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THE ANALYST

Amperometric Titration of Copper and Cadmium in the Presence of Zinc, Cobalt and Nickel with Sodium Diethyldithiocarbamate

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The reaction of some metal ions with diethyldithiocarbamate in 0·1 M sodium hydroxide solution is investigated polarographically. Methods are presented for the amperometric titration of cadmium in the presence of zinc and cobalt, and of cadmium and copper in admixture.

MANY metals, including copper, cadmium and zinc, have been titrated amperometrically with sodium diethyldithiocarbamate.[1] However, because numerous metal ions react with this ligand, the use of masking agents or pH control is necessary to achieve selectivity. For instance, copper(II) can be titrated in the presence of zinc, nickel and iron when EDTA is present[2] or in the presence of manganese(II) and chromium(III) when tartrate is added.[3] Copper can be titrated in the presence of zinc in ammoniacal solution at pH 11, and the zinc can subsequently be titrated after acidification to pH 6 with acetic acid.[4] Other examples are also known.[1]

It has been shown[5] that zinc, manganese(II), nickel and cobalt do not complex with disodium ethylene-1,2-bisdithiocarbamate in 0·1 N sodium hydroxide solution, whereas copper(II), cadmium and lead do form complexes. Thus, under these conditions, it would be possible to titrate copper or cadmium amperometrically in the presence of the non-complexing metals by using sodium ethylene-1,2-bisdithiocarbamate without adding a masking agent. However, aqueous solutions of this reagent are not stable for more than a few hours,[5] so sodium diethyldithiocarbamate was investigated as an alternative titrant. Solutions of this compound in 0·01 M sodium hydroxide solution are stable for at least 3 days.[6]

Polarographic measurements showed that zinc, manganese(II) and cobalt(II) (5×10^{-3} M) did not complex with diethyldithiocarbamate in 0·05 M sodium hydroxide solution. Addition of titrant to the metal-ion solution immediately produced an anodic wave of the ligand. Copper(II) gave the expected 2:1 ligand-to-metal complex. No anodic wave appeared until this ratio had exceeded 2:1. When nickel (5×10^{-3} M) was titrated, the first increment of titrant produced a small anodic wave, but the height of this wave hardly increased until the ligand-to-metal ratio was 2:1. A smaller concentration of nickel (10^{-4} M), however, showed no evidence of complexing with diethyldithiocarbamate in 0·1 M sodium hydroxide solution. Cadmium showed rather more complicated behaviour. When ligand was added to a cadmium solution, the anodic wave of the ligand appeared when the ligand-to-metal ratio was greater than 1·0, whereas in the reverse titration, the ligand wave had disappeared when the ratio was 2·0.

Addition of ligand to cadmium until it was in appreciable excess over cadmium gave the titration graph shown in Fig. 1. It shows that the 1:1 species is converted into the 2:1 complex when a large amount of ligand is present. Moreover, the anodic wave of a 1-fold excess of uncomplexed ligand in the presence of the 1:1 complex decreased to half-height after 30 minutes but showed little change thereafter.

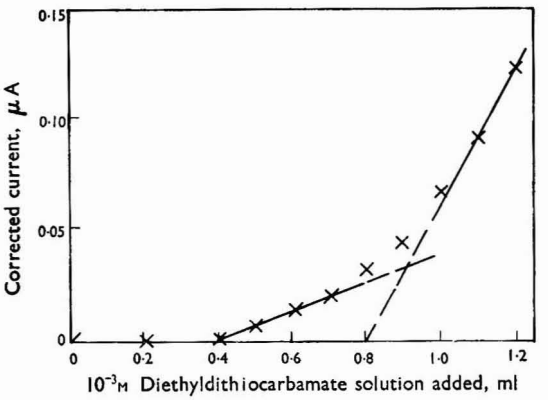

Fig. 1. Amperometric titration of 4×10^{-7} moles of cadmium(II) with 10^{-3}M sodium diethyldithiocarbamate solution

The results can be interpreted on the basis of competition for the metal ions between diethyldithiocarbamate (L^-) and hydroxide—

$$M(OH)_2 + 2L^- \rightleftharpoons ML_2 + 2\,OH^- \text{ (M = Co(II), Mn(II), Cd or Cu)} \quad .. \quad (1)$$

$$\text{or } ZnO_2^{2-} + 2L^- + 2H_2O \rightleftharpoons ZnL_2 + 4\,OH^- \quad .. \quad .. \quad .. \quad .. \quad (2).$$

For cobalt(II), manganese(II) and zinc, the equilibria lie almost completely towards the left-hand side of the equations, whereas for copper(II), it is completely to the right-hand side; nickel is an intermediate example, and small amounts of ligand are insufficient to send the equilibrium towards the right-hand side. These results enable limits for the values of the solubility products of these metal dithiocarbamates to be calculated, on the basis of the

TABLE I

SOLUBILITY PRODUCTS OF METAL DITHIOCARBAMATES

Metal	Cobalt(II)	Manganese(II)	Zinc	Nickel	Copper(II)
log K_{so} ($M(OH)_2$)[7]	−15	−13	−17	−15	−20
log K_{so} (ML_2)	> −16	> −14	> −19	−19·7 ± 0·5	< −28
log K_{so} (ML_2) by Hulanicki[8] ..	—	—	−17	−23	−30

solubility products of the metal hydroxides. These results do not take into account the effect of supersaturation phenomena and possible slow reactions between diethyldithiocarbamate and the metal hydroxides. These values are given in Table I. The reaction of cadmium can best be described as follows—

$$Cd(OH)_2 + L^- \overset{\text{rapid}}{\rightleftharpoons} Cd(OH)L + OH^- \quad .. \quad .. \quad (3)$$

$$Cd(OH)L + L^- \overset{\text{slow}}{\rightleftharpoons} CdL_2 + OH^- \quad .. \quad .. \quad .. \quad (4).$$

Equilibrium in equation (3) is displaced markedly and rapidly to the right-hand side, but equilibrium in equation (4) needs an excess of ligand to drive it to this side. The latter reaction is also slow, and is responsible for the slow decrease in ligand wave-height in the presence of the 1:1 complex. This slow decrease could not have arisen from decomposition of either free or complexed ligand to give sulphide ions because addition of further ligand still gave the 2:1 end-point on extrapolation of the steep part of the titration graph. Complexes of the type Cd(OH)L have also been postulated[7] when L represents Cl^-, PO_4^{3-}, $P_3O_{10}^{5-}$ or nitrilotriacetate.

The investigation indicated that it should be possible to titrate amperometrically cadmium or copper in the presence of zinc, cobalt or manganese, in a hydroxide solution.

Table II shows that the titration of 2×10^{-4} M cadmium was satisfactory in the presence of a 50-fold excess of cobalt(II) and a 5-fold excess of zinc. A 50-fold excess of zinc or manganese(II), however, gave low results, presumably because of co-precipitation of cadmium with manganese hydroxide or as cadmium zincate. It was also possible to titrate copper in the presence of similar amounts of nickel (Table II). Care should be taken, however, not to spatter nickel hydroxide at the top of the polarographic cell during de-aeration, because the precipitate is difficult to dislodge from the glass, and it is likely to entrain copper.

TABLE II

TITRATION OF CADMIUM AND COPPER

Cadmium added, moles $\times 10^7$	Other ions added, moles $\times 10^7$		Cadmium found, moles $\times 10^7$	Other ions found, moles $\times 10^7$	
2·0	—	—	1·9	—	—
2·0	Zn	100	1·5	—	—
2·0	Zn	10	2·0	—	—
2·0	Mn	100	0·1*	—	—
2·0	Mn	10	—†	—	—
2·0	Co	100	2·1	—	—
1·0	Cu	1·0	1·0	Cu	1·0
3·0	Cu	1·0	3·0	Cu	1·0
—	Cu	4·5	—	Cu	4·5
	Ni	0·5			
—	Cu	5·0	—	Cu	5·0
	Ni	5·0			
—	Cu	5·0	—	Cu	5·1
	Ni	10·0			

* Poor graph.
† Non-linear graph, therefore no intercept.

The formation of the two cadmium complexes enabled a method to be devised for the determination of cadmium and copper in admixture. The sum of the metals is first determined by adding increments of titrant in the range appreciably in excess of that required to form CdL_2 and CuL_2. The titration is then repeated, with lesser amounts of titrant so that the inflection for the CdL complex can be detected, as in Fig. 1. This inflection occurs when CuL_2 and CdL have been formed.

EXPERIMENTAL—

The polarographic curves were recorded in a Kalousek vessel with a standard calomel reference electrode and a Cambridge pen-recording polarograph. The titrant was a 10^{-3} M solution of analytical-reagent grade sodium diethyldithiocarbamate in water, freshly made up every 3 days. The total anodic current of the diethyldithiocarbamate was measured at −0·3 volt *versus* S.C.E., except when the concentration of free ligand was sufficiently large ($>10^{-4}$ M) for the adsorption wave to be separate from the main wave,[6] when measurements should then be made at −0·2 volt, so that the total wave-height is measured.

PROCEDURE

DETERMINATION OF CADMIUM, OR COPPER, IN THE PRESENCE OF ZINC AND COBALT—

Add 2 ml of about 10^{-4} M cadmium solution to 8 ml of 0·1 M sodium hydroxide solution in the polarographic cell. De-oxygenate with nitrogen, and titrate with diethyldithiocarbamate in 0·2-ml increments. Correct the wave-height for dilution effects, and plot the corrected wave-height *versus* volume of titrant; extrapolate the straight line to zero wave-height to obtain the end-point.

DETERMINATION OF COPPER AND CADMIUM IN ADMIXTURE—

Dilute a volume of the unknown solution, containing about 10^{-7} moles of cadmium and copper, to 10 ml with 0·1 M sodium hydroxide solution, and proceed as above. The end-point obtained gives the end-point for the formation of CuL_2 and CdL_2. Repeat the titration with five times the volume of unknown solution, by adding the titrant in 0·1-ml increments. The plot of corrected wave-height *versus* volume of titrant (as in Fig. 1) gives

the end-point for the formation of CuL_2 and CdL at the point of first appearance of the anodic current. The concentration of copper and cadmium can readily be calculated.

If the approximate concentration of copper *plus* cadmium is known, the first titration can be omitted, and the two end-points obtained from the second graph. In this instance, the titration should be carried out rapidly, to avoid the slow changes in wave-height with time noted above.

