1							
Fructose	2·1							
Raffinose	0·4							

METHOD

PREPARATION OF SOLUTION—

Weigh 26·000 ± 0·002 g of raw sugar from the prepared sample into a nickel scoop, transfer it to a 100-ml calibrated flask with about 60 ml of boiled distilled water at 20° ± 0·1° C, and dissolve it by agitation without heating. Dilute the solution to the mark with boiled distilled water at 20° ± 0·1° C.

PREPARATION OF THE ADSORBENT COLUMN—

Place a small cotton-wool plug in the lower end of a glass column, 30 cm long and 1·6 cm in internal diameter, with the lower end restricted to a short tip, 0·4 cm in diameter, and with a B19 socket at the top. Tamp the plug, and pour on a layer of Celite 560 approximately $\frac{1}{16}$ inch thick. Tamp down again.

Weigh 2·5 g of Carbon - Celite mixture (1 part of BDH "Charcoal, decolorizing powder" to 3 parts of Celite 560) and transfer it to the prepared column under a light suction from the bottom of the column. Tamp and seal the top of the column with a cotton-wool plug.

CLARIFICATION AND MEASUREMENT OF ROTATION—

Pour 40 to 50 ml of the raw sugar solution into the tube containing the prepared bed. Apply a positive pressure, equivalent to 1 to 2 inches of mercury, to the column by means of an air line and standard-joint adapter at the top of the column. Discard the first 15 ml of effluent. Collect the next 25 to 30 ml of effluent in a dry tube or beaker *taking precaution against evaporation* by covering the mouth of the receiving vessel.

For photo-electric measurement, transfer a portion of the collected effluent to the photo-electric polarimeter cell, and allow the solution to come to thermal equilibrium with the instrument. Allow the instrument to record the rotation of the solution for a further 60 seconds.

For visual measurement, transfer the collected effluent to a dry polarimeter tube. Place the tube in the visual polarimeter and allow the solution to come to thermal equilibrium with the instrument. Read the optical rotation of the solution.

CALIBRATION OF THE PHOTO-ELECTRIC POLARIMETER—

The method of calibrating this instrument has been described in the Proceedings of the XIII Session of I.C.U.M.S.A. 1962[7] and by Mesley.[8] The instrument is standardised by observations on solutions of pure sucrose whose rotations have previously been determined by using a visual instrument. The accuracy of calibration is, therefore, never better than that of visual observation of the standardising solution. In all instances, the solutions were allowed to attain thermal equilibrium with the instrument before any observations were made. The rotation assigned to any one solution was the mean of ten individual observations in the steady state.

COMPARISON OF THE PROPOSED METHOD WITH THE ~~ESTABLISHED~~ TENTATIVE I.C.U.M.S.A. STANDARD METHOD I

Groups of samples of raw cane and beet sugar drawn from lots with a common origin and with the same degree of polarisation were collected together. Several bulk samples representing the various countries of origin and with a range of polarisations were then thoroughly mixed and sieved before use. Polarisation determinations were made by the conventional I.C.U.M.S.A. lead-clarification technique[9] and the proposed carbon-column technique on solutions made from these bulk samples.

COMPARATIVE RESULTS—

Reproducibility—Observations, made firstly on the basis of internal repeatability on any one solution and secondly on the basis of repeatability of results obtained by repetition of the two methods from a common bulk, showed that the two methods differed slightly in this respect. The standard deviation of ten individual observations on a solution prepared by the proposed method was in the range 0·01° to 0·02° S; all prepared solutions were colourless. The established technique showed a wider range of observations on any one solution; this was dependent on the original colour of the sugar and the efficiency of the lead clarification. Most light-coloured sugars gave solutions for which the standard deviation of ten observations varied from 0·01° to 0·02° S, but darker sugars (polarisation generally 95° to 97° S), gave solutions on which the standard deviation of ten observations varied from 0·02° to 0·03° S.

Results obtained by ten replicate analyses from the same common bulk sample showed standard deviations of 0·04° to 0·06° S by both methods.

Comparison of results by I.C.U.M.S.A. Standard Method I and the proposed method—The comparative results for cane and beet sugars by the two methods are set out in Tables III and IV. The results so far obtained in the range 95° to 100° S show no apparent correlaton between the polarisation by standard method I and the difference between the results of the two methods (standard method I minus proposed method). The differences range from +0·33 to −0·16 for cane sugar, and +0·07 to −0·20 for beet sugar. Positive differences are expected owing to the fact that the proposed method eliminates the effects of lead precipitate volume; negative differences may be partially explained by the effects of lead salts on the rotation of certain amino-acid constituents.[10]

The tabulated results are the means of three separate analyses by the two methods, except where indicated as means of ten independent replicates.

A statistical analysis of the pooled results of 168 determinations on 49 samples by both methods showed that the average standard deviation for each method was 0·05° S. The

TABLE III: RESULTS FOR CANE SUGAR

Source	Rotation found by— visual observation on lead-clarified solution (x)	instrumental observation on carbon-clarified solution (y)	Difference, $x - y$
Antigua	97·58	97·53	+0·05
Australia 1*	98·76	98·72	+0·04
Australia 2	98·80	98·85	−0·05
Australia 3	98·64	98·67	−0·03
Australia 4	98·85	98·88	−0·03
Barbados 1	97·23	96·90	+0·33
Barbados 2	97·03	96·79	+0·24
British Guiana 1	98·78	98·76	+0·02
British Guiana 2	98·88	98·77	+0·11
British Guiana 3	98·03	97·95	+0·08
British Guiana 4	97·03	96·91	+0·12
British Honduras 1	97·94	97·81	+0·13
British Honduras 2	98·23	98·23	0·00
Cuba 1	96·57	96·47	+0·10
Cuba 2	98·11	98·03	+0·08
Cuba 3	96·22	96·15	+0·07
Dominica	97·77	97·72	+0·05
Fiji 1	98·63	98·65	−0·02
Fiji 2	98·55	98·59	−0·04
Jamaica 1	97·60	97·54	+0·06
Jamaica 2	96·96	96·83	+0·13
Mauritius	99·01	99·04	−0·03
Peru	97·35	97·34	+0·01
S. Rhodesia	97·56	97·51	+0·05
St. Kitts	97·24	97·18	+0·06
S. Africa 1	98·72	98·88	−0·16
S. Africa 2	98·97	98·99	−0·02
India	97·17	97·18	−0·01
Trinidad 1*	96·94	96·82	+0·12
Trinidad 2	98·47	98·47	0·00
Trinidad 3*	96·56	96·49	+0·07
Trinidad 4	96·95	96·92	+0·03
Trinidad 5	97·04	97·13	−0·09
Trinidad 6	98·26	98·33	−0·07

* Values from 10 repeated observations.

TABLE IV: RESULTS FOR BEET SUGARS

Source	Rotation found by— visual observation on lead-clarified solution (x)	instrumental observation on carbon-clarified solution (y)	Difference, $x - y$
Bury St. Edmunds	98·62	98·56	+0·06
Ely 1	98·78	98·83	−0·05
Ely 2	97·25	97·31	−0·06
Peterborough	98·07	98·11	−0·04
Wissington	98·05	97·98	+0·07
Belgium	97·56	97·55	+0·01
Czechoslovakia	99·96	99·98	−0·02
France	98·04	98·09	−0·05
Poland 1	96·83	96·89	−0·06
Poland 2	97·56	97·73	−0·17
Poland 3	96·69	96·83	−0·14
Poland 4	96·73	96·91	−0·18
Poland 5	97·73	97·93	−0·20
Roumania	99·86	99·89	−0·03
Russia	99·97	99·98	−0·01

TABLE V

RESULTS AT VARIOUS PROBABILITY LEVELS

Number of replicate determinations	Difference between polarisations for significance, °S		
	P = 0·05	P = 0·02	P = 0·01
1	0·14	0·17	0·18
2	0·10	0·12	0·13
3	0·08	0·10	0·11
4	0·07	0·08	0·09
6	0·06	0·07	0·08
9	0·05	0·06	0·06
10	0·04	0·05	0·06

same average standard deviation was obtained for each of the sub-groups, cane and beet sugar, by each method. Variance-ratio tests showed no significant difference between standard deviations at the 1 per cent. probability level.

A summary of the numbers of replicate determinations necessary to establish differences between the two methods at various probability levels is given in Table V. From this it may be seen that for triplicate determinations, differences greater than 0·10° S are significant, and that for the ten-fold determinations, differences greater than 0·05° S are significant and these may be regarded as a measure of the net lead effect.

OTHER METHODS OF ASSESSING POLARISATION

A proposal for determining polarisation by direct observation of unclarified solutions in the photo-electric polarimeter has been reported.[8] This method requires an instrumental calibration for each individual sample, this calibration being required in order to correct the direct reading for light-absorption effects. The calibration involves three separate readings of the photo-electric polarimeter with (*i*) pure sucrose in the rotation cell, (*ii*) pure sucrose in the rotation cell with the sample in a 1-cm cell interposed in the non-polarised part of the light path and (*iii*) an unclarified solution in the rotation cell. Owing to varying optical densities of the raw sugar solutions, the photo-electric instrument shows a variable degree of electronic noise, and individual traces of the pen recorder under conditions (*ii*) and (*iii*) above show ranges of observation on a single solution covering 0·02° to 0·03° S with sugars in the 95° to 100° S range. With lower-grade sugars, variations in individual readings exceeded 0·20° S. The net effect is that the sum of variance for the various observations necessary to obtain a polarisation from an unclarified sugar is such that the standard deviation for ten repeat observations on a single solution is greater than 0·03° S, and this is too large for the method to be useful for measuring small differences given by different techniques.

It was considered, therefore, that this technique was suitable only for examining lightly coloured solutions with optical densities ($E^{26\,\%}_{1\,cm}$ at 540 mμ) of not greater than 0·25.

CONCLUSIONS

The proposed technique of clarification has been demonstrated to be effective when used with raw sugar solutions, and the reproducibility of observation of the rotation of the prepared solution is at least as good as that of the equivalent solution prepared by the standard lead-clarification technique.

The method is therefore proposed as an alternative to the standard lead-clarification technique for use in obtaining the value of the optical rotation of the sugars in a solution prepared from unrefined or partially refined sugar.

I thank the Government Chemist for permission to publish this paper.

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A Concentration-cell Method for the Determination of Trace Amounts of Chloride in Solutions of Lithium Salts

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A concentration-cell technique has been applied to the determination of up to 4 μg of chloride per ml in solutions of lithium salts containing 0·025 g of lithium per ml (3·6 M). The method is without bias and has a standard deviation of 0·06 μg per ml at the 1 μg per ml level. This precision remains sensibly constant over the quoted range. A modification of the method, which involves the removal of the chloride from the sample as hydrogen chloride and subsequent absorption in the concentration cell, gives satisfactory results below the 0·25 μg per ml level with a standard deviation of 0·03 μg per ml.

THE need for a sensitive method for determining trace amounts of chloride in lithium and its salts led to the investigation of a concentration-cell technique that will be referred to as the "large excess" method. This is based on similar principles to those used by Furman and Low[1] and also by Blaedel, Lewis and Thomas,[2] and has the advantage of giving satisfactory results in solutions of high ionic strength.

It was decided to effect the concentration of the chloride content of a large volume of sample solution by a modification of the "large excess" method. To this end it was proposed to convert the chloride to hydrogen chloride by Bergman and Martin's concentration procedure,[3] and to absorb this in the concentration cell.

The advantage of a concentration-cell technique is that the effect of large amounts of neutral salts is balanced out by immersing the electrodes in solutions of the same ionic strength. For chloride determinations, the potential difference, E, is measured between two silver - silver chloride electrodes, one of which dips into the unknown chloride solution (solution 1) and the other into the unknown chloride solution *plus* a known, added amount of chloride (solution 2). The e.m.f. of the cell is then given by—

$$E = k \log_{10} \left[\frac{f_2 (x + a + s_2)}{f_1 (x + s_1)} \right] \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where x is the unknown molar concentration of chloride in the sample solution,
a is the known added molar concentration of chloride,
f_1 and f_2 are the activity coefficients of solutions (1) and (2), respectively,
s_1 and s_2 are the molar concentrations of chloride due to the solubilities of the electrodes in solutions (1) and (2), respectively,
k is 0·0581 at 20° C and
E is the observed e.m.f., in volts.

Since the total ionic strength in each half cell is the same (3·6 M), $f_1 = f_2$ and the liquid-junction potential can be neglected. In the "large excess" method, a is made so large that x and s_2 can be neglected in the numerator and equation (1) becomes—

$$E = k \log_{10} \left(\frac{a}{x + s_1} \right) \quad .. \quad .. \quad .. \quad .. \quad (2)$$

The solubility product, P, of silver chloride can be expressed by the equation—

$$P = s_1 (x + s_1) \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where $(x + s_1)$ is the total chloride concentration and
s- is concentration of silver, which is equal to the concentration of the chloride dissolved from the electrode.

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The elimination of s_1 from equations (2) and (3) gives—

$$E = k \log_{10}\left(\frac{2a}{x + \sqrt{x^2 + 4P}}\right) \quad .. \quad .. \quad .. \quad .. \quad (4)$$

which can be expanded to give—

$$x = \frac{a}{10^{E/k}} - \frac{P10^{E/k}}{a} \quad .. \quad .. \quad .. \quad .. \quad (5)$$

The use of this equation for determining x necessitates the prior determination of P under the conditions of analysis. It is also necessary to subtract from x a blank value (determined separately), which is due to the chloride content of the reagents used.

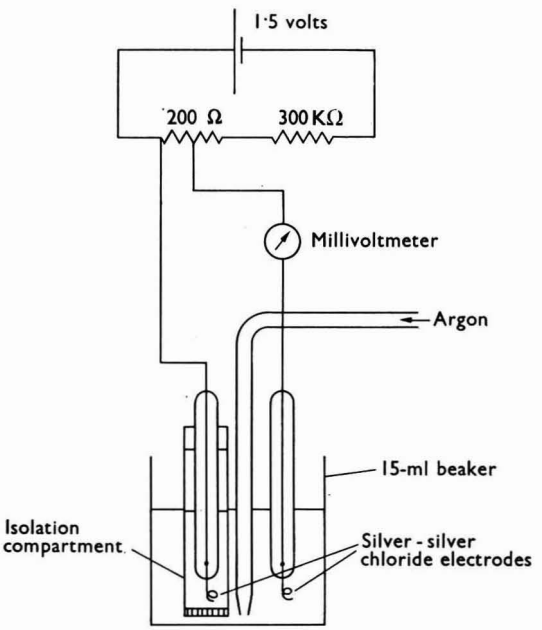

Fig. 1. Diagram of cell assembly

Experimental

Apparatus—

Cell assembly (see Fig. 1)—The cell consisted essentially of an isolation compartment (closed by a G4 sintered-glass disc) immersed in a small beaker. A silver - silver chloride electrode was dipped into each half cell and the potential difference between them was measured by a pH meter used as a millivoltmeter. The application of a small backing voltage was used to compensate for the small asymmetry potential. The solution in the beaker was stirred by argon flowing at a rate of 100 ml per minute through a fine capillary.