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The Rapid Dissolution of Plutonium Dioxide by a Sodium Peroxide - Sodium Hydroxide Fusion, Followed by Determination of the Plutonium Content by Controlled-potential Coulometry

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A method is described for the dissolution of plutonium dioxide, followed by determination of the plutonium content by controlled-potential coulometry. The plutonium dioxide is brought into solution by fusion with a mixture of sodium peroxide and sodium hydroxide at 600° C for 15 minutes in an alumina crucible. The cold melt is leached with water, which is then acidified with sulphuric acid. The solution is heated for 15 minutes to decompose hydrogen peroxide and, after cooling, diluted to a suitable volume. The plutonium content of an aliquot containing about 4 mg of plutonium is determined by controlled-potential coulometry. A potential of +0·30 volt *versus* a S.C.E. is used for reduction to plutonium(III), whereas +0·70 volt *versus* a S.C.E. is used for the quantitative oxidation to the plutonium(IV) state.

Mean recoveries on 100-mg amounts of plutonium dioxide that had been ignited at 850° C were 99·95 per cent., with a coefficient of variation of 0·11 per cent. For the complete dissolution of samples previously ignited at higher temperatures (about 1600° C), an increase in the ratio of the weight of fusion mixture to sample is necessary. Mean recoveries on 50-mg amounts of plutonium dioxide that had been ignited at 1600° C were 99·85 per cent., with a coefficient of variation of 0·54 per cent.

In earlier work from this laboratory a sodium peroxide sinter technique[1] was described for the rapid dissolution of plutonium dioxide, including high-fired material. The plutonium content of the resulting solution was then determined by differential spectrophotometry. Subsequent experience with this method has shown that occasionally inconvenience can result from the existence of small amounts of undissolved sample in the final solution. This problem was traced to the difficulty of obtaining satisfactory mixing of the sodium peroxide and the sample under glove-box conditions. It was considered that this difficulty might disappear on modifying the sinter so that fluid conditions occurred; these conditions would be produced by increasing the temperature to above 500° C. In addition, reduction of the size of the sample required for analysis would help to improve and speed up the dissolution procedure. This objective could be achieved if the differential spectrophotometric method for plutonium was replaced by an electrochemical method. For example, it is possible to determine as little as 1 mg of plutonium to within ±0·2 per cent. by controlled-potential coulometry, as compared with the 30 mg of plutonium needed for a single determination by differential spectrophotometry. Another advantage is that by handling smaller sample weights, the final determination can be carried out in a fume cupboard, instead of a glove-box, with a consequent improvement in the speed of analysis.

It was expected that a peroxide fusion would give a rapid method for the dissolution of plutonium dioxide, either alone or in mixtures with certain other materials. It should, for

example, be much quicker than the ammonium hydrogen sulphate fusion,[2] which takes 4 hours to effect the dissolution of refractory plutonium dioxide. Moreover, it should also be satisfactory for the dissolution of other materials mixed with plutonium dioxide, particularly those which are not dissolved by fusion with ammonium hydrogen sulphate. These materials include ruthenium metal, ruthenium dioxide, silica and chromium oxides. In view of these possible advantages, the peroxide fusion technique has been examined in some detail.

Experimental

Selection of a suitable crucible—

Although a platinum crucible is satisfactory for sodium peroxide sinters carried out at about 450° C, it is not suitable for fusions at temperatures in excess of 500° C. In considering suitable crucible materials, it was thought that a metal crucible would be preferable to one made from a ceramic because of such factors as ease of handling and the absence of porosity, difficulties usually associated with ceramics. Specimens of various metals were, therefore, tested by fusing small pieces with 0·5 g of sodium peroxide *plus* 0·5 g of sodium hydroxide for 15 minutes at 600° C in alumina crucibles. By this means it was hoped to identify those metals undergoing negligible attack in the fusion process, and a summary of the results is shown in Table I. These results indicated that, of the metals examined, zirconium was the most suitable for further study. Several zirconium crucibles were made, therefore, by a cold-drawing process. Initially, they had rough internal surfaces, which led to losses caused by melts creeping up the crucible walls, but this effect was reduced by polishing the internal surfaces of each crucible. Evidence of the removal of some zirconium from the crucible walls by the fusion process was detected. Moreover, this attack caused the formation of a grey deposit on the bottom of each crucible, which proved difficult to remove. At this stage, it was concluded that a satisfactory metal crucible would be difficult to obtain.

In spite of known difficulties with ceramics, crucibles made only from this type of material were left for consideration. Experimental fusions were carried out, therefore, in thoria, magnesia and alumina crucibles. Thoria crucibles were completely unattacked by the peroxide melt but, unfortunately, they had a poor resistance to thermal shock, which resulted in severe cracking. Magnesia crucibles were badly attacked during the fusion, and the melt crept up the wall surfaces. Fortunately, alumina crucibles withstood the fusion much better, and were only slightly attacked, 20 mg being a typical average loss in weight for a 15-minute fusion. Moreover, each crucible appeared to be usable for about four fusions, provided that careful drying of the crucible walls was carried out after each fusion. Also, problems connected with the creeping of the melt did not occur with the crucibles tested. On this evidence, an alumina crucible appeared to be the only satisfactory ceramic crucible readily available. The small amounts of aluminium passing into solution would not cause any interference in the coulometric determination of plutonium. Alumina crucibles of 15-ml capacity, as supplied by Thermal Syndicate Ltd., were, therefore, used exclusively in this investigation.

Dissolution of plutonium dioxide—

Experimental fusions were carried out initially on a plutonium dioxide sample that had been ignited at 850° C. The mesh size of the material was less than 100 B.S.S. The sample (100 mg) was mixed with 0·5 g of sodium peroxide in an alumina crucible, 0·5 g of sodium hydroxide then added and the crucible heated at 600° to 620° C for 15 minutes. After cooling, the melt was leached by the method already described,[1] and the resulting solution acidified by adding it, dropwise, to 10 ml of water *plus* 4 ml of sulphuric acid (sp.gr. 1·84) contained in a 50-ml beaker. After warming for 15 minutes, the solution was cooled and diluted to 50 ml with water. The plutonium content was determined on a suitable aliquot by controlled-potential coulometry. The coulometric determination appeared to be fairly normal, with little trouble arising from any residual peroxide in solution. The only noticeable effect was that a slightly longer than normal reduction time (about 30 minutes) was required to reach a constant background current of less than 10 μA. A total of eight fusion experiments was carried out, and the behaviour in each instance was identical. Clear brown solutions were obtained without any trace of undissolved material. The mean recovery for plutonium from these determinations was 99·95 per cent., with a coefficient of variation of 0·11 per cent.

Experimental fusions were next attempted on a plutonium dioxide sample that had been fired at 1600° C. A series of eight dissolutions with 100-mg portions of material was carried out, followed by coulometric determination of the plutonium. These experiments gave recoveries for plutonium that were up to 2 per cent. low. Further work showed that this bias was not caused by interference in the coulometry, but to slightly incomplete dissolution of the sample. Further dissolutions were then carried out with 50-mg portions of the plutonium dioxide sample to determine whether the higher ratio of fusion mixture to sample would improve the situation. Three fusions were carried out initially, and these gave good recoveries on subsequent coulometric titration. Ten more determinations were completed, and the mean recovery for the thirteen determinations was 99·85 per cent., with a coefficient of variation of 0·54 per cent.

TABLE I

OBSERVATIONS ON FUSING VARIOUS METALS WITH SODIUM PEROXIDE *plus* SODIUM HYDROXIDE

Material	Observations
Nickel	Badly attacked
Manganese - nickel alloy	Badly attacked
Stainless steel	~10% weight loss
Zirconium (clean surface)	2% weight loss
Zirconium (oxidised surface)	4% weight loss
Silver	~30% weight loss
Gold	Badly attacked

METHOD

APPARATUS—

Alumina crucibles—Recrystallised alumina crucibles, 15-ml capacity, as supplied by Thermal Syndicate Ltd.

Muffle furnace—A "Hotspot," obtainable from A. Gallenkamp & Co. Ltd., or similar furnace.

Controlled-potential coulometer[3,4]—This was fitted with a digital voltmeter (Type LM 1010–2, Solartron Laboratory Instruments Ltd., Chessington, Surrey).

Electrolysis cell for coulometry—As described previously.[5]

REAGENTS—

All reagents were of AnalaR grade.

Sodium peroxide.

Sodium hydroxide.

Sulphuric acid, 18, 1 and 0·5 M.

Distilled water.

RADIOCHEMICAL SAFETY—

Operation on dry samples containing plutonium dioxide, up to the point of complete dissolution, should be conducted in a glove-box. Aliquot portions for completion of the analysis can be handled in a fume cupboard with an efficient extraction and filtration system.

PROCEDURE—

Weigh 50 to 100 mg of sample ground to less than 100 B.S.S. mesh size (50 mg for samples of plutonium dioxide that have been ignited at temperatures above 1000° C) and transfer it into an alumina crucible containing 0·5 g of sodium peroxide. Mix by rotating the crucible by hand at an angle of 45°, then add 0·5 g of sodium hydroxide in pellet form. Heat the crucible in a muffle furnace at 600° to 620° C for 15 minutes, keeping the crucible covered with an alumina (or silica) lid. Then remove the crucible from the furnace and allow it to cool. Add 1 ml of water to the crucible and allow the dissolution reaction to proceed for 5 to 10 minutes. Then add a further 0·5 ml of water, and gently swirl the contents of the crucible. If any of the melt is left undissolved, warm the crucible cautiously on a hot-plate, but avoid prolonged heating. (All of the melt should dissolve to give a brown to black-coloured suspension.) Transfer the extract, dropwise, with a Pasteur pipette into a 50-ml

beaker containing 10 ml of water *plus* 4 ml of sulphuric acid. Mix the solution during the addition by gently swirling the contents of the beaker. Wash the crucible by transferring about 2 ml of the acidic solution back into the crucible, and rinse the crucible walls with this solution. Wash the crucible with three further 2-ml portions of 0·5 M sulphuric acid and then with 2 ml of water. Combine the washings with the solution in the beaker, then warm on a hot-plate for 15 minutes, or until all de-gassing has ceased. Cool the solution and dilute to 50 ml with M sulphuric acid. Use suitable aliquots, containing about 4 mg of plutonium, for determination by controlled-potential coulometry.