Electrodes (see Fig. 1)—Silver wire (1 mm in diameter) was cemented into a Pyrex tube so that 1·5 cm projected from one end. Electrical contact was made to the end of the wire within the glass tube by previously spot-welding a copper lead to it. The projecting part of the silver wire was cleaned with ethyl methyl ketone and washed with water. It was then immersed for 1 minute in diluted (1 + 1) nitric acid, withdrawn, and washed well with water. Two such electrodes were made the joint anode in 0·1 N hydrochloric acid, the cathode being a platinum wire, and a current of 1 mA was passed for 1 hour. The electrodes were then washed with water and stored in a sample solution overnight before use.

Gas train (see Fig. 2)—In the modified method, argon was passed through a rotameter, a soda lime tower and an 80 per cent. sulphuric acid solution before entering the concentration cell. The acid was contained in a vessel 20 cm long and 4 cm in diameter and the argon was led into it via a G4 sintered-glass disc.

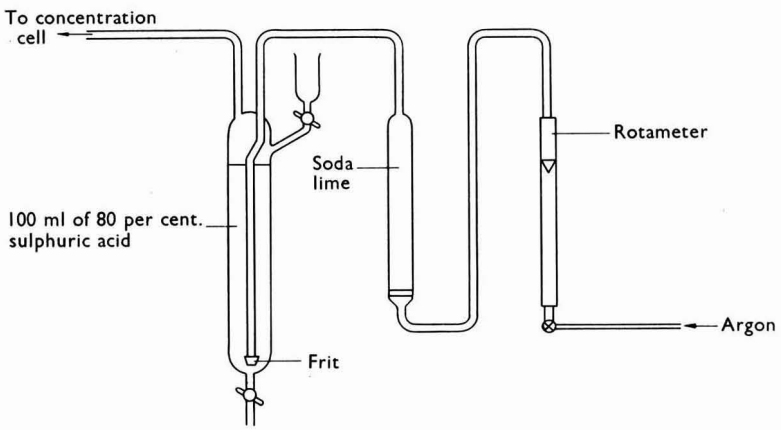

Fig. 2. Diagram of gas train for use in the modified method

REAGENTS—

De-mineralised water—Obtained from an ion-exchange column. All solutions were made up with this water.

Chloride-free nitric acid—This was prepared by distilling analytical-reagent grade nitric acid containing less than 0·7 μg of chloride per ml and rejecting the first and last fraction (each fraction being one-fifth of the whole distillate).

Sulphuric acid—Analytical-reagent grade material (Judactan) containing less than 0·1 μg of chloride per ml.

Standard chloride solution—A solution containing 400 μg of chloride per ml was prepared by dissolving 0·659 g of analytical-reagent grade sodium chloride in de-mineralised water and diluting the solution to 1 litre.

Chloride solution, 0·3 M—This was prepared by dissolving 17·60 g of analytical-reagent grade sodium chloride in water and diluting the solution to 1 litre. (The addition of 0·05 ml of this solution to 5 ml of a sample solution gave a "large excess" solution 300×10^{-5} M with respect to chloride.)

PREPARATION OF THE SAMPLE SOLUTION—

The sample of lithium (0·5 g) was added to a flask fitted with a dropping funnel and inlet and outlet tube, and argon was passed through the flask at about 100 ml per minute. Water, 8 ml, was added cautiously from the dropping funnel, and then 50 per cent. nitric acid was added until all the metal had dissolved and the solution was neutral to phenolphthalein. A further 0·1 ml of the 50 per cent. acid was then added, and the solution made up to 20 ml (equivalent to a 3·6 M solution).

In the modified method, 50 per cent. sulphuric acid was used instead of nitric acid to effect dissolution.

THE DETERMINATION OF THE SOLUBILITY PRODUCTS—

The sample solution, 5 ml, was transferred to the beaker, and a further 2 ml of the same sample solution containing an excess of chloride (300×10^{-5} M $= a$) was added to the isolation compartment. The e.m.f. of the cell, E_1, was noted to the nearest millivolt, and standard chloride solution was added to the beaker from an Agla micrometer syringe pipette so as to increase the chloride content by $2{\cdot}00 \times 10^{-5}$ M and to change the e.m.f. of the cell to E_2.

Simultaneous equations based on equation (5) were then set up and solved for P, the solubility product—

$$x = \frac{a}{10^{E_1/k}} - \frac{P10^{E_1/k}}{a}$$

$$x + 2{\cdot}00 = \frac{a}{10^{E_2/k}} - \frac{P10^{E_2/k}}{a}$$

The mean value of P so obtained was found to be $3{\cdot}2 \times 10^{-10}$ for a solution of 3·6 M lithium nitrate at 20° C. The solubility product was also determined in the same way for the 0·03 N nitric acid, used as a blank solution. The mean value at 20° C was found to be $1{\cdot}48 \times 10^{-10}$. The solubility product, P, was determined once only for a series of similar solutions rather than setting up two simultaneous equations for each analysis.

THE "LARGE EXCESS" METHOD—

The sample solution, 5 ml, was transferred to the beaker, and about 2 ml of the same sample solution containing an excess of chloride (300×10^{-5} M = a) was added to the isolation compartment. The observed e.m.f. of the cell, E, was noted to the nearest millivolt and used in equation (5) for calculating the chloride content of the sample solution, x. From this was subtracted the blank value determined as above (but with 0·03 N nitric acid used as the sample solution) and the appropriate solubility product.

Several synthetic samples were made up by adding known amounts of standard chloride solution to a sample solution to give solutions containing up to 4 μg of chloride per ml, and the chloride determined (see Table I).

It should be noted that all measurements were made in a draught-free, thermostatically controlled room. Any variation in temperature from 20° C was taken into account by using the appropriate value of k. This applies also to the determination of solubility products.

TABLE I

RECOVERIES FROM SAMPLE SOLUTIONS CONTAINING KNOWN ADDED AMOUNTS OF CHLORIDE

Chloride added, μg per ml	Total chloride found, μg per ml	Net chloride recovered, μg per ml	Recovery, per cent.
0	1·38	—	—
0·38	1·81	0·43	113
0·75	2·22	0·84	112
1·06	2·23	0·85	80
1·50	2·92	1·54	103
1·80	3·30	1·92	107
2·08	3·35	1·97	95
2·22	3·57	2·19	99
2·52	3·98	2·60	103

Replicate results obtained for a bulk sample of lithium were 0·93, 1·00, 0·84, 0·93, 0·98, 0·98, 0·85 and 0·92 μg per ml, with a mean value of 0·93 μg per ml and a standard deviation of 0·06 μg per ml.

The results in Table I show that there is no significant bias in the "large excess" method. Table I also indicates that the precision does not vary with the amount of chloride present, up to 4 μg per ml. The time required for one determination if about 10 minutes, but the calculation of the results is rather tedious.

THE MODIFIED "LARGE EXCESS" METHOD—

Argon sweep gas was allowed to flow through the apparatus shown in Fig. 2 for half an hour to remove any chloride impurity from the 80 per cent. sulphuric acid (100 ml). About 2 ml of 0·03 N nitric acid, containing 300×10^{-5} M chloride, was added to the isolation compartment and 5 ml of the 0·03 N nitric acid was added to the main beaker. The initial e.m.f. of the cell was noted, and the sample was added to the 80 per cent. sulphuric acid. Readings of the e.m.f. of the cell were then noted every few minutes until they levelled off

(see Fig. 3, curve A). This usually took less than 20 minutes. In some instances the e.m.f. did not level off (see Fig. 3, curve B), and the determination had to be repeated. This phenomenon was considered to be due to contamination from the connecting tube and was not investigated further; an improvement was effected by replacing all rubber tubing wherever possible with copper tubing. It was decided to repeat all determinations in which the e.m.f. of the cell had not attained a constant value within 30 minutes.

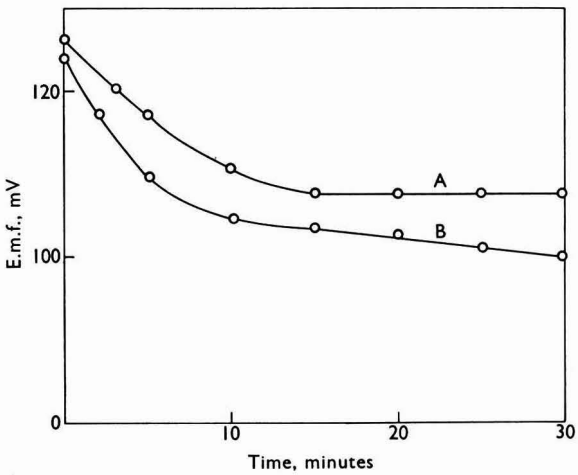

Fig. 3. Graph showing recovery of chloride from 80 per cent. sulphuric acid. Curve A, normal curve; curve B, spurious curve

As a preliminary experiment, a known amount of chloride was added to the sulphuric acid and the final e.m.f. of the cell noted. The initial em.f. of the cell was used to calculate the original chloride content of the 0·03 N nitric acid in the beaker, and this was subtracted from the final chloride content to give the amount absorbed in the cell. The results for recoveries of 4·25 μg of chloride added as standard chloride solution from 80 per cent. sulphuric acid are given below—

Chloride recovered, μg	4·50	5·10	3·90	3·84	4·10
Recovery, per cent.	106	120	92	90	96

Synthetic solutions were made up by adding known amounts of chloride to 20-ml aliquots of a sample solution containing 2·5 per cent. of lithium (as the sulphate). The recoveries from 80 per cent. sulphuric acid were determined as before, the whole of the 20-ml aliquot being added. The results are given in Table II and indicate a mean recovery of 90 per cent. The concentration factor achieved was 4, being the ratio of the volume of the sample solution to the volume of the solution in the concentration cell.

TABLE II

RECOVERIES OF CHLORIDE FROM 80 PER CENT. SULPHURIC ACID ADDED AS STANDARD CHLORIDE IN 20 ML. OF SAMPLE SOLUTION

Solution	Chloride added, μg per ml	Total chloride found, μg per ml	Net chloride recovered, μg per ml	Recovery, per cent.
Sample A	0	0·19	—	—
	0·26	0·39	0·20	77
	0·55	0·65	0·46	84
	1·05	1·20	1·01	96
	2·67	2·70	2·51	94
Sample B	0	0·44	—	—
	0·25	0·59	0·15	60
	0·50	0·97	0·53	106
	1·00	1·42	0·98	98
	1·50	2·00	1·56	104
	2·00	2·31	1·87	94

A series of replicate results were obtained for the same sample solution by adding 20-ml aliquots to the 80 per cent. sulphuric acid and, as before, the hydrogen chloride evolved was measured by the "large excess" method. A blank value, determined by using 20 ml of water instead of the sample, was subtracted, and the replicate results found for the modified "large excess" method were 0·09, 0·11, 0·09, 0·14, 0·17 and 0·16 μg of chloride per ml, with a mean value of 0·13 μg per ml and a standard deviation of 0·03 μg per ml.

Conclusions

A method based on the concentration-cell principle has been developed for determining up to 4 μg per ml of chloride in solutions of lithium salts. The standard deviation at the 1 μg per ml level is 0·06 μg per ml and this appears to be constant throughout the range. The method is without bias and is rapid in operation, but involves a rather lengthy calculation. This could, however, be simplified somewhat by using a nomogram.[2]

A modification of the method involving the prior removal of the chloride as hydrogen chloride has proved to be satisfactory for the analysis of samples containing less than 0·25 μg per ml of chloride, when the standard deviation is 0·03 μg per ml. This modified method, however, is subject to error due to occasional contamination of the apparatus with chloride.

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The Radiometric-Spectrophotometric Determination of Microgram Amounts of Niobium in Rocks and Minerals

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An improved method for the determination of 0·5 to 150 p.p.m. of niobium in silicate rocks and minerals is described. Niobium-95 is used to determine the yield of the chemical separation. The thiocyanate complex is extracted into ethyl acetate and the concentration is measured spectrophotometrically. The standard deviation of replicate analyses is less than 2 per cent. at the 20 p.p.m. level, but increases at lower concentrations and reaches 20 per cent. at 1 p.p.m.

An extremely sensitive spectrophotometric method for determining microgram amounts of niobium in silicate rocks has been described by Grimaldi.[1] This method involves repeated fusion of the sample with sodium hydroxide, together with the repeated precipitation of mixed hydroxides. The final precipitate is ignited, fused with pyrosulphate and the resulting cake extracted with a specified acid mixture and made up to 25 ml. A 15-ml portion of this solution is then treated with ammonium thiocyanate and stannous chloride solutions and extracted at a constant temperature with 20 ml of ethyl acetate. Iron and titanium are then removed from the organic layer by shaking with a specified stripping solution. The final organic layer is made up to 25 ml in a calibrated flask, and the optical density is measured against a blank solution.

The accuracy of Grimaldi's method is largely dependent upon the efficiency of the chemical separation. Grimaldi notes that the niobium thiocyanate complex is only partly extracted by ethyl acetate, but that under given conditions of reagent concentration and temperature the fraction extracted is constant. A 1° C rise in temperature causes a 1 per cent. decrease in the amount of niobium extracted, so that samples and standards must be extracted at the same temperature. Grimaldi recommends the extraction of only one sample and blank solution at a time.

Milner and Smales[2] used niobium-95 radiotracer to measure the chemical yield of niobium separated from low-grade niobium ores. The present work investigates the method described by Grimaldi, modified by adding niobium-95 to follow the chemical separation, and describes a faster, accurate procedure in which the extraction need not be temperature controlled and which allows the simultaneous extraction of five samples and a blank solution.

Experimental

A small, known volume of niobium-95, sufficient to give a few thousand counts per minute in the final solution, was evaporated in a nickel crucible containing 0·3 to 0·4 g of 120-mesh rock powder. The rock was then fused with sodium hydroxide and the resulting melt treated exactly as described by Grimaldi, except that no attempt was made to control the temperature at which the thiocyanate complex was extracted. The distribution of niobium between the subsequent precipitates and filtrates was determined by measuring the γ-ray activities of the filtrates with a scintillation counter. These experiments showed that small amounts of niobium, up to 5 per cent. of the amount present, were lost during the separation before the final solvent-extraction stage. Samples containing more iron tended to suffer smaller losses of niobium, presumably because ferric hydroxide acts as a carrier for niobic acid during precipitation. The chemical yield for the whole analysis, including the extraction step, showed considerable variation, a range of 50 to 60 per cent. being typical for a batch of five samples treated simultaneously. This variation was found to arise mainly at the extraction stage. Unless freshly prepared stannous chloride solution was used in the complex formation, low yields of 25 to 35 per cent. were obtained.

Difficulty was experienced in achieving complete decomposition of 120-mesh rock powders by sodium hydroxide fusions. A hydrofluoric - sulphuric acid decomposition, and a modified version of the chemical separation described by Grimaldi, was found to be more suitable.

By extracting the whole of the solution of the pyrosulphate melt, instead of the portion taken by Grimaldi, it was found possible to determine 2 p.p.m. of niobium in a 0·3- to 0·5-g sample of silicate rock, and 0·5 p.p.m. in 1 g of limestone. The correct conditions for the final extraction were maintained by adjusting the amounts of acid in the leach solution (see below).

METHOD

REAGENTS—

Use analytical-grade reagents.

Stock standard niobium solution—Fuse 0·1 g of Specpure niobium pentoxide with 2 g of potassium hydrogen sulphate in a silica crucible. Dissolve the cake in the crucible by heating with 10 ml of sulphuric acid, sp.gr. 1·84. Cool the solution, transfer it to a 200-ml calibrated flask and dilute to the mark with 33 per cent. v/v sulphuric acid. Store in a desiccator. This solution contains 0·5 mg of niobium pentoxide per ml.

Dilute standard niobium solution—Dilute 5 ml of stock niobium solution to 100 ml with 33 per cent. v/v sulphuric acid. Prepare freshly as required. This solution contains 25 μg of niobium pentoxide per ml.

Ammonium thiocyanate solution, 25 per cent. w/v, aqueous—This solution is stable for 1 month.

Potassium hydrogen sulphate, fused.

Leach solution—Mix 20 ml of 25 per cent. w/v aqueous tartaric acid with 65 ml of hydrochloric acid, sp.gr. 1·18, and 115 ml of water.

Niobium-95 tracer solution—Dilute 1 mC of the carrier-free oxalate complex (available from the Radiochemical Centre) with water so that 2 to 5 ml gives about 20,000 c.p.m.

Stannous chloride solution—Prepare a fresh 40 per cent. w/v solution of stannous chloride dihydrate in hydrochloric acid, sp.gr. 1·18, for each batch of samples.

Sodium hydroxide solution, 10 per cent. w/v, aqueous.

Stripping solution—Make sufficient for six samples by mixing together 80 ml of water, 40 ml of hydrochloric acid, sp.gr. 1·18, 30 ml of 25 per cent. ammonium thiocyanate solution and 3 ml of 40 per cent. stannous chloride solution. Prepare a fresh solution immediately before use.

PROCEDURE—

Weigh 0·3 to 0·5 g of 120-mesh rock powder into a 40-ml Teflon dish. Add a known volume (2 to 5 ml) of tracer solution (also set aside for reference an equal volume of tracer made up to 25 ml in a calibrated flask), 5 ml of 40 per cent. w/w hydrofluoric acid, 5 ml of 50 per cent. v/v sulphuric acid and 1 ml of nitric acid, sp.gr. 1·42. Evaporate to strong fumes of sulphuric acid. Cool, add 10 ml of water, 10 ml of hydrochloric acid, sp.gr. 1·18, and heat to dissolve most of the salts. Transfer the solution to a 150-ml beaker, rinsing the dish with hydrochloric acid and water. Add a Whatman accelerator, heat the solution and make it just alkaline with strong ammonia solution. Digest the mixture for 15 minutes, filter the solution through a No. 541 filter-paper and wash the precipitate with hot 2 per cent. ammonium chloride solution. Flush the precipitate into the original beaker with a jet of water, wash the paper with 10 ml of 50 per cent. hydrochloric acid and then water, collecting the washings in the beaker. Heat to dissolve the precipitate and stir in 50 ml of hot 10 per cent. sodium hydroxide solution. Digest the mixture for 10 minutes, filter the solution through the same filter-paper and wash the precipitate with 5 per cent. aqueous ammonia solution.

Dissolve the precipitate as before, and repeat the precipitation with ammonia solution, washing the precipitate with hot 2 per cent. ammonium chloride solution. Ignite the paper and precipitate in a silica crucible (this is conveniently carried out overnight in a muffle furnace set to rise slowly to 500° C).

Add 1 g of potassium hydrogen sulphate, weighed to the nearest 0·02 g, and fuse the mixture gently until a clear melt is obtained. Cool, add 0·4 ml of sulphuric acid, sp.gr. 1·84, and heat the mixture strongly on a sand-bath to disintegrate the cake. Transfer by pipette

20 ml of leach solution into a 50-ml beaker, transfer about 10 ml of this solution to the cooled crucible and heat gently on a sand-bath to dissolve the paste. Transfer the solution to the beaker and wash the crucible with 1 ml of water. Heat the solution to incipient boiling and filter it through a small No. 41 filter-paper into a 50- to 100-ml separating funnel. Prepare a reagent blank solution consisting solely of 1 g of potassium hydrogen sulphate, fused, dissolved and filtered as for the samples.

To the cold filtered solutions add in the following order, by pipette and with mixing, 5 ml of 25 per cent. ammonium thiocyanate solution, 0·5 ml of 40 per cent. stannous chloride solution and 20 ml of ethyl acetate. Shake for 1 minute and prepare sufficient stripping solution as described above. Run off and discard the aqueous layer. Shake the organic layer for 1 minute with 15 ml of stripping solution, discard the aqueous layer and repeat with a further 10 ml of stripping solution. Make the ethyl acetate layer, noticeably yellow if the sample contains more than about 10 μg of niobium, up to 25 ml in a calibrated flask. Without delay (the optical density decreases by 2 per cent. in 1 hour), measure the optical densities of the solutions against the extracted blank solution in 4-cm cells at a wavelength of 385 mμ. It was found convenient to form the thiocyanate complex, extract and measure five samples and a blank solution simultaneously. Calculate the chemical yields by comparing the γ-ray activity of portions of the sample extracts with an equal volume of the diluted tracer reference solution set aside at the beginning.

Determine the niobium concentration in the extracts from a calibration curve prepared by evaporating suitable portions of a niobium solution, together with tracer solution, in silica crucibles and fusing the residue with potassium hydrogen sulphate, etc., and determining the chemical yield as described above. The author used as standards 0·1-, 0·2-, 0·3-, 0·4- and 0·5-ml portions of the dilute niobium standard solution dispensed from a micrometer-syringe burette. In 4-cm glass cells, an optical density of 0·1 was obtained from a solution containing 1·366 μg of niobium per 25 ml of solution. With the spectrophotometer used, the calibration curve remained constant for many months, and was a straight line in the range used (0 to 10 μg of niobium per 25 ml of solution).

Results and discussion

This method has been used for determining niobium in the 0 to 40 p.p.m. range in some eighty samples of igneous and metamorphic rocks and several sandstones, limestones and oceanic oozes. Some alkali lavas were found to contain 40 to 150 p.p.m. of niobium. It is hoped to publish detailed geochemical results elsewhere, but Table I shows results obtained for the U.S. Geological Survey standard rocks W–1 and G–1. It is apparent from Table I

Table I

Niobium contents, p.p.m., of the standard rocks G–1 and W–1

	Found by Grimaldi[1] for		Found in present work for	
	G–1	W–1	G–1	W–1
Average	21·7	9·6	23·1	10·2
Standard deviation	0·9	0·6	1·5	1·1
Number of samples	10	10	13	14
Range	20 to 23	9·1 to 11	20·7 to 25·0	8·9 to 12·2

that the reproducibility obtained in the present work is not as good as is shown by Grimaldi's results. The author attributes this to the fact that his values for G–1 and W–1 were obtained singly, as one of batches of ten other rocks, during a period of about 12 months. The greater precision of the results obtained when triplicate analyses of three German granites were made in one batch is indicated below—

Sample	W6	Me	C7
Niobium found, p.p.m.	26·0, 26·2, 26·1	18·6, 18·7, 18·3	29·7, 29·5, 29·8

The proposed method has been found to be applicable to a wide range of geological material and is relatively fast, one person being able to carry out a dozen accurate niobium determinations in 3 days. It was found to be free from interference by other elements, except those listed by Grimaldi, which are quoted in Table II.

TABLE II

INTERFERENCES LISTED BY GRIMALDI[1]

Substance	Amount present	Error caused
Oxides—		
CaO, MgO, Fe_2O_3, Al_2O_3	50 mg each	Nil
Y_2O_3, Ce_2O_3	25 mg each	Nil
CoO, Cr_2O_3, ZnO, NiO, Sc_2O_3, Sb_2O_3, PbO, Bi_2O_3, ThO_2	10 mg each	Nil
TiO_2	4mg	Nil
	10 mg	+0·3 μg of Nb_2O_5 per ml in 1 to 10 μg of Nb_2O_5 per ml
ZrO_2	2·5 mg	Nil
	3 mg	−0·2 μg of Nb_2O_5 in 10 μg of Nb_2O_5 per ml
	5 mg	−0·6 μg of Nb_2O_5 in 10 μg of Nb_2O_5 per ml
Ta_2O_5	2 mg	Nil, if extraction and measurement is carried out within 20 minutes of complex formation
Sulphates—		
SO_4^{2-}	—	Decreases intensity of colour of complex
$K_2S_2O_7$*	0·3 g	−2 per cent. in 1 to 10 μg of Nb_2O_5 per ml
	0·6 g	−7 per cent. in 1 to 10 μg of Nb_2O_5 per ml
Ions—		
Re^{VII}	4·6 μg per ml	Equivalent to 1 μg of Nb_2O_5 per ml
W^{VI}	9·5 μg per ml	
Pt^{IV}	11 μg per ml	
V^{V}	28 μg per ml	
Mo^{VI}	60 μg per ml	
Cu^{II}	880 μg per ml	
Au^{III}	1100 μg per ml	
U^{VI}	1700 μg per ml	

* This effect can be eliminated by using the same weight of pyrosulphate or potassium hydrogen sulphate for the samples and blank solutions.

I am indebted to Professor E. A. Vincent for his critical reading of the manuscript.

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The Detection and Estimation of Aflatoxin in Groundnuts and Groundnut Materials

Part IV.* Routine Assessment of Toxicity due to Aflatoxin B_1

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A sensitive, high-resolution thin-layer chromatographic method is described for determining aflatoxin B_1 in groundnut kernels, groundnut meals and peanut butter by fluorescence. Kieselgel G is used as absorbent and is shown to offer advantages over alumina. Aflatoxin levels are determined by a dilution technique and expressed in terms of categories.

Various methods have been described for determining aflatoxin in groundnuts and their products. In several of these, the general principle is that the extracted toxin is separated either by paper[1,2] or thin-layer[2,3,4,5] chromatography and estimated subjectively by observation of the fluorescence of the chromatogram spot in ultraviolet light of wavelength 365 mμ. In one group of methods,[1,2,3] the final determination is based upon the smallest weight of toxin giving a detectable fluorescence under carefully controlled conditions: thus, on alumina the visual limit of sensitivity has been given as 0·003 μg of aflatoxin B_1, but it was later shown that with certain groundnut extracts run on this adsorbent there was a variable "enhancement" effect and consequent overestimation of the aflatoxin level. Other methods[4,5] involve comparison with standards of pure toxin run alongside, but their applicability is limited by the difficulty of obtaining pure aflatoxin and the undesirability of frequent handling of this potentially hazardous substance.[6,7,8,9,10] Procedures based on quantitative ultraviolet spectrophotometry[11,12,13] of the aflatoxins are also extant[14,15,16]; these are more accurate than the subjective methods, but are lengthier and less suitable for routine use.

This paper describes a method suitable for the detection of both aflatoxins B_1 and G_1 in groundnut kernels, groundnut meals and peanut butter. The assessment of possible biological effects of suspect materials in terms of aflatoxin B_1 and the more definite determination of aflatoxin contents of samples without recourse to the use of pure comparison standards are also discussed.

Experimental

When Kieselgel G thin layers (508 $\pm$ 10 μ thick)† and the conditions of fluorescent excitation already prescribed were used,[3] the smallest weight of aflatoxin B_1 giving an observable fluorescence was determined as 0·0004 μg. Similarly, the visual limit of sensitivity for aflatoxin G_1 was 0·0003 μg. With this adsorbent, dilution of sample extracts to the visual extinction of the fluorescence point can still result in over-estimation of their aflatoxin contents; but the "enhancement" effect remains approximately the same between samples, as shown in the trials described below. Extracts prepared from three different samples of groundnut kernels, previously shown to contain less than 5 μg of aflatoxin B_1 per kg, were dosed with this pure metabolite at two different levels. The aflatoxin B_1 contents of the dosed sample extract were then determined by dilution to extinction of fluorescence on both alumina chromatoplates by the technique already described,[1,3] and by the present method, with Kieselgel G chromatoplates: the results are shown in Table I.

Table I shows that aflatoxin B_1 contents determined by the dilution technique with Kieselgel G thin layers are less subject to variation between samples and concentrations than those obtained with alumina. Over-estimates of the actual aflatoxin content nevertheless still occur with this adsorbent, and in the sample extracts instanced in the Table, the actual aflatoxin level is over-estimated by a factor of 1·2 to 1·5 (based on the individual

* For details of earlier parts of this series, see reference list, p. 496.

† This thickness corresponds to a 0·020-inch feeler gauge.

replicates). Numerous further determinations carried out in these laboratories on two samples of homogenised groundnut meals (particle size, less than 250 μ), whose aflatoxin B_1 contents have been determined[16] by ultraviolet spectrophotometric assay as 10,200 $\pm$ 190 μg per kg and 6500 $\pm$ 200 μg per kg, gave results of 12,500 to 14,000 and 6000 to 8000 μg per kg, respectively.