Coulometric determination—Transfer the aliquot of sample solution to the coulometer cell, and add sufficient M sulphuric acid to cover the working electrode. Remove oxygen from the solution by passing a stream of nitrogen through it. Then reduce the plutonium to the tervalent state by electrolysing at a potential of +0·30 volt *versus* a S.C.E. until the current attains a low constant value (10 μA or less). After adjusting the coulometer to zero, carry out the quantitative oxidation of plutonium to the quadrivalent state by electrolysing at +0·70 volt *versus* a S.C.E. until the cell current reaches its previous low value (10 μA or less). Correct the digital-voltmeter reading, Q, for a blank determination, carried out in exactly the same way with an aliquot of solution from a blank sodium peroxide - sodium hydroxide fusion. Calculate the weight of plutonium from the expression—

$$\text{plutonium, mg} = \frac{Q\ (\text{corrected}) \times F \times 239{\cdot}1 \times V}{96{,}487 \times A}$$

where F is the calibration factor in millicoulombs per millivolt for the coulometer range used, A is the volume of the aliquot taken for analysis and V is the total volume of the sample solution.

Results

The procedure was tested on two samples of plutonium dioxide, one having been ignited at 850° C and the other at 1600° C. The results, which are shown below, are expressed as percentage recoveries, assuming $PuO_{2\cdot00}$ stoicheiometry. Any error in this assumption is very small and is negligible relative to the precision obtained.

Temperature of sample ignition 850° C; sample weight 100 mg; recovery, per cent. 99·83, 100·16, 100·04, 100·05, 100·01, 99·89, 99·84 and 99·93 (mean 99·95); coefficient of variation 0·11 per cent.

Temperature of sample ignition 1600° C; sample weight 50 mg; recovery, per cent. 99·95, 100·23, 99·70, 99·15, 100·95, 100·04, 99·50, 100·29, 99·05, 99·60, 100·25, 99·27 and 100·08 (mean 99·85); coefficient of variation 0·54 per cent.

Conclusions

The sodium peroxide - sodium hydroxide fusion technique is a rapid and simple method for the complete dissolution of refractory plutonium dioxide, and it is by far the most rapid method for dissolving high-fired material. The sulphuric acid solution obtained on dissolving the melt is suitable for the direct determination of the plutonium content by controlled-potential coulometry. Although a glove-box is necessary for the fusion and dissolution of the melt, the resultant solution can be transferred into a fume cupboard and the determination completed there because of the small amount of plutonium needed for coulometry. This method represents an improvement and a simplification over the method involving a sodium peroxide sinter followed by differential spectrophotometry. The analysis by this latter method must be carried out entirely in a suite of glove-boxes because of the high concentration of plutonium involved.

We thank Mr. D. Wicks for carrying out some of the coulometric determinations.

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A Stable, Solid-state, High Voltage Source for Electrode Polarisation

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A simple, cheap, versatile, solid-state, high voltage source is proposed as a replacement for high tension battery supplies. It is capable of providing up to 70 μA at 1000 volts, with drift and noise less than 0·1 per cent., and can be used for electrode polarisation, radiation counting tube supplies and other small-current applications.

THE obsolescent high tension radio battery of 120 volts has been commonly used in high voltage sources for the supply of small currents for polarisation of electrodes in techniques such as differential electrolytic potentiometry.[1] The high cost and bulk of, *e.g.*, a 1000-volt source built up from such batteries, and the short shelf life and increasing scarcity of the tapped batteries, have initiated a search for a simple, cheap alternative. A Cockroft - Walton voltage multiplier driven by a square-wave generator[2] offered a promising start.

SOURCE VOLTAGE AND CURRENT STABILITY—

In differential electrolytic potentiometry and other constant-current techniques, the electrode potential is a function of current density, and if the potential difference between the electrodes is E_Δ volt, the source voltage is V_S volt, the stabilising resistance in series with source and cell (the ballast resistance) is R_B, and the cell resistance *plus* the internal resistance of the source is R_{int}, then the current is given by—

$$I = \frac{V_S - E_\Delta}{R_B + R_{int}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1).$$

In a differential electrolytic potentiometric titration, for instance, E_Δ will vary from zero to a maximum value dependent on I and, if the electrode processes are fast, on the Q of the titration.[3] A maximum E_Δ of 100 mV would require a V_S of 100 volts to restrict variation of I to 0·1 per cent., while a maximum E_Δ of 1 volt would require a 1000-volt source for the same stability. R_B must be correspondingly large, not only to minimise the effect of any change in R_{int}, but also to give the proper value of I. Partial differentials emphasise the dependences

$$-\frac{\partial I_\Delta}{\partial E_\Delta}(V_S,R_B,R_{int}) = -\frac{\partial I_\Delta}{\partial V_S}(E_\Delta,R_B,R_{int}) = \frac{1}{R_B + R_{int}} \quad .. \quad (2), (3)$$

so, plainly, the larger R_B the better. Ballast load (the product $V_S \times R_B$) is a significant parameter in differential electrolytic potentiometry, and partial differentiation with respect to this product—

$$\frac{\partial I_\Delta}{\partial V_S R_B}(E_\Delta,R_{int},V_S,R_B) = -\frac{(V_S - E_\Delta)}{V_S(R_B + R_{int})^2} \quad .. \quad .. \quad .. \quad (4)$$

shows that the larger V_S and the ballast load the better within the limits of Johnson noise. As E_Δ changes rapidly in the equivalence point region and also changes greatly with current density, inadequate stabilisation leads to erratic and drifting potentials. In view of these requirements it was decided to turn attention to a variable voltage source capable of supplying up to 50 μA at 1000 volts.

CIRCUIT PRINCIPLES—

The circuit, shown in Fig. 1, consists of an inverter and a voltage multiplier. The inverter is a push - pull amplifier with two driver transformers. The output is fed back via C_1 to the primary of T_1 at such a level as to overdrive the transformer, giving a clipped waveform that approaches a fast rise square wave. The output of T_2 carries a nominal 100-volt peak a.c. signal and is isolated, so that consistent earthing of positive or negative battery terminals can be used. The multiplier is a conventional series of voltage doublers, and the number of stages can be reduced or increased if a lower or higher maximum output voltage is required. Any silicon diodes capable of handling 200 peak inverse volts can be used, and the capacitors should be generously rated. Regulation is effected by connecting miniature neons across any suitable taps. As shown in Fig. 1, three 70-volt neons in series are connected across adjacent 200-volt taps, and give a nominal 1000-volt output in steps of 100 volts. Connected over two taps (*e.g.*, −300 to −700) the output would be 500 volts in steps of 50 volts, while a single neon in place of three as in Fig. 1 would give a 350-volt output in steps of 35 volts. The unit consumes less than 500 mW and is conveniently powered by small dry batteries, of from 6 to 15 volts; the output voltage will vary with supply voltage.

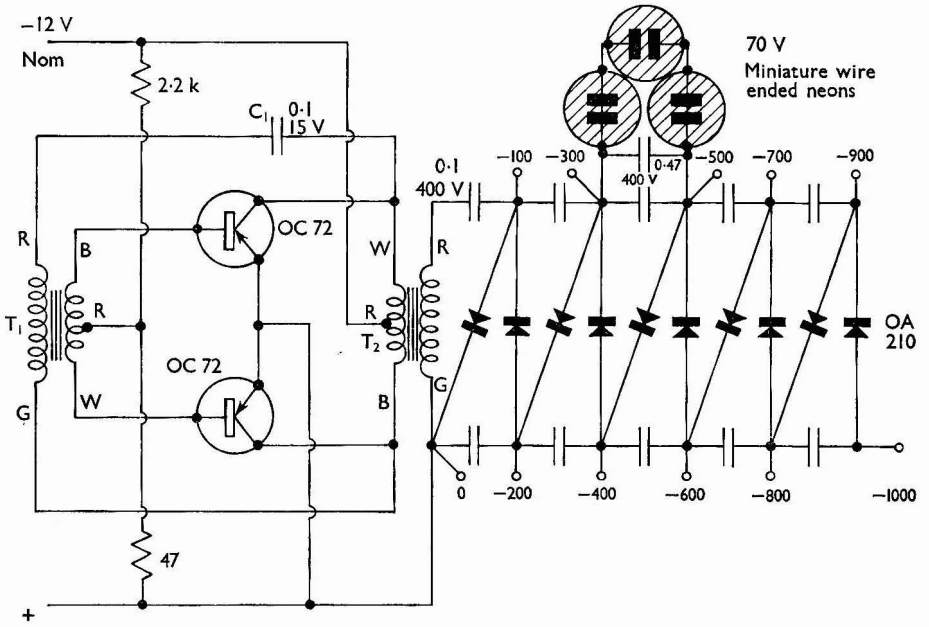

Fig. 1. Circuit diagram of inverter multiplier. T_1 and T_2 are Rex LT44, 20KΩ/1KΩCT obtained from Alpha Radio Ltd. Leads: R red, B black, W white, G green. Details of the circuit are given in the text

CONSTRUCTION—

The circuit can be laid out as in the circuit diagram and occupies about 4 × 2 inches on a piece of drilled paxolin. The board can be mounted in a small box, together with the battery and a ceramic wafer switch connected to the various taps so that ten steps of output voltage can be selected. A small square electrical conduit joint box makes a convenient container, and reduces the effect of temperature variations. The neons serve as indicator lamps, and glow steadily when operating satisfactorily; near overload they flicker, while if too large a current is drawn the lamps are extinguished.

PERFORMANCE—

For a 12-volt supply a typical maximum output is 70 μA at 900 volts. Stability was examined by connecting the output to a ballast resistor and a standard (±0·01 per cent.)

resistor in series, monitoring the voltage drop across the standard resistor by means of an E.I.L. 39A pH meter, and recording the difference from a standard voltage on a Honeywell 513 X17 strip chart recorder. The stability of the output is a function of the stability of the supply to the inverter. With a raw 12 volt d.c. laboratory supply, produced by a

TABLE I

OUTPUT STABILITY OF 1000-VOLT SOURCE

Current, μA	R_B, Ω	R_S, Ω	Duration, minutes	Drift *plus* noise, per cent.
1	10^9	10^5	100	$<0{\cdot}1$
2	5×10^8	10^5	60	0·08
5	2×10^8	10^5	35	0·04
10	10^8	10^5	100	0·06
20	5×10^7	10^4	120	0·09
0·5*	2×10^9	10^5	70	2·0
2*	5×10^8	10^5	75	1·5

* Raw 12-volt d.c. supply $\pm$ 10 per cent., 200 to 400-mV noise. Remainder with battery or regulated d.c. supply.