TABLE I

RECOVERY OF AFLATOXIN B_1 FROM DOSED SAMPLES

Actual aflatoxin B_1 content of dosed extract, μg per kg*	Aflatoxin B_1 content determined†— on alumina thin layers, μg per kg	Aflatoxin B_1 content determined†— on Kieselgel G thin layers, μg per kg
1000	1900	1250
500	1100	600
1000	2500	1250
500	1100	600
1000	5600	1250
500	1100	600

* In conformity with international trends, results are now expressed as μg per kg, rather than as parts per million (p.p.m.). Division by 1000 converts μg per kg to p.p.m.
† Results quoted represent means of duplicate determinations.

The method described below involves de-fatting of the groundnut material, extraction of the toxin with methanol, the preparation of a purified chloroform solution of the toxin and subsequent thin-layer chromatography of three portions of the chloroform extract on Kieselgel G. Examination of the resulting chromatogram in ultraviolet light of wavelength 365 mμ permits the toxicity level of the sample to be classified as very high (greater than 1000 μg per kg), high (250 to 1000 μg per kg), medium (50 to 250 μg per kg) or, low or negative (less than 50 μg per kg). For edible-grade products, the lower limit of sensitivity may be extended to include material within the range 5 to 50 μg of aflatoxin B_1 per kg. Assignment to fairly broad categories suffices for most purposes, any over-estimation through the "enhancement" effect erring on the side of safety, although the variability of this effect makes difficult the attempt to determine precise aflatoxin levels. The use of pure comparison standards run alongside does not overcome this difficulty.

Since the solvent system described in this paper does not effect the separation of aflatoxins B_1 and B_2 in a single development, the contribution of aflatoxin B_2 to the total aflatoxin B fluorescence should be known, if toxicity estimates based on aflatoxin B_1 alone are to be reliable. In methanolic solution, the fluorescence of aflatoxin B_2 is eight times that of B_1.[17] On Kieselgel G thin layers, under the prescribed[3] conditions of excitation, the smallest weight of aflatoxin B_2 giving an observable fluorescence has been determined as 0·0003 μg, compared with 0·0004 μg for aflatoxin B_1. Since the amount of aflatoxin B_2 in both naturally and artificially produced toxins is negligible,[13,15] it follows that the contribution of aflatoxin B_2 to the total aflatoxin B fluorescence on these thin layers may be ignored, particularly as aflatoxin B_2 has only one quarter the toxicity of aflatoxin B_1.[17]

METHOD

APPARATUS—

The glass apparatus detailed below must be thoroughly cleaned with dichromate mixture and washed free from acid before use. Standard interchangeable-joint glassware may be used when appropriate.

Soxhlet extractor—Capacity, 100 ml.
Coil-condenser—Effective length, 150 mm.
Boiling-flasks—Short necked, 250-ml capacity.
Water-bath, 6-hole—To accept 250-ml flasks.
Separating funnels—Conical, 250-ml capacity.
Filter-funnels—Diameter, 8 cm.
Calibrated flasks—Capacity, 25 ml and 50 ml.
Pipette—Capacity, 5 ml.
Drummond microcaps—Capacity, 5 μl, 10 μl and 20 μl.
Apparatus for coating plates with thin layers. (The "Camag" apparatus is suitable.)

Chromatographic tank—Size, 8 × 5 × 3 inches.

Fluorescent lamp—Philips type HPW, 125 watts, (wavelength 365 mμ), in a suitable starter unit.

Extraction thimbles.

REAGENTS—

All reagents should be of analytical-reagent grade.

Light petroleum—Free from aromatic hydrocarbons, boiling-range 40° to 60° C.

Methanol.

Diethyl ether.

Chloroform.

Sodium sulphate, anhydrous.

Chromatographic silica gel—Kieselgel G, obtainable from E. Merck & Co. Inc.

SAMPLING—

Careful sampling is an essential preliminary to the method, and with whole groundnuts this operation is exceptionally difficult, since the toxicity may be associated with a very small proportion of affected kernels. The analytical sample (20 g) must be representative of a trade sample, which may weigh up to 5 kg (trade samples of less than 0·5 kg are unlikely to be truly representative), and this analytical sample should be obtained by a process of comminution and quartering.

PREPARATION OF THIN LAYERS OF SILICA GEL—

Stir 100 g of Kieselgel G with 220 ml of water for 20 minutes. The resultant slurry is sufficient to coat 18 to 20 glass plates (20 × 10 × 0·15 cm). Coat the glass plates to a thickness of 508 ± 10 μ by means of a suitable coating apparatus, and leave the coated plates on the bench at room temperature in a dust-free atmosphere for 1 hour. Transfer the plates to a forced-draught oven for 1 hour at 100° C, and finally cool them at room temperature in a dust-free atmosphere for 30 minutes before storing them in a desiccator over "tell-tale" silica gel.

PROCEDURE—

(*a*) *De-fatting*—Extract 20 g of comminuted (see Note 1) groundnut material with light petroleum in a 100-ml Soxhlet extractor (syphon-rate, 10 to 12 changes per hour) for the appropriate period according to the material (see Note 2).

(*b*) *Extraction of the toxin*—Dry off residual solvent from the thimble containing the de-fatted material in a forced-draught oven at 65° C for 30 minutes. Replace the thimble in a clean 100-ml Soxhlet extractor and extract the material with methanol for 4 hours (syphon-rate, 6 changes per hour). Concentrate the methanolic extract to 50 ml and transfer it to a 250-ml separating funnel. Rinse the extraction flask with 25 ml of water and add the rinsing to the separator. It may be necessary to add a further 5 ml of water to the extraction flask if an appreciable amount of solid material remains in the flask; transfer this rinsing to the separator as before.

Wash the flask with 25 ml of chloroform, transfer the washing to the separator and shake it thoroughly; allow the chloroform layer to separate, and run off this layer through a bed of 10 g of sodium sulphate (see Note 3). Repeat the extraction three more times with 25-ml portions of chloroform and combine the extracts. Concentrate the combined extracts, and make up to 25 ml; this is solution A. Dilute 5 ml of solution A to 50 ml with dry chloroform; this is solution B.

(*c*) *Thin-layer chromatography of the toxin*—Spot (see Note 4) 10 μl of solution A and 5 and 20 μl of solution B 1 cm from one edge of a chromatoplate, and develop it in subdued light with 5 per cent. of methanol in chloroform until a solvent path-length of 10 cm from the base-line has been obtained (see Note 2 for procedure to be followed when no preliminary de-fatting procedure has been carried out).

(*d*) *Assessment of toxicity levels*—Examine the dry, developed chromatoplate at a distance of 30 cm from a Philips fluorescent lamp, and observe the presence or abscence of a purple-blue fluorescent spot of R_F value 0·5 to 0·55 (see Note 5) corresponding to aflatoxin B_1. The toxicity level of a sample may now be classified, in terms of aflatoxin B_1, on the basis of the presence or absence of fluorescence of spots of R_F value 0·05 to 0·55 as shown in Table II.

TABLE II

CLASSIFICATION OF AFLATOXIN B_1 LEVELS

Size of portion loaded	Concentration of aflatoxin, μg per kg: no fluorescence observed	Concentration of aflatoxin, μg per kg: fluorescence observed	Aflatoxin B_1 level if fluorescence is observed
5 μl of solution B.. ..	1000	1000	Very high
20 μl of solution B.. ..	250	250 to 1000	High
10 μl of solution A.. ..	50*	50 to 250	Medium

* Samples containing less than 50 μg of toxin per kg are classified as of low or zero aflatoxin B_1 levels.

(*e*) *Assessment of aflatoxin B_1 levels below 0·05 p.p.m.*—If greater sensitivity is required, as in the examination of edible grade groundnuts or peanut butter, proceed as described below—

Concentrate 10 ml of solution A to 2 ml, load 20 μl on to a chromatoplate and develop and examine the chromatogram as described above. If no fluorescence is observed at this concentration, the sample under examination contains less than 5 μg of aflatoxin B_1 per kg.

(*f*) *Location of other toxic metabolites of* Aspergillus flavus *on developed chromatoplates*—The chromatograms of certain samples may exhibit a second greenish-blue fluorescent spot of R_F value 0·45 to 0·50, corresponding to aflatoxin G_1. No samples of naturally infected groundnut material appear to contain this toxic metabolite in the absence of the more highly toxic aflatoxin B_1, and, further, aflatoxin G_1 is usually present in much smaller proportion.

NOTES—

1. It is recommended that representative samples of groundnut meal or expeller cake should be ground in a laboratory-type hammer-mill until the ground material passes a British Standard 18-mesh sieve. De-corticated whole and broken groundnuts should be comminuted in a vegetable slicing and shredding machine until the ground material passes a 10-mesh sieve.

2. Groundnut meals and expeller cakes should be extracted for 2 hours. Crushed groundnuts will require a 4-hour extraction period. Solvent-extracted meals, which normally contain less than 2 per cent. of oil, need not be de-fatted at this stage, the small amount present being extracted with the toxin by methanol. Its later removal may be effected conveniently by developing the loaded chromatoplates over a 12-cm path-length in diethyl ether before development in the recommended methanol - chloroform system. De-fatting of peanut butter may be effected by the procedure described below—

Weigh 20 g of peanut butter into a 250-ml beaker and add 75 ml of light petroleum. Warm gently on a water-bath to 35° to 40° C, with constant stirring for 5 minutes. Place a 7-cm filter-paper (Whatman No. 1) in a Buchner funnel surmounting a 1-litre flask, and add 6 g of Hyflo Supercel to form a flat filtration bed in the funnel. Filter the light-petroleum slurry through the Hyflo Supercel bed using gentle suction until the light petroleum has been removed. Wash the beaker and the filtration bed with a further 25 ml of light petroleum, removing with gentle suction as before. Continue suction until the layer of partially de-fatted groundnut material just detaches from the sides of the Buchner funnel. Break up the dry groundnut material and the filter aid in the funnel with a spatula, and return it to the 250-ml beaker. Add 75 ml of light petroleum and warm gently at 35° to 30° C with constant stirring for further 5 minutes. Place a further 6 g of Hyflo Supercel in the Buchner funnel and repeat the filtration and drying process as before, finally drying the de-fatted groundnut material and filter aid in an oven at 65° C for 15 minutes. The dry groundnut material and filter aid is then transferred to an extraction thimble for the methanolic extraction.

It is recommended for peanut-butter samples that the loaded chromatoplates be developed in diethyl ether over a 12-cm path-length before development with the recommended methanol - chloroform solvent system.

3. Experience has shown that if the volumes of water and methanol quoted in the method are strictly adhered to, little difficulty should occur with emulsion formation during the chloroform-extraction stage. However, if emulsions are encountered, they may be broken by adding either a few drops of methanol or of water, as the circumstances may indicate. Exceptionally, continuous liquid - liquid extraction may be preferred, but this increases the over-all time of the method, since up to 6 hours' running may be needed.

4. When portions are being applied to the coated surface of a chromatoplate, the solvent should only be allowed to spread over an area defined by 0·5 to 0·7 cm in diameter.

5. If more than one spot is to be examined at one time, it is recommended that each spot be viewed individually (beginning with that of least concentration) while the other spots are covered with black paper.

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NOTE—References **1**, **2** and **3** are to Parts II, I and III of this series, respectively.

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SHORT PAPERS

The Determination of Selenium in Soils and Sediments with 3,3′-Diaminobenzidine

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The use of 3,3′-diaminobenzidine for determining selenium was described by Cheng,[1] and applied by Luke[2] to the analysis of copper and lead after the selenium had been separated by co-precipitation with arsenic. The procedure described below necessitated only minor modifications of Luke's method and has been applied successfully to soils and sediments over the concentration range 0·1 to 500 p.p.m.

In the hope of avoiding a separation, the dithizone method of Mabuchi and Nakahara[3] was attempted, but with no success. There was appreciable interference from copper at 100 p.p.m., and the selenium complex decomposed within 30 minutes, thus precluding the use of a standard series for visual colour comparison.

Method

Reagents—

Use analytical-reagent grade materials whenever possible.

Acid mixture—Mix 800 ml of nitric acid, sp.gr. 1·42, with 200 ml of 60 per cent. w/w perchloric acid.

Hydrochloric acid, 6 M—Prepare from hydrochloric acid, sp.gr. 1·18.

Arsenic solution—Dissolve 250 mg of arsenic trioxide and 2 g of sodium hydroxide pellets in 200 ml of water.

Hypophosphorous acid solution, 50 per cent. w/w, aqueous.

EDTA (disodium salt) solution, 5 per cent. w/v, aqueous.

m-*Cresol purple indicator solution*—Prepare a 0·1 per cent. w/v aqueous solution of the sodium salt.

Ammonia solution, 10 M—Prepare from ammonia solution, sp.gr. 0·88.

Formate buffer solution—Dissolve 60 g of ammonium formate in about 600 ml of water, add 200 ml of formic acid, sp.gr. 1·22, and 6 M hydrochloric acid until the pH is about 1·6. Dilute the solution to 1 litre with water.

3,3′-*Diaminobenzidine solution*—Prepare a 0·5 per cent. w/v aqueous solution of the tetrachloride. Prepare the solution afresh each day and keep it in a refrigerator when not in use. It is desirable to avoid contact between this reagent and the skin.

Benzene—Crystallisable grade.

Standard selenium solutions—Dissolve 50 mg of selenium in 10 ml of nitric acid, sp.gr. 1·42, boil the solution gently to expel brown fumes, cool, and then dilute it to 500 ml in a calibrated flask. From this solution prepare a dilute solution containing 5 μg of selenium per ml.

Procedure—

Weigh 1 g of sieved sample into a 50-ml beaker, add 10 ml of acid mixture, set the beaker aside until any effervescence has subsided and add a further 10 ml of acid mixture. Evaporate the solution to fumes of perchloric acid and remove the beaker when the volume of solution is between 1 and 2 ml. Allow it to cool, add 10 ml of 6 M hydrochloric acid and heat to boiling. Filter the hot solution through a 9-cm Whatman No. 540 filter-paper into a 50-ml beaker and wash the residue with 6 M hydrochloric acid. Add 2 ml of arsenic solution to the filtrate, mix, add 5 ml of hypophosphorous acid and boil the mixture gently until the precipitate has flocculated. Filter through a 9-cm Whatman No. 540 filter-paper and wash the precipitate with 6 M hydrochloric acid. Place the filter-paper and precipitate in a 50-ml beaker, add 20 ml of acid mixture so that the paper is completely immersed and heat to destroy the paper. When oxidation is complete,

evaporate the solution to fumes until its volume is between 1 and 2 ml. When cold, transfer the solution to a test-tube, add 1 ml of EDTA solution, 0·05 ml of indicator solution and 10 M ammonia solution until the colour of the indicator is orange. Dilute the solution to 10 ml with water, add 2 ml of formate buffer solution and 0·5 ml of 3,3′diaminobenzidine solution. Mix, and set it aside for 30 minutes. Add 2 ml of 10 M ammonia solution and extract the selenium complex by shaking the mixture vigorously for 30 seconds with 2 ml of benzene. Compare the colour with a standard series. If the intensity of colour from the sample is greater than that of the highest standard, dilute the solvent phase with benzene and again compare with the standard series.

Prepare a standard series containing 0, 1, 2, 3, 4, 6, 8, 10, 15, 20, 30, 40, 60, 80 and 100 μg of selenium. Keep this series in the dark when not in use.

Discussion

According to Cheng,[1] 5 mg each of chromium(III), molybdenum(VI), nickel(II), copper(II), and iron(III) have no effect on the reaction between 3,3′-diaminobenzidine and selenium in the presence of EDTA. However, for the present application, a far greater tolerance towards iron was necessary, and since 100 mg caused serious interference, co-precipitation with arsenic was adopted as a convenient means of separation. Of the elements that may accompany the arsenic and selenium, there is no interference from tellurium, antimony, bismuth, lead, silver and tin, and copper is masked by EDTA. In any case, these elements are unlikely to be present in the sample at more than trace concentrations.

Serious loss of selenium may occur both during the sample attack and dissolution of the arsenic precipitate if at either stage, after evaporation to perchloric acid fumes, heating is continued to dryness.

The optimum range of pH for formation of the selenium complex is 1·5 to 2·7, and that for its extraction with benzene is 4·3 to 10·3. The use of a formate buffer solution facilitates both pH adjustments, permitting the second one to be achieved by adding a fixed volume of dilute ammonia solution. It was confirmed that the minimum period for colour development at ambient temperature is 30 minutes, there being no change in subsequent colour intensity when the solution is set aside for up to 2 hours. The complex in benzene solution has an absorption maximum at 400 mμ, and although extraction is not complete in one treatment, the percentage extracted is consistent between samples and standards treated under similar conditions.

Cheng[1] found that in toluene solution both the reagent and its selenium complex have an absorption peak at 420 mμ. However, the absorption peak for the reagent may be attributed to its impurities, since 3,3′-diaminobenzidine tetrahydrochloride can vary greatly in quality; a good batch will give a pale-yellow aqueous solution, with no absorption peak at 400 mμ when extracted with benzene. This solution will be stable for at least 1 day if stored in a refrigerator, and will yield a colourless zero standard, but if stored at room temperature the colour of the reagent solution deepens to red and this solution gives a brown zero standard. The standard series, if kept in the dark when not in use, will be stable over an 8-hour day.

For sample extracts more intense than the top standard, the solvent phase is diluted with benzene until a suitable intensity of yellow is obtained, and the solution can be compared with the standard series without further extraction. This dilution technique is satisfactory for at least 500 μg of selenium.

The method has also been applied without alteration to vegetation samples, by using 1 g of milled material. Natural waters may be analysed by evaporating 500 ml of sample with 20 ml of acid mixture in a 600 ml beaker; when the perchloric acid is fuming, the solution is cooled and transferred to a 50-ml beaker and the procedure followed as described for soils. Larger amounts of sample may be taken by adding successive 500-ml volumes to the concentrated sample solution.

Results

A standard deviation of ±8·3 p.p.m. was obtained for a sample with a mean value of 62 p.p.m., and ±0·7 p.p.m. for one at 2 p.p.m. The recovery of selenium, obtained when standard solutions were subjected to the whole procedure, was 85 to 90 per cent. for 20 μg and 93·3 per cent. for 60 μg. Results obtained by the proposed method are compared in Table I with those by distillation and visual comparison of elemental selenium using the method of Ward *et al.*[4]

An average rate of 25 samples per 8-hour day is achieved by one analyst. To improve sensitivity, sample weights up to 10 g have been taken without changing the procedure, but this results in loss of productivity.

TABLE I

COMPARISON OF SELENIUM RESULTS OBTAINED BY THE PROPOSED PROCEDURE AND BY DISTILLATION AND SUBSEQUENT VISUAL COMPARISON OF ELEMENTAL SELENIUM

Sample number	Description of sample	Selenium, p.p.m., found by— proposed procedure	distillation
1	Peat-rich, stream sediment	86	80
2	Sandy, stream sediment	21	30
3	Sandy, stream sediment	4	<10
4	Sandy, stream sediment	4	<10
5	Peaty soil	52	50
6	Sandy, clay loam	6	10
7	Ferruginous, peaty mud	80	70
8	Fine sand and silty stream sediment	2	<10
9	Peaty mud	18	15
10	Peaty mud	140	125

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Determination of Chloride in Beer by Radioactivation Analysis

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NEUTRON-ACTIVATION analysis has been used by several investigators for the quantitative determination of chlorine in various materials.[1,2,3] As a contribution to these studies, chloride has been determined in beer by using radiochemical separations.

Chloride represents one of the predominating factors governing the quality of beer, affecting mainly its gustatory properties. High concentrations of chloride in beer give it a bad taste and smell.

When any material is irradiated with neutrons, various nuclear reactions take place. The reaction of interest in the chloride determination[4] is—

$$^{37}\mathrm{Cl}\ (\mathrm{n},\gamma)\ ^{38}\mathrm{Cl}$$

which is produced by thermal neutrons. The properties associated with the reaction are—

Abundance of chlorine-37 = 24·6 per cent.; half-life of chlorine-38 = 37·29 minutes.

Cross-section of chlorine-37 = 560 ± 120 mb; radiation produced = multiple β-particles, as well as γ-rays at 1·60 and 2·15 MeV.

Possible interference is caused by the reactions—

$^{38}\mathrm{A}\ (\mathrm{n},\mathrm{p})\ ^{38}\mathrm{Cl}$—in an argon matrix,
$^{41}\mathrm{K}\ (\mathrm{n},\alpha)\ ^{38}\mathrm{Cl}$—in a potassium matrix,

and from the second-order reaction—

$$^{36}\mathrm{S}\ (\mathrm{n},\gamma)\ ^{37}\mathrm{S} \xrightarrow{\beta} {}^{37}\mathrm{Cl}\ (\mathrm{n},\gamma)\ ^{38}\mathrm{Cl}$$—in a sulphur matrix.

Method

Reagents—

Use materials of analytical-reagent grade.

Standard ammonium chloride solution—Prepare an aqueous solution of ammonium chloride to contain 2 mg of chlorine per ml.

Ammonium chloride carrier solution—Prepare an aqueous solution of ammonium chloride to contain 10 mg of chlorine per ml.

Sodium nitrate hold-back carrier solution—Prepare an aqueous solution of sodium nitrate to contain 10 mg of sodium per ml.

Sulphuric acid, sp.gr. 1·84.

Nitric acid, 2 and 0·1 N.

Silver nitrate solution, 1 per cent., aqueous.

Sodium nitrate solution, 1 per cent., aqueous.

Ethanol, absolute.

Diethyl ether.

Irradiation—

Transfer 12 ml of beer by pipette into a specially designed polythene snap-closure tube.[5] Use another small polythene snap-closure tube (10 × 40 mm) containing 1·2 ml of standard ammonium chloride solution as a standard, place it in the central tube and stopper the latter at its base. Put the target in a waterproof polythene bag.

Irradiate the target for 30 minutes in a neutron flux of 6×10^{10} neutrons per sq. cm per second. (We used the "Democritus" swimming-pool reactor.)

Chemical separation[6]—

After irradiation, quickly open the sample behind a shield. Take a 5-ml portion of the beer (sampling in duplicate) and transfer it by pipette to a 100-ml spherical flask containing 1 ml of ammonium chloride carrier solution, 1 ml of sodium nitrate hold-back carrier solution and a few pieces of porcelain (to prevent violent foaming of the beer during distillation). Add 10 ml of sulphuric acid and stopper the flask immediately (to prevent loss of the small amount of hydrogen chloride produced) with a stopper having two side tubes, one being used for introducing air from an air-pump and the other being used as an air-condenser for cooling the hydrogen chloride produced.[7] Immerse the air-cooled tube in a 50-ml centrifuge tube containing 20-ml of 0·1 N nitric acid, 7·5 ml of silver nitrate solution and 1 ml of sodium nitrate hold-back carrier solution. Start the distillation of hydrogen chloride by gently heating the flask with a bunsen burner, and stop heating when white fumes of sulphur trioxide appear; the duration of the distillation procedure is about $7\frac{1}{2}$ minutes. Heat the precipitate of silver chloride that will have formed in the 50-ml centrifuge tube for 1 minute, and then filter it under suction through a pre-weighed 15-mm diameter Whatman No. 42 filter-paper by using a Hirsch G-45 filter funnel. Wash the silver chloride precipitate with three 5-ml portions of 2 N nitric acid, then with 1 per cent. sodium nitrate solution, water, absolute ethanol and finally with diethyl ether. Transfer the filter-paper and precipitate into a pre-weighed culture tube, and count the γ-ray emission. After counting, heat the tube and precipitate at 110° C, cool it and re-weigh until constant weight is obtained (which gives 90 per cent. of the chemical yield).

Take a 0·5-ml aliquot of the standard ammonium chloride solution in duplicate and subject it to the procedure above, as a standard.

Determination of radioactivity—

Make the radioactivity measurements by using a well-type scintillation crystal connected to a single-channel analyser adjusted to count γ-rays of energy greater than 1 MeV.

Results

Identification and control of radiochemical purity of chlorine-38—

In order to identify chlorine-38 and to be assured of its radiochemical purity, a γ-ray spectrometric examination and half-life determination were made on the radioisotope in the isolated precipitate of silver chloride.

The γ-ray spectrometric examination was made to find whether or not there was any γ-ray contaminant in the isolated precipitate of silver chloride coming from the analysed sample. Thus,

20 minutes after withdrawal from the reactor, the silver chloride was quantatively examined by scintillation counting with a 256-channel pulse-height analyser. The energy spectrum examined showed no indication of any γ-ray-emitting radionuclide except chlorine-38 at 1·60 and 2·15 MeV.

The half-life of the isolated radioelement was determined by plotting a decay curve for the isolated precipitate of silver chloride in both the standard and beer samples on "semilog" graph paper. The half-life was obtained from the slope of the straight line, which was calculated by the method of least squares. A value of 38·5 minutes, with a standard error of $\pm$0·1 minute was found. This served as an additional indication of the radiochemical purity of chlorine-38.

Comparison of methods—

Quantitative results for the chloride content of beers of different types and origins have been obtained by the radiochemical method and the classical gravimetric method, which involved precipitating and weighing the silver chloride precipitate from a 50-ml sample of beer. The results are given in Table I. The correlation coefficient for the two series of results was computed to ascertain the agreement between the two methods. This indicated a very good correlation at the 1 per cent. significance level. However, a *t*-test of significance on the differences between the results obtained by the two methods indicates that the results by the conventional gravimetric method are significantly higher than those obtained by radioactivation analysis. This is possibly due to the occlusion of the silver chloride precipitate with other elements present in the beer samples. During the activation analysis, however, the distillation procedure eliminated such interfering elements.

Table I: Analysis of beer samples

Sample	Concentration of chloride, p.p.m.,* found by— neutron-activation analysis	classical gravimetric analysis
Greek beer, 330 g	148	160
Greek beer, 500 g	230	300
Greek lager	242	263
Greek beer (Pilsner type) ..	200	250
Greek beer (Pilsen type) ..	183	170
Danish beer	155	150
Danish beer	138	178
Dutch beer	140	178
Dutch beer	261	265

* Each determination was made at least in duplicate.

Discussion

The analytical method proposed uses radiochemical separations, and results in remarkable purity of the isolated precipitate of silver chloride. Experimental values are reproducible with a relative error of less than $\pm$2 per cent. The sensitivity of this method reaches the value 0·001 p.p.m. if a higher neutron flux is used (10^{13} neutrons per sq. cm per second). The determination of chloride in beer by a non-destructive technique, without a chemical separation of chloride, was not possible, owing to the presence of traces of manganese in beer, since the energy peaks of the radionuclides formed, chlorine-38 and manganese-56, are very close.

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The Absorptiometric Determination of Antimony: Extinction Coefficient for the Tetraiodoantimonate(III) Ion

By R. A. WASHINGTON

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During the analysis of lead samples for antimony, it was necessary to prepare a standard curve of the percentage transmission of hydrogen tetraiodoantimonate(III) at 425 mμ. The results obtained can be used for calculating the extinction coefficient for the tetraiodoantimonate(III) ion, and because of the sparsity of this information in the literature, it was thought that the present results should be reported briefly.

The analytical method used was essentially that described by Elkind, Goyer and Boltz,[1] and by McChesney,[2] and summarised in "Tables of Spectrophotometric Data."[3] Known amounts of standard antimony solution were added to a 50-ml calibrated flask, 25 ml of potassium iodide reagent was added, and the solutions were diluted to the mark with dilute sulphuric acid to make the final acid concentration 2·5 to 3·5 N. The standard antimony solution was prepared by dissolving 0·274 g of antimony potassium tartrate in water, adding 160 ml of sulphuric acid, and diluting to 1 litre with water. For small amounts of antimony, this solution was diluted by a factor of 10 with 3 M sulphuric acid. The percentage transmission was measured at 425 mμ in 1-cm cells. Readings were made against a blank solution prepared by diluting 25 ml of potassium iodide reagent to 50 ml, and by using dilute sulphuric acid to make the final acid concentration about 3 N.