3-phase transformer rectifier, regulated to 10 per cent. and containing 200 to 400 mV of noise, an output stability of 1 to 2 per cent. was obtained. With batteries or a regulated d.c. supply (Solartron AS 1411) regulation of the output was 0·1 per cent. or better, and high frequency noise was of the same order. Typical results are given in Table I.

The author thanks Mr. M. Riley who carried out the performance tests.

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A Semi-automatic Timed End-point Karl Fischer Titrator

By B. COPE

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The construction, operation and evaluation are described of a low-cost instrument for providing semi-automatic analysis of the water content of liquids and gases by means of a Karl Fischer titration.

The Karl Fischer titration is usually carried out manually by adding small volumes of titrant at regular intervals to the titrand until a given current is flowing between the indicating electrodes. If the end-point current is held for a given time, then the titration is complete. However, if the current falls below the given value during this period, then more titrant is added until a permanent end-point is reached.

The instrument described does this automatically.

Description—

The instrument operates by means of a standard Karl Fischer dead-stop indicator circuit,[1] with a moving-coil relay replacing the micro ammeter. The moving-coil relay operates as a switch, in conjunction with a second relay, to de-activate (at the end-point), and to activate (when water is present) an automatic titrant dispenser, which dispenses a given volume of titrant into the titrand each time it is activated.

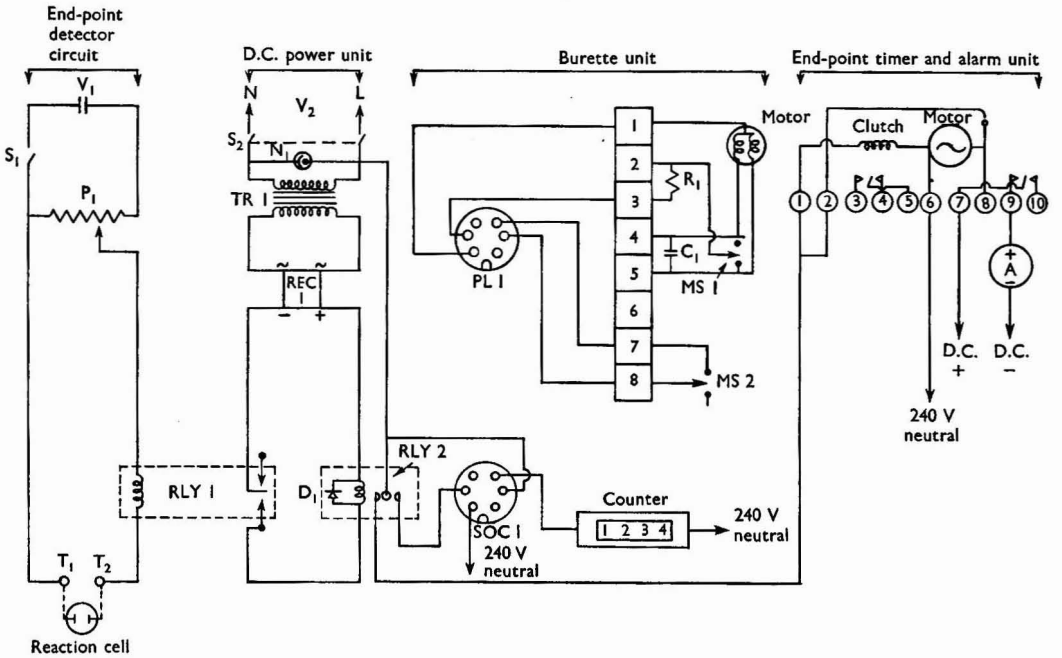

Fig. 1. Semi-automatic Karl Fischer water-content titrimeter circuit diagram

In the end-point condition, the given interval of time allowed for the end-point is automatically measured, and an audible alarm energised when this condition is fulfilled. An impulse counter fitted to the dispenser mechanism counts the number of additions of titrant dispensed, from which the amount of water present in the titrand can be calculated. A circuit diagram is given in Fig. 1, the location of the components is shown in Figs. 2 and 3 and the components are listed in the Appendix.

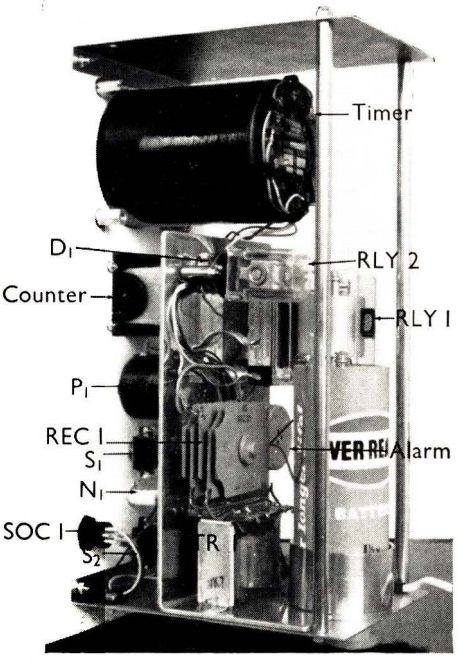

Fig. 2. Location of components, rear view

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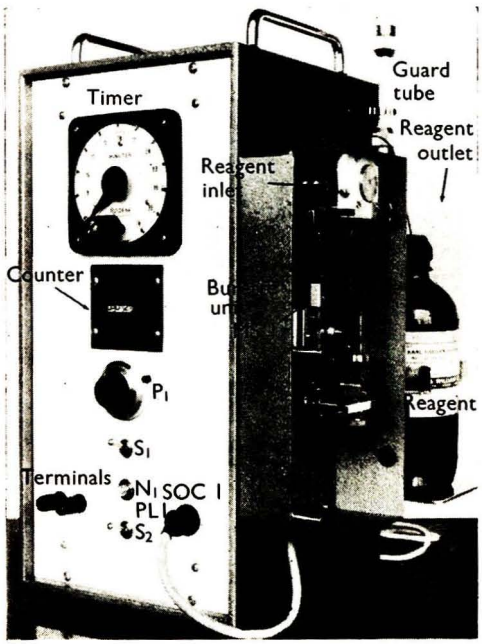

Fig. 3. Location of components, front view

To face page 437]

END-POINT DETECTOR CIRCUIT—

A standard Karl Fischer "dead-stop" circuit is used, except that the micro ammeter normally used to detect the end-point current is replaced by a moving-coil relay, fitted with a "make" contact at 90 μA.

This relay, RLY1 (Fig. 1), provides a means of switching the reagent dispenser on and off via the second relay, RLY2, by breaking the electrical supply to the motor-driven syringe.

THE D.C. POWER UNIT—

The d.c. power unit consists of a transformer, TR1, and rectifier, REC1, to provide 12-volt d.c. for the operation of the relay, RLY2, and the "Bleeptone" alarm, A.

THE BURETTE UNIT—

The burette unit is made from a Fison's automatic dispenser with several modifications.

ELECTRICAL MODIFICATIONS—

The control unit provided with the dispenser has been built into the burette unit as shown in Fig. 1. The microswitch, MS2, has been modified to provide an impulse source for the burette counter (Fig. 1).

DISPENSER-VALVE MODIFICATIONS—

The dispenser valve, as supplied by the manufacturer, is made from polytetrafluoroethylene (PTFE) and stainless steel. However, as Karl Fischer reagent attacks the metal portion of the valve, this has been replaced by a PTFE section. PTFE inlet and outlet lines have been fitted to the valve, and full details are shown in Fig. 4.

The unit is fitted with 1-ml tuberculin syringe, with Luer fitting, and set to deliver about 0·05 ml per stroke.

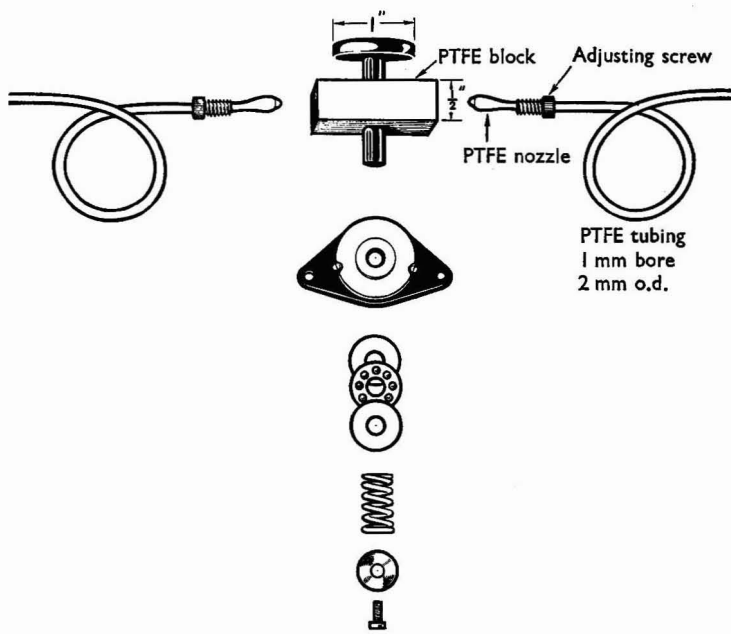

Fig. 4. Burette valve construction

END-POINT TIMER AND ALARM UNIT—

The end-point timer and alarm are connected as shown in Fig. 1. The timer is automatically re-set to the given end-point time each time the burette is activated. If the burette is not activated and the timer allowed to traverse its cycle, then the "Bleeptone" alarm is activated signalling the completion of the titration.

OPERATION—

The following procedure for the determination of water in methanol is given to illustrate the mode of operation of the instrument.

APPARATUS—

Karl Fischer semi-automatic titrimeter.
Titration cell (Fig. 5).
Syringe, 10 μl.

REAGENTS—

Karl Fischer reagent (1 *ml* ≃ 5 *mg of water*).
Water.