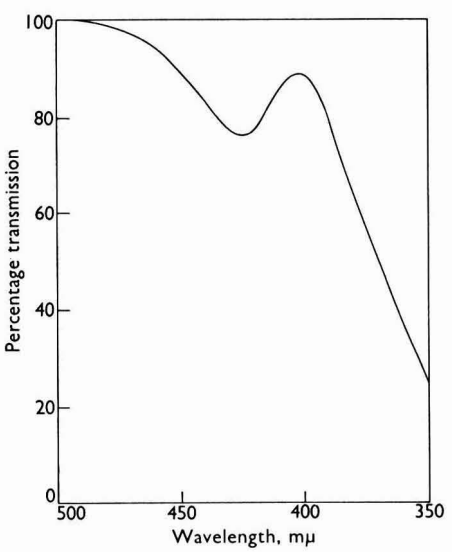

Fig. 1. Absorption spectrum of the tetraiodoantimonate(III) ion

A typical absorption spectrum is shown in Fig. 1. The results are summarised in Table I. The values of the extinction coefficient were calculated from the equation—

$$\log \frac{I}{I_0} = -\epsilon cl$$

where $\frac{I}{I_0}$ is the transmission, c is the concentration in moles per litre and l is 1 cm.

TABLE I

RESULTS FOR EXTINCTION COEFFICIENT OF TETRAIODOANTIMONATE(III) ION

Concentration of antimony, moles per litre	Percentage transmission	Extinction coefficient
$3{\cdot}28 \times 10^{-6}$	97·5	$3{\cdot}36 \times 10^{3}$
$8{\cdot}20 \times 10^{-6}$	93·0	$3{\cdot}84 \times 10^{3}$
$1{\cdot}64 \times 10^{-5}$	86·0	$3{\cdot}99 \times 10^{3}$
$2{\cdot}46 \times 10^{-5}$	76·0	$4{\cdot}85 \times 10^{3}$
$3{\cdot}28 \times 10^{-5}$	69·5	$4{\cdot}82 \times 10^{3}$
$8{\cdot}20 \times 10^{-5}$	40·8	$4{\cdot}57 \times 10^{3}$
$1{\cdot}64 \times 10^{-4}$	17·8	$4{\cdot}57 \times 10^{3}$
	Mean $(4{\cdot}31 \pm 0{\cdot}58) \times 10^{3}$	

Application of Chauvenet's criterion to the results showed that none of the values should be rejected. The error indicated in the mean value is the calculated standard deviation.

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The Detection of Thiophosphate Insecticides on Paper Chromatograms with Congo Red

BY A. IRUDAYASAMY AND A. R. NATARAJAN

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SEVERAL methods[1 to 8] involving the use of more than one spray reagent have been reported for locating organo-phosphorus insecticides on paper chromatograms. Recently, Dutt and Seow[9] have discussed the relative merits of the various reagents used in the detection of esters of thiophosphoric acid. These authors have also described the use of Metanil yellow, Yellow R.F.S. or methyl orange as a single spray reagent for the detection of some thiophosphate insecticides with a detection limit of 1 to 2 μg, but the spots so developed on the chromatograms were found to fade away overnight.

In a search for a more sensitive single spray reagent that would give relatively stable spots, the use of several dyes were investigated by us. It was found that if, after exposure to bromine vapour, the chromatogram was sprayed with Congo red, blue spots appeared on a red background colour. The proposed method is much simpler and more sensitive than the other existing methods, and gives adequate resolution of the insecticide studied. When a series of twelve determinations was made on each of four insecticices, the R_F values had a standard deviation of less than ± 4 per cent. The results are given below—

Insecticide	Baytex	Diazinon	Malathion	Parathion
R_F value	0·81	0·56	0·90	0·71

EXPERIMENTAL

Separation of the esters of thiophosphoric acid was accomplished by reversed-phase chromatography. Impregnated paper for the work was prepared by soaking Whatman No. 1 chromatographic paper strips in a 15 per cent. v/v liquid Paraffin B.P. in light-petroleum (boiling-range 60° to 80° C) solution, and then allowing the solvent to evaporate at room temperature for 1 hour. The treated papers were spotted with 1 or 2 μl of an ethanolic solution of the insecticide and then developed with the solvent running in the machine-direction of the paper by the ascending-solvent technique at the ambient temperature, with dimethylformamide - n-butanol - water (14 + 1 + 5) mixture as the mobile phase over a 9-cm run ($\sim$3 hours). It was not found necessary to equilibrate the mobile with the stationary phase.

After the chromatogram had been developed, the paper was thoroughly dried by letting it stand at room temperature (about 27° C). The paper was exposed for about 20 seconds to bromine vapour from a 10 per cent. v/v solution of bromine in carbon tetrachloride. The paper was then allowed to stand in air for a few minutes to remove the final traces of bromine from the paper. Residual bromine in the paper should be avoided, as the heavy background colour formed will obscure the spots. The paper was then sprayed with a 0·4 per cent. v/v solution of Congo red in 50 per cent. v/v aqueous ethanol.

Results and discussion

The Congo red spray reagent gives clear blue spots against a red background. The mechanism of the reaction is that the phosphorothioate ion reacts with bromine under anhydrous conditions to form the sulphenyl bromide,[10] which is readily hydrolysed by the aqueous ethanol of the spray to yield hydrobromic acid. The resultant hydrobromic acid turns the Congo red blue. The spots are stable for over 10 days if protected from light; otherwise the spots are stable only for about 4 days, and tend to fade away thenceforth. Locally available thiophosphate insecticides, Baytex (*OO*-dimethyl-*O*-[4-(methylthio)-*m*-tolyl]-phosphorothioate), diazinon, malathion and parathion gave responses at the 0·5-μg level.

The technique has also been applied to the detection of the above-mentioned phosphorothioates in samples of vegetable and animal origin. The co-extractives from animal tissues and vegetable matter such as apples, grapes, oranges, tomatoes, potatoes or cabbage cause blue spots at the points of origin of the chromatogram. The co-extractives, which are retained by the stationary solvent phase, exhibit little or no tendency to move with the mobile phase. The co-extractives at various levels are well tolerated, and their presence was found not to alter appreciably the R_F values of the thiophosphate insecticides.

We thank Bayer (India) Ltd., Tata Fison Ltd., Cyanamid India Ltd. and the Monsanto Chemicals of India Private Ltd. for their generous gifts of the pesticides.

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A Method of End-point Location in Constant-current Coulometry

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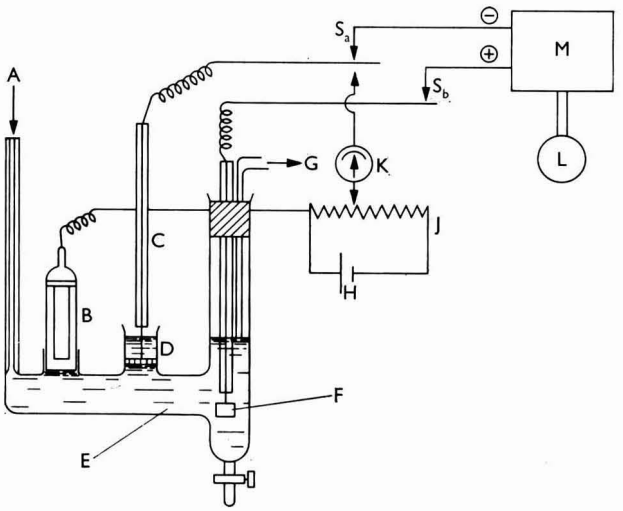
A preliminary investigation has been made of a coulometric technique, in which the end-point of the titration is determined by noting the voltage change between a polarised working electrode immersed in the solution under test and a reference electrode, at a measured time after interruption of the constant-current supply. Such interruptions of current and the voltage readings are made at intervals, and a graph is then plotted of the readings against time of current flow. The voltage changes thus observed are changes in the reversible electrode potential of the working electrode *plus*, possibly, a contribution due to changes in the partly decayed overpotential of this polarised electrode.

Present coulometric practice usually requires one or two indicating electrodes besides the working electrode to be accommodated in the solution under examination, and one of the advantages of this technique is that the size of the electrolysis half-cell can be reduced, since it is required to contain only one electrode (which can be a platinum wire) that functions as a working and indicating electrode alternately.

This process is at the moment empirical, in that the readings thus determined have not yet been precisely related theoretically to concentration changes. Nevertheless, the maximum rate of voltage change found experimentally appears to coincide with the maximum rate of change of the reversible electrode potential for the systems that have been examined.



A = Inlet for sodium sulphate solution
B = Calomel (or other) reference electrode
C = Cathode
D = Cathode compartment
E = Electrolyte
F = Anode
G = Outlet for sodium sulphate solution
H = 3-volt dry battery
J = High-resistance calibrated potentiometer
K = Null-point galvanometer
L = Clock
M = Constant-current supply
S_a,S_b = Switches

Fig. 1. Diagram of apparatus

Experimental

Apparatus—

The apparatus is illustrated in Fig. 1. M denotes the constant-current supply unit with associated clock. Current supply and clock can be started and stopped simultaneously by a switch on the unit (not shown). Any source of constant current can be used; the present work was carried out with a one-current (about 7 mA) supply version of the circuit used by Glass and Moore.[1] The current is fed through the switch, S, to the cathode, C, and anode, F. The half-cell consists of the small (about 1·5-ml capacity) compartment, D, containing the cathode, joined with a sintered-glass base to the main unit. B is a calomel (or other) reference electrode. The inlet, A, allows a solution of sodium sulphate (about 2 M) to flow steadily through the anode-containing compartment; this solution escapes at G. The purpose of the flowing solution is to maintain an anolyte of constant composition. The tube, G, is attached to a suction pump, and its height adjusted so that solution is not sucked from, nor forced into, D. The voltage of the cell comprising B and C, when the constant-current supply is cut off and the switch S operated, can be read on a valve voltmeter (the millivolt scale on a pH meter) or as shown in Fig. 1, where K is a null-point galvanometer and J is a high-resistance (about 50 KΩ) calibrated potentiometer, activated by the large-size 3-volt dry battery, H. The use of this simple equipment is described.

Operation—

A measured amount of the substance (in these preliminary experiments about 5×10^{-5} equivalents was convenient) is introduced into C together with about 0·5 ml of saturated potassium chloride solution. Water may be added to effect dissolution if necessary, the total volume being about 1 ml. The solution is stirred by bubbling through it a stream of nitrogen (not shown in Fig. 1), and the current and clock switched on. After a minute or so, current and clock are switched off, and precisely 10 seconds later, S is operated, breaking contact with the current-supply unit

and making contact with J and K. J is rapidly adjusted until zero deflection occurs, and potentiometers and clock readings are recorded. After 10 seconds, the rate of change of voltage becomes small, and consequently fluctuations in the time spent in manipulating the potentiometer are of little importance. S is then re-set in the position shown, and clock and current re-started. After a further minute or so, the cycle of operations is repeated, and this is done (noting time and potentiometer readings on each occasion) until the titration is complete; this is shown by the sudden large change in the potentiometer readings.

Coulometric titrations tested

Titration of acid—

The electrode reactions are—

$$(a)\ 2H^+ + 2e = H_2 \text{ or } (b)\ 2H_2O + 2e = H_2 + 2OH^-$$

The titration is carried out as a direct titration, and some typical results are shown in Fig. 2, curves A and B.

Titration of base—

In this instance, a back-titration is done after addition of a known excess of acid.

Titration by generation of iron(II) ions—

A titration of dichromate ions by generation of iron(II) ions is shown in Fig. 2, curve C.

In these titrations, the capacity of the unit was $4{\cdot}23 \times 10^{-6}$ equivalents per minute.

The recoveries in most instances are low compared with those obtained by conventional methods.

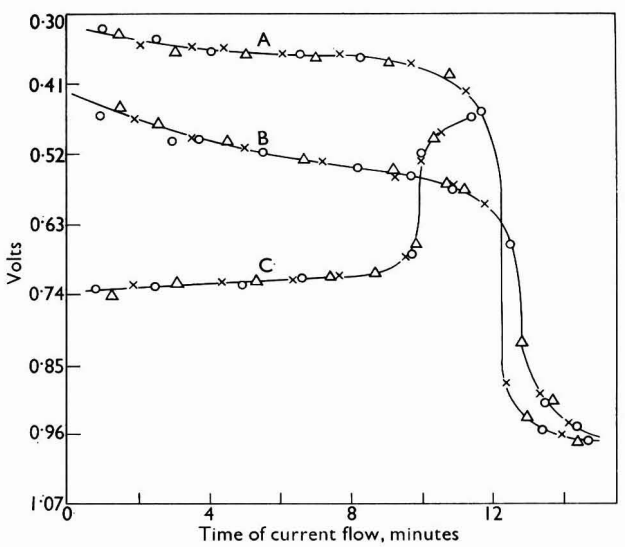


Fig. 2. Titration curves for triplicate determinations (o, △, x) of 0·5-ml portions of hydrochloric acid, acetic acid and potassium dichromate—

Curve	Sample	Mean titration time found, minutes	Equivalents taken	Equivalents found	Recovery, per cent.
A	0·1023 N hydrochloric acid	11·95 ± 0·02	$5{\cdot}12 \times 10^{-5}$	$5{\cdot}06 \times 10^{-5}$	98·8
B	0·1069 N acetic acid	12·50 ± 0·02	$5{\cdot}35 \times 10^{-5}$	$5{\cdot}29 \times 10^{-5}$	98·9
C	0·0818 N potassium dichromate*	9·60 ± 0·02	$4{\cdot}09 \times 10^{-5}$	$4{\cdot}06 \times 10^{-5}$	99·2

* Determinations made by generating iron(II) ions at the cathode from a solution of 0·5 ml of potassium dichromate added to 1 ml of fully oxidised M sulphuric acid - 0·1 M orthophosphoric acid - 2 M ferric chloride mixture.

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The Determination of Sodium Nitroprusside

A Special Application, The Determination of Small Amounts of Sodium Nitroprusside Impregnated on Cellulose Strips

By BRIAN A. SEWELL

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Current methods for the quantitative determination of sodium nitroprusside impregnated on cellulose[1,2,3] are based on the Gmelin reaction, and involve aqueous extraction of sodium nitroprusside from the cellulose (paper) strip and subsequent reaction with sodium sulphide solution to form a magenta-coloured complex[4,5,6]—

$$S^{2-} + Na_2[Fe(CN)_5NO] \longrightarrow Na_2[Fe(CN)_5NOS]^{2-}$$

Results obtained with this method were low and inconsistent. Errors were shown to be due to two main factors, the slow decomposition of sodium nitroprusside in aqueous solution during the extraction procedure (see Fig. 1) and the rapid and serious decomposition of the coloured complex (see Fig. 2).