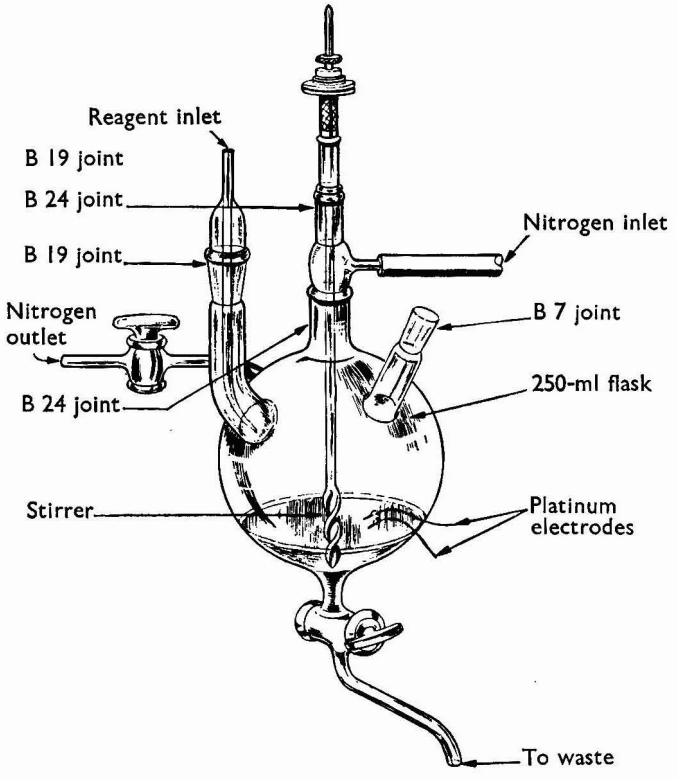

Fig. 5. Karl Fischer titration cell

PROCEDURE—

Introduce 100 ml of dry methanol into the titration cell, switch on the stirrer and set the variable resistance, R, of the titrimeter at 125 ohms and the end-point timer at 2 minutes. Switch on cell current, S_1, and burette power supply, S_2. Allow the titrator to operate until the "Bleeptone" alarm sounds.

CALIBRATION—

Set the burette counter at zero and add 5 μl of water to the titrand, by means of the syringe. Allow the titrator to operate until the "Bleeptone" alarm sounds, and note the burette counter reading.

$$5\ \mu\text{l of water} = 5\ \text{mg of water} = C\ \text{counts.}$$

SAMPLE ANALYSIS—

Set the burette counter at zero and transfer by pipette a volume of sample (V ml) into the titration cell. Allow the titrator to operate until the "Bleeptone" alarm sounds and note the burette counter reading (C_1 counts).

$$\text{Percentage w/v of water in sample} = \frac{C_1 \times 5 \times 100}{C \times V \times 1000}.$$

EVALUATION—

The instrument was evaluated with the above technique.

Volumes of water in the range 1 to 10 μl were introduced into the cell, under steady conditions, and the number of injections of Karl Fischer reagent dispensed recorded. The results are shown in Table I.

TABLE I

EVALUATION OF SEMI-AUTOMATIC KARL FISCHER TITRIMETER

Volume of water added, μl	Number of injections dispensed
1	6, 5, 6, 6, 6, 5
2	10, 10, 10
3	16, 15, 16, 15
4	21, 21
5	27, 27, 27, 27, 27, 26, 27, 28, 27, 27
6	32, 33
7	37, 37
8	44, 43, 43
10	54, 54

The standard deviation of the number of injections is 0·23 injection, *i.e.*, $\pm$0·46 injection at 95 per cent. probability. As less than 1 injection is not practicable, the error of a single determination must be 0 or 1 injection. This corresponds to better than 99·9 per cent. confidence limits. For the Karl Fischer reagent used in this evaluation (1 ml $\simeq$ 5 mg of water), 5·4 injections are equivalent to 1 μl of water and the coefficient of variation (99·9 per cent. + probability), based on an error of $\pm$1 count, is given by—

$$\frac{100}{5{\cdot}4 \times W}$$

where W mg is the amount of water present in the titrand.

For a 5 per cent. coefficient of variation, at least 4 mg of water should be present in the titrand under the conditions quoted. To determine smaller amounts of water at this accuracy, a dilute Karl Fischer reagent should be used.

PERFORMANCE—

A determination of the type illustrated takes 5 minutes, during which time the operator is available to carry out other work, such as preparation of samples.

The instrument can also be used for the determination of moisture in gases. For example, in the method of Reid and Turner,[2] for the determination of water in plastics, the water present in the polymer is vaporised into a nitrogen stream and swept into a Karl Fischer cell. Whereas previously the titration of the water was carried out manually, it may now be carried out automatically.

The instrument has been in use in the laboratories of this Company for more than a year, and has given satisfactory service during this period. The cost of components for this instrument is about £100 and the over-all cost £200.

Appendix

Components list

(Instrument assembly by I.C.A.M. Ltd., Northop, Mold, Flintshire)

Item	Description	Manufacturer
S1	Switch SPDT	Radiospares Limited, London, W.1
S2	Switch SPDT	
T1 and T2	Insulated terminals	
N1	Panel, neon clear, 240-volt	
TR1	Transformer, Hygrade, 240-volt 50 cycles 2 × 6·3-volt	
REC1	Rectifier Rec 20	
RLY2	Relay, type 1, 12-volt d.c., 120 ohms	
D1	Diode 10 DE type, REC50A	
P1	Potentiometer, Model A, 10 turns, 500 ohms and Duo-Dial Model RB	Beckman, Glenrothes, Scotland
RLY1	S170 d.c. relay make at 90 μA, resistance 3300 ohms, Specification S170/1/457	Sangamo Weston, Enfield, Middlesex
PL1 and SOC1	6-Pin plug and socket, Part No. P194	A. F. Bulgin, Barking, Essex
MS1 and MS2	Microswitch, type HA1	Crouzet Ltd., Brentford, Middlesex
V1	1·5-Volt d.c. battery	—
V2	240-Volt a.c. supply	—
A	Audible alarm, "Bleeptone", 12-volt d.c.	A. P. Besson Ltd., Hove, Sussex
Burette unit	Fisons automatic dispenser includes R1 4700-ohm resistor and C1 0·4-μF condenser	Fisons Ltd., Loughborough, Leicestershire
PTFE valve block	—	I.C.A.M. Ltd., Northop, Mold, Flintshire
Counter	Re-set vending counter, Part No. KK1441	Veeder-Root, Croydon, Surrey
Timer	Chronoset CF, 0–36 minutes direct-clutch model	Technical Representations Ltd., Stockport, Cheshire
Case	Type DA 40168	Bedco Ltd., Harpenden, Herts.

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The Rapid Determination of Tungsten in Ores by X-ray Fluorescence Analysis

By K. G. CARR-BRION and K. W. PAYNE
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An X-ray fluorescence powder method involving the use of tungsten Kα radiation enables tungsten to be rapidly determined in ores of different mineralogical composition. Good agreement with chemical assays is obtained.

When tungsten is determined in powdered ores by X-ray fluorescence analysis, marked heterogeneity effects[1] can occur with the particle sizes produced by normal grinding methods. These effects are caused by tungsten being found as two minerals with markedly different X-ray absorption coefficients in the tungsten L wavelength region. The minerals are scheelite ($CaWO_4$) and wolframite ($(Fe,Mn)WO_4$). The difference in tungsten Lα intensity per unit concentration from scheelite to wolframite containing samples was found to be about 20 per cent. in the middle of the concentration range examined. If the ratio of the two minerals is known and remains effectively constant, analyses can still be carried out with tungsten L radiation by using mineral powder standards. However, if the ratio of the two minerals varies, considerable errors can result. These effects can be overcome by fusion or minimised by ultra-fine grinding. Fusion is time consuming and may prove difficult with some ores, for instance, those containing large amounts of arsenic. The fineness of grinding required to eliminate these effects is beyond the range of rapid conventional grinding equipment: the samples examined had already been ground to less than 300 mesh in a "swing mill." A third method of overcoming the effect is to use tungsten Kα radiation for the determination. The much lower X-ray absorption coefficients encountered enable the determination to be made directly on the powdered ores without measurable heterogeneity effects. The use of hafnium as an internal standard guards against any matrix effects with both the tungsten L and K radiations.

Experimental

Instrumental conditions—

A Phillips P.W. 1212 X-ray spectrometer, equipped with a gold tube operating at 100 kV, 20 mA and a lithium fluoride 220 analysing crystal was used: 2θ angles used were tungsten $K\alpha_{1,2}$ 8·39°, hafnium $K\alpha_{1,2}$ 8·92° and background 8·09°: counting times for each position, 30 seconds (in two increments of 15 seconds).

Sample preparation—

Samples were ground to less than 300 mesh in a "swing mill." Those expected to contain more than 5 per cent. of tungsten were diluted 1 + 7 w/w with a potassium sulphate buffer powder containing 5 per cent. of hafnium dioxide; those expected to contain less than 5 per cent. of tungsten were diluted 1 + 1 with the buffer. Two-gram buffered samples were examined directly by hand tamping in the sample holders.

Results

Some typical results obtained are shown in Table I.

Table I

Examination of analysed samples

Sample	Tungsten found by X-ray method, per cent.	Accepted tungsten concentration, per cent.
6516	53·1	52·5
5543	43·5	42·8
5544	16·3	15·8
6371	2·07	1·92
8344	0·84	0·73
4618	0·52	0·51

Reproducibility and accuracy—

The reproducibility obtained on successive sampling was better than 1 per cent. relative. The error relative to the composition determined by standard chemical methods was 1·8 per cent. for ten samples in the concentration range 16 to 60 per cent. of tungsten, and 6 per cent. for six samples in the concentration range 0·5 to 2 per cent. The exact mineralogical composition of these samples was unknown, but wolframite and scheelite standards gave equal intensities per unit concentration. Few samples were examined in the intermediate range.

Conclusions

The proposed method enables tungsten in ores of widely varying mineralogical composition to be determined rapidly. The concentration range covered is between 0·5 and 60 per cent. of tungsten. The sensitivity is limited by the relatively poor signal-to-background ratio found in the tungsten K region of the spectrum. The use of tungsten L radiation would give a much lower limit of determination, but would require the use of a fusion technique to eliminate heterogeneity effects.

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A Rapid Method for the Determination of Malathion in Wheat Grains

By E. WEISENBERG, S. GERTNER and J. SCHOENBERG

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A simple method for the determination of malathion in wheat grains is described. The insecticide was extracted with chloroform and the extract treated with a Celite - Nuchar - sodium sulphate mixture to absorb impurities selectively. The hydrolysis was carried out with ethanolic sodium hydroxide solution and the copper complex extracted into cyclohexane. The method is applied to amounts of 50 to 250 μg.