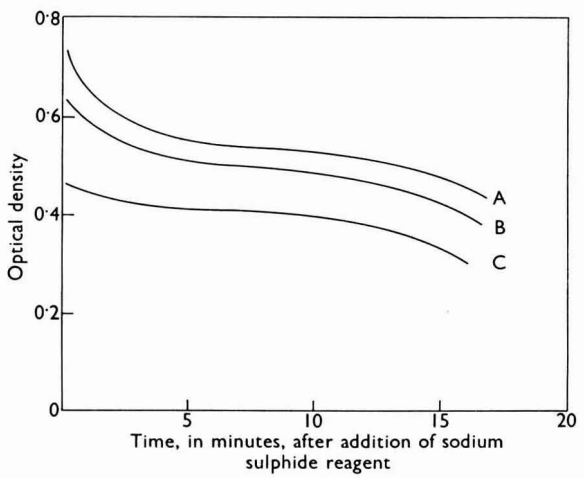

Fig. 1. Graphs showing decomposition of 10 ml of a 0·01 per cent. w/v aqueous solution of sodium nitroprusside revealed by colour formation with 2 ml of a 10 per cent. w/v aqueous solution of sodium sulphide. Curve A, fresh solution of sodium nitroprusside; curve B, 30 minutes old solution of sodium nitroprusside; curve C, 60 minutes old solution of sodium nitroprusside

The method described reduces to a minimum both of the decomposition effects occurring in aqueous solutions. Dimethylsulphoxide is used for extracting sodium nitrprusside from the paper matrix and the sulphide - nitroprusside complex is developed in a mixture of dimethylsulphoxide and isopropanol with a 2 per cent. aqueous solution of sodium sulphide. The complex obeys Beer's law and may be measured colorimetrically, a maximum absorption being obtained between $3\frac{1}{2}$ and 12 minutes (see Fig. 2).

Method

Apparatus—

Photo-electric colorimeter—An Evelyn colorimeter, or similar instrument, fitted with cells of path length 20 mm, and a 515-mμ (green) filter.

Reagents—

Dimethylsulphoxide.
Dimethylformamide.

Isopropanol.

Sodium sulphide reagent—A freshly prepared 2 per cent. w/v solution of sodium sulphide in water.

PROCEDURE—

Cut a measured area of the paper strip, or strips, known from preliminary experiments to contain about 1 to 2 mg of nitroprusside, and shake the sample with 10 ml of dimethylsulphoxide in a 25-ml stoppered cylinder for 15 minutes. Filter the extract by suction into a Buchner tube, washing the paper residue with 4 ml of isopropanol. Transfer the filtrate to a 25-ml calibrated flask, aiding complete transfer with a further 4 ml of isopropanol. Add 5 ml of sodium sulphide reagent and dilute to the mark with isopropanol, mixing well. Five minutes after the sodium sulphide reagent has been added, measure the optical density of the solution with the colorimeter, against a blank solution of 10 ml of dimethylsulphoxide, 10 ml of isopropanol and 5 ml of sodium sulphide reagent.

Construct a calibration graph for sodium nitroprusside as follows—

Weigh accurately 4, 8, 12, 16 and 20 mg of sodium nitroprusside and dissolve each separately in 100 ml of dimethylsulphoxide in 100-ml calibrated flasks. Transfer by pipette 10 ml of each solution into five 25-ml calibrated flasks. Add to each, 8 ml of isopropanol and 5 ml of sodium sulphide reagent. Dilute to the mark with isopropanol and measure the optical density after 5 minutes.

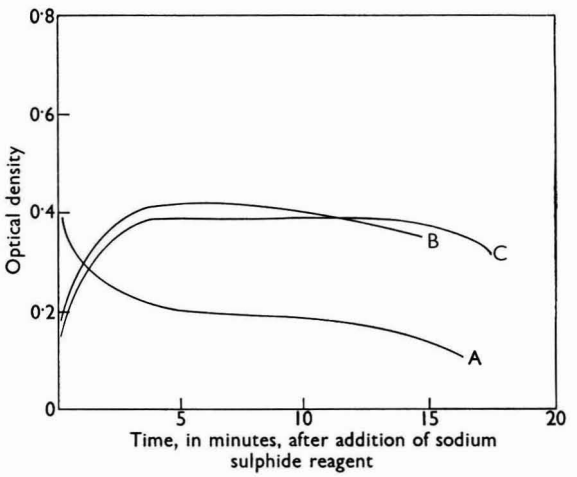

Fig. 2. Graphs showing stability, under various conditions, of coloured complex formed between 5 ml of sulphide reagent and sodium nitroprusside solution—

Curve	Strength of sodium nitroprusside solution	Addition
A	0·01 per cent. aqueous	10 ml of water
B	0·01 per cent. w/v in dimethylformamide	10 ml of isopropanol
C	0·01 per cent. w/v in dimethylsulphoxide	10 ml of isopropanol

An alternative solvent is dimethylformamide. Owing to the slightly reduced stability of the complex in this solvent (see Fig. 2), the results obtained are rather less consistent.

Dimethylsulphoxide and dimethylformamide readily absorb water from the atmosphere; therefore, care must be taken that the solvents are not left in open vessels, for any length of time, before or during use.

RESULTS

Recoveries were determined by applying to blank paper strips, by using a Hamilton syringe, 5, 7·5 and 10 μl of a 2 per cent. w/v solution of sodium nitroprusside in dimethylformamide to

give 0·10, 0·15 and 0·20 mg, respectively, per strip. After gentle evaporation of the solvent, the paper strips, in sample batches of 10, were subjected to the assay procedure.

TABLE I

RECOVERY OF SODIUM NITROPRUSSIDE

Weight of sodium nitroprusside added per strip, mg	Weight of sodium nitroprusside found, by using dimethylsulphoxide method, mg	Recovery, per cent.
0·0100	0·091	91·00
	0·099	99·00
	0·090	90·00
	0·091	91·00
	0·089	89·00
0·150	0·161	107·33
	0·155	103·33
	0·169	112·67
	0·149	99·33
	0·133	88·67
0·200	0·144	96·00
	0·193	96·50
	0·209	104·50
	0·197	98·50
	0·197	98·50

The reproducibility of the method was determined by using different batches of a nitroprusside-impregnated cellulose test strip. For each determination, the reactive areas of 10 strips were used (known to contain about 1 to 1·5 mg of nitroprusside in total). The results are shown in Table II.

TABLE II

REPRODUCIBILITY OF THE METHOD

Batch	Weight of sodium nitroprusside, mg, found in replicate determinations on test strips by using dimethylsulphoxide			
1	0·105	0·101	0·092	0·097
2	0·114	0·101	0·085	0·080
3	0·093	0·080	0·107	0·114
4	0·107	0·105	0·094	0·101
5	0·132	0·140	0·127	—
6	0·123	0·144	0·127	0·112

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Determination of Potassium Chromate and Hydrogen Peroxide in the Presence of Each Other

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A SPECTROPHOTOMETRIC method developed by the authors[1] for determining hydrogen peroxide based on the reaction—

$$H_2O_2 + 2K_3Fe(CN)_6 + 2KOH \longrightarrow 2K_4Fe(CN)_6 + 2H_2O + O_2$$

is applicable to the direct determination of hydrogen peroxide in a basic solution containing potassium dichromate.

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Determination of hydrogen peroxide

A known volume of an alkaline solution containing chromate and hydrogen peroxide is mixed with a known volume of potassium hydroxide solution of required strength. Subsequently, a known volume of potassium ferricyanide solution of desired concentration is added. The resultant mixture is diluted to a definite volume with water. The final solution should be molar with respect to potassium hydroxide; and the concentration of potassium ferricyanide must be more than double the exact concentration of hydrogen peroxide; the concentration of chromate should not exceed 1400 μM, otherwise chromate interferes with the analysis. The solution is allowed to stand for 15 minutes and its optical density is measured at 418 mμ against M potassium hydroxide as the blank solution. The optical density is corrected for the optical density due to chromate at 418 mμ measured in a separate experiment. The corrected optical density divided by 2ϵ ($\epsilon = 959$) and multiplied by the dilution factor gives the concentration of hydrogen peroxide in the original solution. In the presence of potassium dichromate at a concentration of 709 μM and for concentrations of hydrogen peroxide of 39 to 551 μM in the final solution, the standard deviation of the analysis was $\pm 4{\cdot}2$ per cent.

Hydrogen peroxide at concentrations up to 8000 μM in 0·1 M potassium hydroxide does not interfere with the spectrophotometric determinations of chromate at 373 mμ. The extinction coefficient for chromate is 4690 and it is constant above pH 9.

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First received *February 24th*, 1964
Amended *January 22nd*, 1965

Book Reviews

Theorie und Praxis der gravimetrischen Analyse. Part II. Bestimmung der Metalle. By Dr. László Erdey. Pp. 802. Budapest: Akadémiai Kiadó. 1964. Price $18.00; 129s.

Following the excellent theoretical introduction to the principles, methods and techniques of gravimetric analysis (previously reviewed in *The Analyst*, 1964, **89**, 564), this major volume, Part II, is concerned with the actual methods that are available for determining metal ions by precipitation after the addition of hydroxide ion, sulphide ion, oxine or other organic precipitants and so on, or by electrolytic precipitation, *i.e.*, electro-deposition at electrode surfaces or by internal electrolysis.

The treatment is comprehensive and is, in my opinion, most praiseworthy. Each cation is considered separately, and they are arranged in order of their precipitation according to the classical schemes of qualitative analysis based on the use of hydrogen sulphide. This arrangement preserves the unity of monographic treatment and yet shows up the comparative chemical properties of the cations in relation to each other. Within each chapter there are considered first the general principles underlying the precipitation of the principal weighing forms for the element, and second, in a separate section, the determination of the metal in relation to other metal ions, *i.e.*, methods of separation based on selective precipitation, electrolysis, etc. The separation methods are, of course, related to the precipitation - weighing forms in the earlier part of the chapter and there is a very efficient cross-indexing system maintained throughout. The book is liberally supplied with excellent thermogravimetric and derivatographic curves and there are also some very useful pH - ionic form curves for elements such as aluminium, arsenic and bismuth. There is a wealth of practical know-how scattered throughout these pages and, of course, complete working instructions for the application of the recommended procedures.

This volume ends with full author and subject indexes. Undoubtedly this is a very worthwhile book, which can be recommended in the very highest terms. It is to be hoped that we will soon be supplied with as excellent an English translation as we had previously of Volume I.

T. S. West

THEORIE UND PRAXIS DER GRAVIMETRISCHEN ANALYSE. Part III. BESTIMMUNG DER NICHTMETALLE. By Dr. LÁSZLÓ ERDEY. Pp. 340. Budapest: Akadémiai Kiadó. 1964. Price $13.00; 93s.

This volume appears at first sight to follow a slightly different pattern from Volume II, although it maintains the same general unity of monographic treatment and arrangement within each chapter. This difference is, of course, principally related to the somewhat more divergent ionic species in which the non-metals are encountered, *e.g.*, chlorine as chloride, chlorate, perchlorate, hypochlorite or as covalently bound chlorine, and sulphur as sulphide, sulphite, sulphate, persulphate and the polythionic acids. Also in this volume the author is naturally much more concerned with the determination of these elements in organic compounds and with the consequent need to discuss apparatus and techniques for the "mineralisation" of the element before application of the normal processes of aqueous inorganic gravimetric methods. Yet again, in the separation processes, the text is much more concerned with the application of "evolution" and distillation techniques than in Volume II. Once more, extensive illustration of thermogravimetric and derivatographic curves are given, and there are excellent tabular summaries of information relating to precipitation forms, contamination phenomena, line diagrams relating to distillation and organo-decomposition apparatus, etc.

Volume III ends with a tabular summary of solubility products, a very few of which may be of little practical use to the laboratory worker, and with an enormous table (pp. 38) of gravimetic conversion factors covering all the weighing forms described in Volumes II and III. For the precipitation of "nitron nitrate" factors are given, for example, for conversion to HNO_3, KNO_3, $NaNO_3$, NO_3^- and N_2O_5, and for sodium chloride as a weighing form, there are factors for conversion to forty-five "sought" forms from sodium itself, through salts such as Na_2HAsO_4 to $Na\ \{Mg[UO_2(CH_3COO)_3]_3\}\ 6{\cdot}5\ H_2O$.

This Volume III is a worthy conclusion to a first-rate compilation on the theory and practice of gravimetric analysis. One can but once more re-echo the hope that an English translation will soon become available.

T. S. WEST

THE SOLVENT EXTRACTION OF METAL CHELATES. By JIŘÍ STAŘY. Edited by Professor H. IRVING. Pp. xiv + 240. Oxford, London, Edinburgh, New York, Paris and Frankfurt: Pergamon Press. 1964. Price 60s.

This book is undoubtedly an "original," and as such one can only hail it with delight and a sense of relief that someone has at last had the knowledge and perseverance to write it. The teamwork between Dr. Stařy and Professor Irving, who has acted as editor and who is, of course, well known for his activity in this area, has provided us with a most worthwhile and stimulating book.

The text is arranged according to the plan—

(*i*) Introduction; concerning the distribution law and the relationship of solubility and extractability.

(*ii*) Comparison and Stability of Metal Chelates; a mathematical analysis of the equilibria of different types of metal chelate compounds.

(*iii*) Theory of Solvent Extraction; a semi-quantitative discursive treatment of the variables in extraction processes in general, *e.g.*, pH, reagent concentration and kinetic factors.

(*iv*) Analytical Applications; an examination of the background to the analytical utility of these reactions.

(*v*) Systems; an account of various chelating agents arranged according to the analytico-functionality of the reagents themselves.

(*vi*) Selective Extraction Procedures for Individual Metals; an account of extraction processes arranged according to the metal ions that may be extracted.

The book closes with an appendix on the solubilities and dissociation (protolysis) constants of the chelating agents described in the text, and of their partition coefficients. There is a bibliography, sub-classified according to authors' initials, and a very good subject index. The book is well turned out and is of excellent format; the price is moderate.