The use of malathion, which has been widely used for the control of pests in stored grains, has been based on its relatively strong pesticidal qualities and its low toxicity to mammals. The method described by Norris, Easter, Fuller and Kuchar,[1] based on the alkaline decomposition of malathion, has been recommended for the determination of residues in cereals and oil seeds.[2] Bates, Rowlands and Harris[3] used chromatography on Fuller's earth for the purification of the plant extracts before analysis. In a later publication, Bates and Rowlands[4] found that acceptable recoveries were being obtained without the preliminary use of an absorbent column. Upham[5] reported a modification of this method for the determination of malathion in formulations, in which carbon tetrachloride was replaced by cyclohexane, and the colour stability was found to have improved. In an attempt to improve the accuracy and precision of the method, a critical study was made by Orloski.[6] We adapted and modified this method in order to determine amounts as low as 50 μg. Chloroform was selected as the extracting solvent; according to Upham,[5] the descending order of solvent power for malathion was found to be chloroform, carbon tetrachloride and carbon disulphide. As a preliminary purification of the chloroform extract was found necessary, we effected selective absorption of the impurities by Celite - Nuchar - sodium sulphate as recommended by Koivistoinen, Karinpää, Könönen and Roine.[7] This treatment eliminated the need for repeated washings of the plant extract, which were found to be critical.[2]

Method

Reagents—

Chloroform, B.P. quality.

Acetonitrile, analytical-reagent grade.

Cyclohexane, analytical-reagent grade.

Ethanolic sodium hydroxide solution, about 0·5 N—Dissolve 1 g of analytical-reagent grade sodium hydroxide in 50 ml of absolute ethanol by heating under reflux.

Iron(III) chloride - hydrochloric acid solution—Dissolve 0·2 g of iron(III) chloride in 8 ml of hydrochloric acid (sp. gr. 1·18) and make up to 1 litre with water.

Copper sulphate solution, 1 *per cent. w/v.*

Absorbent for clean-up—This consisted of 1 part of Celite 545 (Johns-Manville), *plus* 2 parts of Nuchar C–190–N (West Virginia Pulp and Paper Co., Covington, Virginia) *plus* 1 part of anhydrous analytical-reagent grade sodium sulphate.

Ethanol, absolute, analytical-reagent grade.

Procedure—

Shake mechanically 150 mg of coarsely ground wheat grains with 300 ml of chloroform for 2½ hours. Filter the mixture through a Buchner funnel and wash the residue in the funnel with three 50-ml portions of chloroform. Evaporate the extract and washings to 200 ml in a Rinco vacuum evaporator. Transfer a 100-ml aliquot into a 250-ml Erlenmeyer flask, add 4 g of the Celite - Nuchar - sodium sulphate mixture and shake it for 5 minutes.

Filter through paper, wash the first flask and the residue on the filter six times with 10-ml portions of chloroform and add these washings to the filtrate, then evaporate the filtrate in a vacuum evaporator to about 10 ml. Transfer it to a small beaker, evaporate to dryness with a current of hot air and dissolve the residue in 8 ml of ethanol; transfer to a 125-ml separating funnel and wash the beaker with 5 ml of cyclohexane and add to the ethanol. To the contents of the separating funnel add 0·2 ml of acetonitrile and 1 ml of ethanolic sodium hydroxide solution; swirl the funnel gently (do not shake) for 5 to 10 seconds and let it stand for 2 minutes. Add 25 ml of iron(III) chloride solution (cooled to 10° C), mix well by swirling it for 10 seconds and let it stand for 5 minutes to allow the phases to separate; discard the cyclohexane. Add exactly 10 ml of cyclohexane and 1 ml of copper sulphate solution and shake the separating funnel immediately for 1 minute. Allow the phases to separate and, as soon as separation occurs, discard the aqueous phase and filter the cyclohexane solution through a small filter containing 0·1 g of anhydrous sodium sulphate. Measure the optical density of the yellow solution, within 15 minutes from the beginning of the hydrolysis, at 420 mμ in a cell of 1-cm path length.

Prepare a standard graph from pure malathion to cover the range of 50 to 250 μg of malathion. The line has a slope of 1·48 optical density units for 1 mg of malathion.

Results and discussion

In the samples of wheat grains analysed the malathion content was found to vary from 0 to 4 p.p.m.

The reliability of the method was studied by recovery tests performed on an untreated sample, and the results are presented in Table I.

Table I

Recovery of malathion from wheat grains

Amount added, p.p.m.	Amount found, p.p.m.	Recovery, per cent.
1·04	0·96	92
2·08	2·03	97
4·16	3·96	95
6·24	5·73	92
8·67	8·33	96

From these results we found that satisfactory recoveries were obtained.

We found that the hydrolysis with ethanolic sodium hydroxide in the presence of cyclohexane was advantageous. The last traces of coloured impurities remained in the cyclohexane layer after the addition of the iron(III) chloride solution, and the aqueous solution remained colourless and clear. The stability of the copper complex in cyclohexane has been found satisfactory. It is possible that the stability of the colour is connected with the fact that in our method a clear solution free from impurities was obtained directly after the hydrolysis. The calibration graph was found to be reproducible and, therefore, it was unnecessary to run daily standards.

It is proposed to study the applicability of the method to a range of foodstuffs in which malathion residues may be present.

We gratefully acknowledge the contribution made by Mrs. S. Gershson, in adapting the Orloski method for the determination of micro amounts of malathion.

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A One-step Extraction and Clean-up Procedure before Gas - Liquid Chromatographic Determination of Some Organochlorine Pesticide Residues in Blood

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An apparatus and a method are described for the extraction and clean-up of organochlorine pesticides, *e.g.*, DDT, DDE, α- and γ-BHC (lindane) and dieldrin, from heparin-treated and lyophilised blood samples for gas-chromatographic determinations.

Extraction and clean-up are carried out in one step on a suitable column with amounts of solvent as small as 25 to 30 ml. Two types of column are used according to the type of organochlorine pesticide to be determined; for acid-stable substances a sulphuric acid - diatomaceous earth column is used, and for alkali-stable pesticides an alkaline column, on which saponification of fat takes place simultaneously with extraction. The method has been applied successfully in worker as well as in general population surveys.

The accumulation and storage of organochlorine pesticides, particularly DDT, in the human organism is well established.[1,2,3,4] Investigation of this phenomenon, however, has been carried out mainly on human fatty tissue and milk samples.

Methods for the identification and determination of organochlorine residues are extensively reviewed by Beynon and Elgar.[5] In general, extraction and clean-up of the active agent are carried out separately. A one-step procedure for the two operations on a conditioned Florisil column was recently described by Langlois, Stemp and Liska.[6]

Experimental results recording the occurrence and level of organochlorine residues in human blood are scarce. Moreover, the few investigations of this kind that have been carried out were concerned primarily with alkali-stable active agents in relation to occupationally exposed people.[7,8] However, DDT and DDE could not be determined separately by the methods used in these investigations.

For the assessment of organochlorine pesticides in the blood of experimental animals, a method was published by Jain, Fontan and Kirk,[9] and hexane-extractable organochlorine insecticides in human-blood samples were determined by Dale, Curley and Cueto.[10] In these tests, as well as in the experiments of Radomski and Fiserova-Bergerova,[11] no clean-up was used before analysis.

For the determination of organochlorine pesticide residues in blood by gas - liquid chromatography we tried to develop a procedure with which substantially lower pesticide levels in blood originating from nutritional intake could be assessed, not only in occupationally exposed people but also in the general population. For this purpose, efficient clean-up of the blood extracts seemed necessary, particularly as the sensitivity of the tritium-foil electron-capture detector, which was also used in our investigations, becomes seriously impaired by lipid co-extractives present in blood extracts.[12]

The present paper describes an apparatus and a method by which the extraction of small amounts of organochlorine (DDT, DDE, α- and γ-BHC and dieldrin) residues in heparin-treated and lyophilised human-blood samples, and the clean-up of the extract, can be achieved in a single step by using only 25 to 30 ml of solvent. The resulting extract is suitable for gas - liquid chromatographic analysis.

The addition of the anti-coagulant to the blood samples became necessary because in this method, even when only incipient clotting occurs, the extractability of the organochlorine pesticides considerably decreases and, generally, these compounds cannot be extracted at all from clotted blood.

Lyophilisation in pesticide residue analysis has been recently mentioned by Brandenberger and Müller.[13] Also, in the course of other investigations in this Institute, good results

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have been obtained by using lyophilised samples in various types of analysis. The procedure had already been successfully applied to the investigation of organochlorine residues in foods, as well as to the determination of other agents, *e.g.*, phosphate esters.

Experimental

Reagents—

All materials should be of analytical-reagent grade, unless otherwise stated.

Florisil, 60 to 100 mesh—This was washed with light petroleum, air-dried and conditioned by the method of Langlois, Stemp and Liska.[6] It was kept for 10 to 12 hours at 140° C, then mixed with 5 per cent. of water; it may be stored for 1 week in a glass-stoppered bottle.

Sodium sulphate, anhydrous.

Diatomaceous earth, commercial grade.

Sulphuric acid mixture—Prepare by mixing equal volumes of concentrated sulphuric acid and fuming sulphuric acid containing 20 per cent. of sulphur trioxide.

Potassium hydroxide.

Alumina (Merck)—This was kept at 450° C for 3 hours and then mixed with 10 per cent. of water.

Sodium chloride.

Heparin solution.

Saline (sodium chloride) solution, 0·9 per cent.

Hexane, re-distilled, boiling-range 62° to 64° C.

Light petroleum, re-distilled, boiling-range 32° to 37° C.

Methylene chloride, re-distilled, boiling-point 42° C.

Benzene, re-distilled, boiling-range 80° to 81° C.

Olive oil.

Lyophilisation of sample—

Freshly drawn blood (2 to 10 ml), depending on the expected amount of pesticide, is placed into a glass tube containing 1 drop of heparin solution. By centrifuging the blood sample, plasma and red blood cells can be examined separately. For this purpose the cellular components must be washed thoroughly with saline and the washings added to the plasma.

After mixing the sample with 50 per cent. of air-dried Florisil, lyophilisation occurs. In our experiments, an apparatus (supplied by Labor Co., Budapest, Hungary) operated by an oil vacuum pump was used, the schematic representation of which can be seen in Fig. 1.

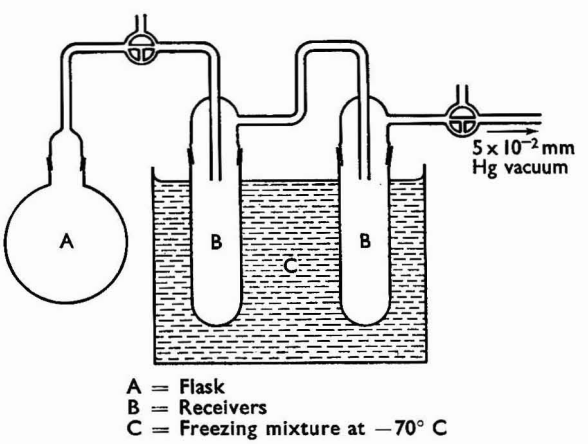


Fig. 1. Schematic representation of the apparatus for the lyophilisation of blood samples

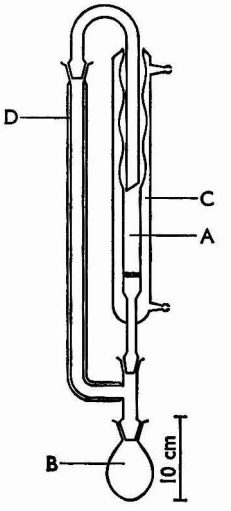
The Florisil - blood mixture is spread on the inner wall of flask A so that a uniform layer is formed. The flask is then immersed in a freezing mixture consisting of solid carbon dioxide and light petroleum until the layer becomes frozen. During this procedure a thin layer of ice is formed on the outer wall of the flask. For subsequent lyophilisation, flask A

is placed into the apparatus and the vacuum pump started. Vapours of sublimating ice are condensed in receiver B, which is cooled to —70° C by the freezing mixture, and thus do not reach the vacuum pump. Lyophilisation is completed when the layer of ice on the outer wall of flask A melts away. The water-free layer adhering to the inner wall of the flask is now scraped off and pulverised.

The vacuum used in this experiment was 5×10^{-2} mm of mercury. Under these conditions, no organochlorine residues, except lindane (see Table I), could be extracted in recovery experiments by methylene chloride (which is used generally in this laboratory in water analysis[14]), in determinable amounts from the ice accumulated in the receiver after thawing, indicating that DDT, DDE and dieldrin do not escape from blood during lyophilisation. In some instances, however, when a higher vacuum is needed, it may become essential to re-check the lyophilisation procedure for eventual loss of pesticide residues.

EXTRACTION AND CLEAN-UP OF SAMPLE—

For extraction and clean-up, column A, in the apparatus shown in Fig. 2, is filled with adsorbent (Fig. 3).



A = Column containing adsorbent
B = Flask
C = Condenser
D = Insulated tube

Fig. 2. Apparatus for extraction of organochlorine pesticides and clean-up of the extract

With acid-stable active agents, such as DDT and its metabolite DDE, or BHC, a modified Davidow column[15] (Fig. 3—Acid) can be used, and with alkali-resistant dieldrin an alkaline column (Fig. 3—Alkaline), developed by the authors, on which saponification of fat takes place simultaneously with extraction. An alkaline column of different composition has been described by Albert.[16]

In the apparatus shown in Fig. 2 a small amount of light petroleum, containing 20 per cent. of methylene chloride for dieldrin, circulates through the lyophilised sample placed on top of the adsorbent in column A. The amount of solvent used (25 to 30 ml) should be such that, after percolating through the column, about 6 to 8 ml would accumulate in flask B. Water is then circulated in condenser C. The water jacket around column A, and condenser C (Fig. 2), prevents the vapour pressure of the low-boiling solvent from disrupting the column, which would counteract the continuous circulation of the solvent. The solvent is made to circulate by gently warming flask B on a suitably regulated water-bath. The solvent vapours enter through insulated tube D into condenser C, where condensation occurs, and the condensed solvent again percolates through the sample and column. The warming of flask B is regulated so that above the sample there should always be a solvent layer of 0·5 to 1·0 cm. With correct column filling the solvent flow-rate is about 3 ml per minute.

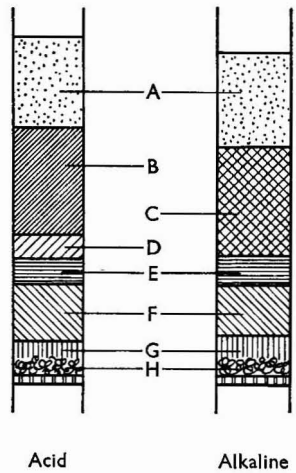


Fig. 3. Acid and alkaline columns: A, lyophilised blood sample; B, sulphuric acid - diatomaceous earth mixture (3·5 ml of fuming sulphuric acid and 4 g of diatomaceous earth); C, 3 g of pulverised potassium hydroxide, wetted with methylene chloride saturated with water; D, 0·5 g of diatomaceous earth; E, 0·5 g of alumina; F, 3 g of Florisil, conditioned; G, 0·5 g of sodium sulphate; H, glass-wool

After 3 hours' circulation the contents of flask B are transferred into a conical centrifuge tube and the solvent evaporated. The residue is dissolved in 0·25 to 1·0 ml of hexane and is ready for gas - liquid chromatographic analysis. With alkaline column filling the extract in flask B is transferred into a separating funnel, washed twice with water and dried over anhydrous sodium sulphate.

GAS - LIQUID CHROMATOGRAPHIC ANALYSIS OF PURIFIED EXTRACTS—

A Perkin-Elmer gas chromatograph, Model 452, was used, and a Pyrex-glass column, 2 feet long and $\frac{1}{4}$-inch diameter, filled with Chromosorb W, 60 to 100 mesh, with 2·5 per cent. of Apiezon L and 0·75 per cent. of Epikote resin 1001.

The conditions used were: carrier gas, nitrogen; inlet pressure, 1·4 kg per cm^2; flow-rate, 200 ml per minute; column temperature, 170° C; injection temperature, about 230° C; detector, electron capture and applied potential, 45 volts; amplifier gain, $\frac{1}{16}$th to $\frac{1}{64}$th; and injection syringe, Hamilton microlitre syringe, 701-N.

In experiments reported here, 10-μl aliquots of the purified and dissolved extract were injected into the gas chromatograph and chromatograms obtained by selecting the appropriate amplifier gain according to active-agent content.

Insecticides were identified by comparing their retention times with those of compounds used for reference.* Thin-layer chromatography, as described by Kovacs,[17] was used as a complementary technique.

The quantitative determination of the pesticide was carried out by multiplying the peak height in the chromatogram by the peak width at half the peak height.

Calibration graphs were prepared by injecting 1 to 10 μl of the standard solutions into the gas chromatograph containing 0·5 to 1·0 μg of pure pesticide per ml of solvent.

Amounts of blood extracts and reference substances must be chosen so that comparative measurements can be carried out with the same amplifier gain. For DDT, with both test and reference substances, the ratio of the peak heights of DDT, and of DDD originating from a minor decomposition of DDT under the working conditions already described, must be considered.

* All organochlorine compounds used for reference were recrystallised several times and controlled by their melting-points.

RECOVERY FROM COLUMNS—

The recovery of known amounts of organochlorine pesticides in pure solution from the columns described has been checked. It was found that on both types of column, acidic as well as alkaline, 94 to 96 per cent. of the active agents added in amounts of 0·05 μg (equivalent to 0·01 p.p.m. of agent in 5 ml of blood) was recovered after 10 minutes' circulation. After 20 minutes the recovery was 98 to 100 per cent.

EXTRACTION TIME—

To determine the extraction time necessary to obtain the maximum of extractable residual material, extraction times of 1 to 4 hours were carried out. It was found that whereas 2 hours were insufficient for optimum results, the highest residue yields could be achieved with certainty in 3 hours. With the solvent flow-rate already described, extraction for 3 hours with only 25 to 30 ml of solvent corresponds to a conventional extraction carried out with 550 to 600 ml of solvent.

RECOVERY OF ADDED PESTICIDES—

Because of lyophilisation, it was necessary to devise special techniques of adding various known amounts of pesticides to blood. The reference substances could be added to blood only in those solvents that do not interfere with the freezing of the Florisil - blood mixture before lyophilisation. In addition, the complete incorporation of the added material into the sample was necessary to avoid substantial evaporation under vacuum. All of these requirements could be met by dissolving the reference substances in benzene and by mixing known portions of the benzene solution with olive oil containing (from previous experiments) no appreciable amounts of organochlorine residues.

One microgram of each pesticide, *viz.*, DDT, DDE, lindane and dieldrin, dissolved in 1 ml of benzene containing 5 drops of olive oil, was added to 10 ml of blood (equivalent to 0·1 p.p.m. of pesticide in the blood). The mixture was vigorously shaken and then allowed to stand for a few hours, during which period the shaking was frequently repeated. Subsequently, an amount of Florisil equal to 50 per cent. w/w of the blood sample was added and the mixture lyophilised. Recoveries are shown in Table I.

TABLE I

RECOVERY OF ORGANOCHLORINE PESTICIDES ADDED TO BLOOD

Pesticide	Amount of pesticide in blood, p.p.m.	Amount of pesticide in blood after adding 0·1 p.p.m. of reference substance, p.p.m.	Recovery, per cent.
DDT	0·0104	0·105	95·2
		0·107	97·1
		0·105	94·7
DDE	0·0172	0·119	102·0
		0·116	98·8
		0·113	96·3
Lindane	0·0051	0·025	20·0
		0·025	20·5
		0·017	11·9
Dieldrin	<0·0001	0·094	94·5
		0·097	97·2
		0·098	98·3

It is noted that although the recovery of added DDT, DDE and dieldrin was almost complete, that of lindane was only partial. This might arise from the considerable solubility in water and volatility of lindane, in consequence of which most of this substance, when added to blood samples, escapes during lyophilisation and can be recovered in receiver B. Of the lindane originally present, however, no trace could be detected in the receiver, and we found no explanation for this, but the assumption of Dale, Curley and Hayes,[18] according to which organochlorine pesticide residues in blood are probably bound to proteins, deserves attention. Therefore, the results must be evaluated with respect to recovery experiments.

Results and discussion

With the apparatus described, extraction of organochlorine pesticides (DDT, DDE, dieldrin, lindane and α-BHC) from blood, and purification of the extract before gas - liquid chromatographic examination can be performed in one single step with a minimum of solvent.

The clean-up of extracts containing acid-stable or alkali-stable pesticides is carried out by using columns with different fillings. With an alkaline column, a partial decomposition of methylene chloride present in the solvent mixture can occur. Our experiments, however, proved that neither did interfering peaks appear on the chromatogram, nor did interference with quantitative extraction or recovery of added reference substance occur as a consequence of this decomposition.

Under these experimental conditions, clear-cut peaks appeared in the course of gas - liquid chromatographic determinations separately carried out with DDE, dieldrin, lindane and α-BHC, indicating that no decomposition occurred. DDT, however, became slightly decomposed to DDD in the gas - liquid chromatographic procedure, thus giving two peaks.