I have already given my general assessment of the text, but would like to single out for special recommendation the arrangement in Chapters (more appropriately, parts) 5 and 6, firstly according to reagent, and secondly according to metal ion. This treatment is identical to that used so

successfully by Morrison and Freiser in their well known "Solvent Extraction in Analytical Chemistry" of 8 years ago. It is also pleasing to find considerable attention focused on the interesting "substoicheiometry" technique—a treatment that, to the best of my knowledge, is the first one in a textbook. Undoubtedly this is a very fine book for students of analytical chemistry and for all who are concerned with separation processes in inorganic analysis. It is vitally important, however, that both categories of reader, particularly the latter, should bear in mind the monographic nature of the text, which is concerned with chelate systems only. Ion-association systems, which are also of outstanding value in analytical separations, and which make available many possibilities that cannot be realised by chelate systems, are not discussed in Chapter 6, which must not, therefore, comprehensive as it is, be regarded as an exclusive guide to solvent-extraction separation processes.

I do, however, feel disposed to criticise some of the contractive terminology used for reagents, *e.g.*, HEDTA for *trans*-1,2-diaminocyclohexane-*NNN'N'*-tetra-acetic acid, instead of the almost universally accepted CDTA, CyDTA or DCTA and so easily confused with HEEDTA (*N*-hydroxyethylethylenediamine-*NNN'N'*-triacetic acid; HAA for acetyl acetone, usually AcAc; HTTA for 2-thenoyltrifluoroacetone, usually TTA. One appreciates the reason for the incorporation of H in the last two, but it is not incorporated in EDTA (H_2EDTA, H_4EDTA?) and HEDTA becomes even more illogical. However, these are minor blemishes, largely of a subjective nature perhaps, on an altogether first-class book.

T. S. West

Analysis of Calcareous Materials. Pp. viii + 481. London: Society of Chemical Industry. 1964. Price 90s.

This S.C.I. Monograph No. 18 comprises mainly a series of papers given at a Symposium held last year in London. The preliminary pages contain a compilation of basic methods of analysis, essentially to provide a basis for the proceedings of the Symposium so that details of widely accepted analytical procedures might be avoided by contributors of papers. In the main this purpose has been fulfilled, but some of the methods appear to be unnecessarily involved, *e.g.*, the determination of carbonate and moisture. Elsewhere, reference to more up-to-date procedures would have been appropriate, *e.g.*, no mention is made of a hydropyrolytic method for determining fluorine. Even so, this is a useful collection of well established methods, and provides an admirable prologue to the proceedings.

Section I of the proceedings deals with sampling, and this is followed by a section on gravimetric, colorimetric and titrimetric procedures in which the use of EDTA figures prominently. The difficulties in recognising indicator colour changes in determining calcium and magnesium with EDTA provided a lively discussion, but it is surprising that there was not wider acceptance of the advantages of automatic or spectrophotometric methods in these titrations.

Sections III and IV, dealing with physical methods for determining elements and compounds, will undoubtedly prove most interesting to the majority of readers. In the first of these sections passing reference is made to atomic-absorption spectrophotometry, but it is suggested, somewhat unexpectedly, that this has little advantage over flame-emission spectroscopy for determining strontium and magnesium. Subsequent papers in this section deal with the application of X-ray fluorescence to the analysis of cements and clays, and stress the difficulties of sample preparation encountered in the determination of the lighter elements.

In the final section, the description of the use of X-ray diffraction studies combined with digital techniques, as applied mainly to cement analysis, is highly topical. Likewise the appearance of papers on thermogravimetry and differential thermal analysis is particularly opportune, now that more refined equipment is available for studies in both these fields.

A publication such as this is likely to have a restricted appeal, but it contains something to interest all who are in any way associated with the analysis of calcareous materials.

J. M. Skinner

Analysis of Essential Nuclear Reactor Materials. Edited by Clement J. Rodden. Pp. xiv + 1280. Washington, D.C.: U.S. Government Printing Office. 1964. Price $4.25 (paper cover); $6.25 (cloth cover).

During the past 20 years the number and variety of reactors and the analytical-chemistry problems of the materials used in them have increased considerably. The object of this volume is to collate published and unpublished information that could be of assistance to those engaged in the analysis of essential reactor materials.

The main text is divided into 16 chapters, each of which is written by workers in the appropriate field. Nine sections deal fairly comprehensively with specific materials, *e.g.*, uranium, plutonium, thorium, beryllium, graphite, alloys and ceramics, coolants, heavy water and expended reactor fuels. The importance of boron in nuclear technology has earned this element a chapter to itself. One chapter is devoted to trace elements, and the remaining sections contain reviews of information relating to specific analytical disciplines.

A good attempt has been made to provide the analyst with the necessary information to carry out many of the analytical techniques cited without a further literature search. This is no mere collection of recipes however, as the book contains a wealth of background information in very readable form, which will be of value to the expert and the novice.

The volume is well illustrated with numerous diagrams and photographs and much useful information is summarised in tabular form. The bibliographies at the end of each section contain some 3,300 references and the book is well indexed.

The authors have undertaken a monumental task and have succeeded in producing a valuable book of reference. The book is of extremely low cost in relation to the tremendous amount of very useful information it contains. The paper used in its production is of good quality, but it is feared that the paper binding will not stand up to the large amount of use this book deserves to receive. It is considered worthwhile to spend the two extra dollars and obtain the cloth-bound edition.

M. S. W. WEBB

COINCIDENCE TABLES FOR ATOMIC SPECTROSCOPY. By JOSEF KUBA, LUDVÍK KUČERA, FRANTIŠEK PLZÁK, MILOSLAV DVOŘÁK and JAN MRÁZ. Pp. xxxii + 1136. Amsterdam, London and New York: Elsevier Publishing Company. 1965. Price 130s.

The prime objective of this collection of about 100,000 wavelength values, covering the range 2000 to 10,000 Å is to simplify the interpretation of complex spectra.

In addition to the rare-earth elements, which are dealt with collectively, over 70 elements are referred to in detail.

An average of about twelve characteristic lines have been chosen for each element, and under each wavelength-heading the wavelengths of elements that lie in close proximity are listed together with the name of the element. For example, under silver, 15 wavelengths, ranging from 2246·41 to 5465·49 Å, are considered individually. Under the first heading (2246·41 Å) appears a list of arc and spark line-intensity values, ranging from those of ruthenium at 2246·38 Å, to those of tin at 2251·15 Å, and added information is indicated by the size and type of the numerical values given.

Data on those regions of the spectrum where the lines are almost coincident with the parent line should prove useful when a spectrograph with a dispersion of, say, 0·5 to 5 Å per mm is used, but this is not a limitation of the book, because the over-all wavelength-coverage is adequate to facilitate the interpretation of spectra from smaller dispersion instruments. There are exceptions to this coverage, for example, spectra of the rare-earth elements, but a large dispersion instrument is usually used when spectra of this complexity are involved.

The authors have made a thorough examination of available data on the subject, and this has resulted in the publication of a first-class reference book, with collected information simply and concisely presented.

W. T. ELWELL

HUMIDITY AND MOISTURE: MEASUREMENT AND CONTROL IN SCIENCE AND INDUSTRY. Editor-in-Chief ARNOLD WEXLER. Volume One. PRINCIPLES AND METHODS OF MEASURING HUMIDITY IN GASES. Editor ROBERT E. RUSKIN. Pp. xvi + 687. New York: Reinhold Publishing Corporation; London: Chapman & Hall Ltd. 1965. Price $30.00.

This is the first volume of a four-volume work that will record the proceedings of the Firts International Symposium on Humidity and Moisture Control, held in Washington in 1963. It is claimed that this first volume forms a complete reference work on instrumentation for measuring water vapour and deals with all modern methods for its determination. Sixty-eight chapters are spread among six general sections on psychrometry, dew-point hygrometry, electric hygrometry, spectroscopic hygrometry, coulometric hygrometry and miscellaneous methods. The book is sponsored by the National Bureau of Standards, Washington, D.C., the U.S. Weather Bureau, the American Society of Heating, Refrigerating and Air-Conditioning Engineers and the Instrument Society of America. It does, in fact, cover its subject thoroughly and authoritatively.

K. A. WILLIAMS

THIN-LAYER CHROMATOGRAPHY: PROCEEDINGS OF THE SYMPOSIUM HELD AT THE ISTITUTO SUPERIORE DI SANITA, ROME, 2–3 MAY, 1963. Edited by G. B. MARINI BETTÒLO. Pp. xii + 232. Amsterdam, London and New York: Elsevier Publishing Company. 1964. Price 65s.

This symposium on thin-layer chromatography was organised by the Istituto Superiore di Sanita in order to promote extended discussion of the rapidly expanding technique. The plenary lectures delivered at the symposium are recorded in this book, together with original papers from the Institute and from other sources represented at the symposium. Discussions of the papers are not included.

The book covers many aspects of thin-layer chromatography. The papers are introduced by a contribution from E. Stahl dealing with the general development of the subject, and they deal with lipids, steroids, sterol acetates, stilboestrol, alkaloids, aromatic hydrocarbons, aliphatic carbonyl compounds, isomeric oximes, proteins, food colours, anti-fermentatives, and cations and other inorganic ions. Materials for use as thin films are described, and considerable attention is paid to the technique of the subject. K. A. WILLIAMS

NEW METHODS OF ANALYTICAL CHEMISTRY. By RONALD BELCHER, Ph.D., D.Sc., F.R.I.C., F.I.C.I., and CECIL L. WILSON, Ph.D., D.Sc., F.R.I.C., F.I.C.I., in association with T. S. WEST, Ph.D., D.Sc., F.R.I.C. Second Edition. Pp. xvi + 366. London: Chapman and Hall Ltd. 1964. Price 60s. in U.K. only.

In this second edition the senior authors have been joined by Dr. T. S. West and have produced what is virtually a sequel to the first edition. Almost all the text is new; some of the methods included in the first edition have now found their way into text-books, others have proved illusory and have been discarded. Apart from some material reproduced from the first edition in order to give a balanced account, the main content is a selection of the more promising methods that have been published since 1955.

The general presentation is as before and the subjects covered are: titrimetric standards; indicators; titrants; organic reagents (for metals); inorganic reagents; selective spectrophotometric reagents; precipitation from homogeneous solution; solvent extraction; miscellaneous methods. Again, the analyses described are almost entirely inorganic and restricted to "classical" methods. This term has been taken to include spectrophotometry and a few electro-titrations, but apparently not other electrochemical methods or ion exchange or chromatography. The topics selected reflect very largely the predilections of the authors, and it is an amusing exercise to compile combinations of authors (however unlikely) that would result in an entirely different book.

Cynics (like the reviewer) may well consider that some of the methods described do not constitute an advance, especially some of the redox procedures, but the task of selection is a difficult one, and by and large it has been well done. Within the very narrow limits that the authors have set, they have provided a useful guide through the maze of recent literature, the sections on metallochromic indicators and on miscellaneous methods being particularly helpful. All analysts should find a study of this book stimulating and worthwhile. J. F. HERRINGSHAW

THE SOCIETY FOR ANALYTICAL CHEMISTRY

FOUNDED 1874. INCORPORATED 1907.

THE objects of the Society are to encourage, assist and extend the knowledge and study of analytical chemistry and of all questions relating to the analysis, nature and composition of natural and manufactured materials by promoting lectures, demonstrations, discussions and conferences and by publishing journals, reports and books.

The Society includes members of the following classes:—(*a*) Ordinary Members who are persons of not less than 21 years of age and who are or have been engaged in analytical, consulting or professional chemistry; (*b*) Junior Members who are persons between the ages of 18 and 27 years and who are or have been engaged in analytical, consulting or professional chemistry or *bona fide* full-time or part-time students of chemistry. Each candidate for election must be proposed by three Ordinary Members of the Society. If the Council in their discretion think fit, such sponsorship may be dispensed with in the case of a candidate not residing in the United Kingdom. Every application is placed before the Council and the Council have the power in their absolute discretion to elect candidates or to suspend or reject any application. Subject to the approval of Council, any Junior Member above the age of 21 may become an Ordinary Member if he so wishes. A member ceases to be a Junior Member on the 31st day of December in the year in which he attains the age of 27 years. Junior Members may attend all meetings, but are not entitled to vote.

The Entrance Fee for Ordinary Members is £1 1s. and the Annual Subscription is £4. Junior Members are not required to pay an Entrance Fee and their Annual Subscription is £1 1s. No entrance Fee is payable by a Junior Member on transferring to Ordinary Membership. The entrance Fee (where applicable) and first year's Subscription must accompany the completed Form of Application for Membership. Subscriptions are due on January 1st of each year.

Scientific Meetings of the Society are usually held in October, November, December, February, April and May, in London, but from time to time meetings are arranged in other parts of the country. Notices of all meetings are sent to members by post.

All members of the Society have the privilege of using the Library of The Chemical Society. Full details about this facility can be obtained from the Librarian, The Chemical Society, Burlington House, Piccadilly, London, W.1.

The Analyst, the Journal of the Society, which contains original and review papers, information about analytical methods and reviews of books, and has a world-wide distribution, and *Proceedings of the Society for Analytical Chemistry*, in which are reported the day-to-day activities of the Society, are issued monthly to all Ordinary and Junior Members. In addition, all Ordinary Members receive *Analytical Abstracts*, providing a reliable index to the analytical literature of the world.

Forms of application for membership of the Society may be obtained from the Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

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THE North of England, Scottish, Western and Midlands Sections were formed to promote the aims and interests of the Society among the members in those areas.

Specialised Groups within the Society are concerned with the study of various branches of analytical chemistry of specialised or topical interest. Groups dealing with such topics as Microchemical Methods, Biological Methods, Thin-Layer Chromatography, Atomic-Absorption Spectroscopy, Thermal Analysis, Automatic Methods and a Special Techniques Group, covering very new developments and specialised physical methods, are at present active—and further Groups are formed from time to time as the need arises.

Non-members of the Society may be non-voting members of a Group.

The Sections and Groups hold their own meetings from time to time in different places. There is no extra subscription for membership of a Section or Group. Application for registration as a member should be made to the Secretary of the Society.

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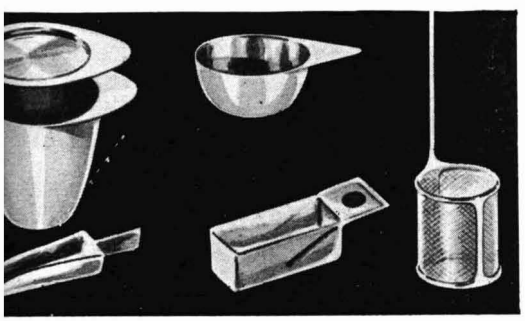

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