Identification of the small DDD peak was achieved by using DDD as a reference substance. When establishing the DDT calibration graph, the peak area of DDT and the small one of the DDT breakdown product were considered. In measurements of the peak areas of the sample, DDT and DDD were compared with corresponding peak areas in the chromatogram of pure DDT solutions used for the preparation of the calibration graph.

The decomposition of DDT depends also on the condition of the gas-chromatographic column. With a freshly filled column increased decomposition was observed, and the rate of decomposition is also affected by the absolute amount of DDT present, for instance, a larger proportion of 1 ng than of 10 to 15 ng is decomposed. Therefore, comparison of sample and reference DDT must be made as far as possible with similar amounts, with the same amplifier gain and also by preparing sample and reference chromatograms in rapid succession.

In Fig. 4 the gas chromatogram of the acid-treated blood extract of a healthy person, with only the usual environmental exposure to insecticides, is shown, together with the thin-layer chromatogram of the same extract and of reference compounds.

Although the presence of β-BHC is revealed by the thin-layer chromatographic plate, the peak of this compound cannot be identified in the gas chromatogram, because, under the conditions used for these measurements, the peak of β-BHC and other unidentified peaks partially overlap. It is noteworthy that the small, unidentified peak after the DDE peak can be seen only in the chromatogram of acid-treated blood extracts and was not noticed, for instance, in fat extracts. In the chromatogram of blood samples other peaks also appear, the identities of which are not yet established.

Fig. 5 shows the gas and thin-layer chromatograms of the blood extract of a worker who was employed on a plant that produced aldrin-treated fertiliser. The blood extract was purified by an alkaline column before gas - liquid chromatography, and the thin-layer chromatogram clearly shows two spots corresponding to dieldrin and DDE, the latter including also DDT converted into DDE by the alkaline treatment. Dieldrin is a metabolite of aldrin formed in the organism.

The method has also been used in general population surveys for the determination of the DDT, DDE, α- and γ-BHC (lindane) and dieldrin contents of blood, and in monitoring the dieldrin concentration of the blood of workers engaged in the production of aldrin-treated fertilisers.

Table II

Organochlorine pesticide residue levels in the blood of the general population in Budapest, Hungary, 1967

Results are expressed in p.p.m.

Serial number	DDT	DDE	α-BHC	γ-BHC (lindane)	Dieldrin
1	0·0104	0·0172	0·0004	0·0051	<0·0001
2	0·0085	0·0061	0·0007	0·0063	<0·0001
3	0·0081	0·0052	0·0003	0·0041	<0·0001
4	0·0124	0·0462	0·0002	0·0032	<0·0001
5	0·0255	0·0253	0·0001	0·0035	<0·0001

Table II shows results from material continuously collected by us from among the general population; the presence of a considerable amount of lindane is remarkable. It is pointed out by Hayes[19] that the presence of lindane in blood is generally a consequence of some unusual exposure to this substance. Our findings, however, show that with the method

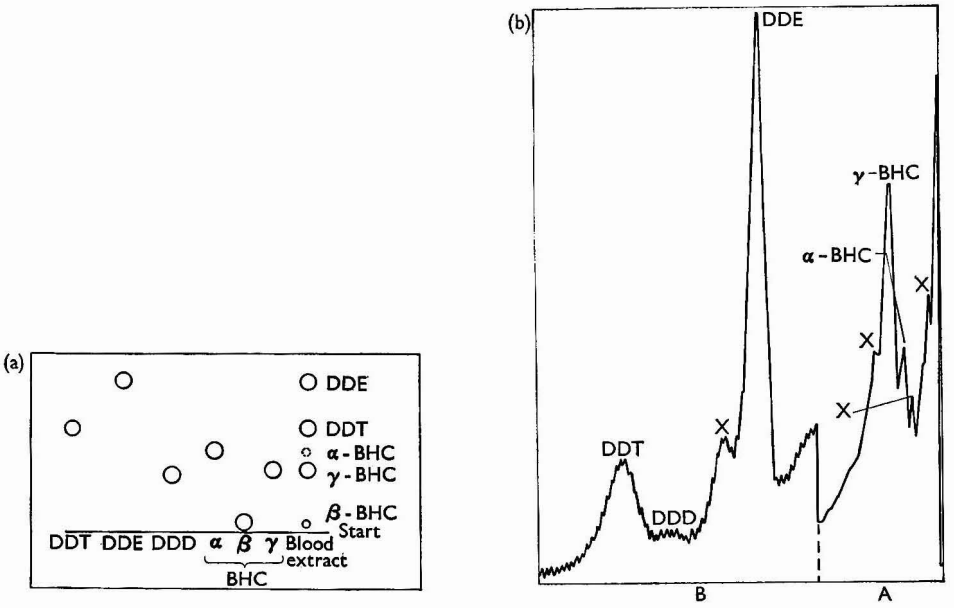


Fig. 4. (*a*) Thin-layer chromatogram and (*b*) gas chromatogram of an acid-treated blood extract (general population blood sample). X = unidentified peak. On gas chromatogram, section A was recorded at 30 inches per hour, with an amplifier gain of $\frac{1}{16}$th and section B at 15 inches per hour with an amplifier gain of $\frac{1}{64}$th. The diagram is reduced to one third of the original size

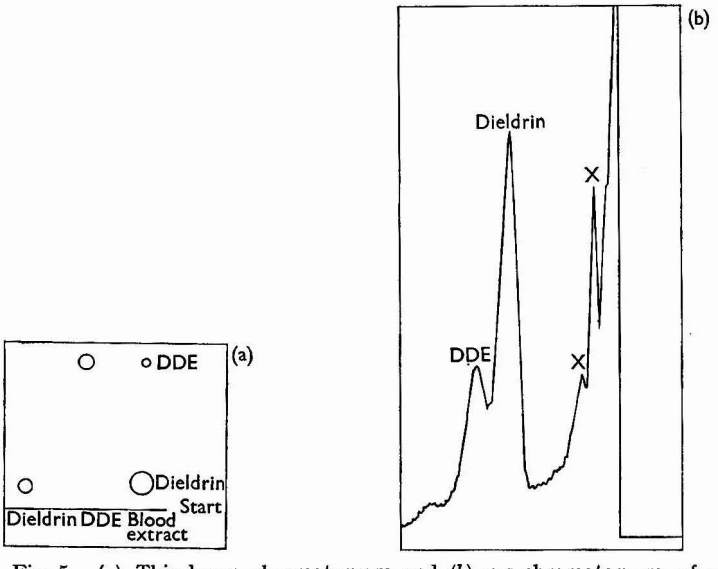


Fig. 5. (*a*) Thin-layer chromatogram and (*b*) gas chromatogram of an alkali-treated blood extract (aldrin-exposed worker's blood), X = unidentified peak. The gas chromatogram was recorded at 15 inches per hour, with an amplifier gain of $\frac{1}{16}$th. The diagram is reduced to one third of the original size

described above, 0·01 p.p.m. of lindane can be detected regularly in the blood of the general population at present in Hungary.

In Table III some representative results are shown, as selected from material collected among workers on a plant producing aldrin-treated fertiliser, corresponding to two different levels of exposure. The first three values relate to transport personnel working in the store-house of the plant, the other three to workers operating the mixing machine inside the plant. It can be seen that the difference between heavy and moderate exposures also reveals itself in the dieldrin levels in blood.

TABLE III

DIELDRIN LEVELS IN THE BLOOD OF WORKERS EMPLOYED IN A PLANT PRODUCING ALDRIN-TREATED FERTILISER

Serial number		Dieldrin in blood, p.p.m.
1	Transport workers	0·017
2		0·024
3		0·020
4	Workers in the mixing room	0·195
5		0·103
6		0·123

In Table IV some results are presented relating to the distribution of DDT and DDE in plasma and erythrocytes in the blood of the general population.

TABLE IV

DDT AND DDE CONTENTS IN PLASMA AND RED BLOOD CELLS OF PEOPLE NOT OCCUPATIONALLY EXPOSED TO PESTICIDES, BUDAPEST, 1966

	1		2		3		4	
	In plasma p.p.m.*		In red blood cells, p.p.m.*		In whole blood, as calculated from 1 and 2, p.p.m.		In whole blood, as determined, p.p.m.	
Serial number	DDT	DDE	DDT	DDE	DDT	DDE	DDT	DDE
1	0·005	0·016	0·002	0·007	0·007	0·023	0·006	0·021
2	0·008	0·025	0·004	0·009	0·012	0·034	0·010	0·032
3	0·008	0·039	0·003	0·011	0·011	0·050	0·010	0·046
4	0·007	0·030	0·003	0·016	0·010	0·046	0·009	0·051
5	0·003	0·018	0·003	0·007	0·006	0·025	0·006	0·028

* Calculated to whole blood.

The results reported in this paper show the suitability of the method for the purposes outlined above. Results and evaluation of our monitoring work will be published elsewhere.

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Determination of the Number of Oxygen Substituents of Steroids by Chromatography

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ΔR_m values obtained by difference of R_m values in the presence and absence of formaldehyde are shown to group in a manner determined by the number of polar functional groups and, to a lesser degree, by the nature of the steroid skeleton. It is proposed that determination of the ΔR_m value provides a means of characterising steroids from natural sources.

Partition chromatography gives ΔR_m values that are constant for each substituting functional group in a particular family of solvent mixtures (reviewed by Bush[1] and Edwards[2]). The present observations stem from the use of formaldehyde in the stationary phase in an attempt to cause strong association with hydroxylic and ketonic substituents, and thus to alter the ΔR_m values.

Paper chromatography of steroids with mixtures of aqueous formaldehyde, light petroleum and benzene gave excessive tailing of the chromatographed spots. Incorporation of methanol eliminated tailing, and the mixture effectively became a modification of the Bush[3] system.

Experimental

The general apparatus, methods, reagents and details of steroid chromatography are described elsewhere[2] and only special points will be elaborated.

Reference steroids—

These were generally purchased from Steraloids Ltd., Croydon, Surrey, and were checked for correctness of melting-point, chromatographic properties and chemical reactions. Other steroids were kindly provided by Professor W. Klyne from the Medical Research Council reference steroid collection.

Conditions of chromatography—

Whatman No. 2 paper was used at 37° C, with 3 hours' equilibration. The solvent front was allowed to proceed 40 cm by descent, and steroids were located by chemical and physical procedures.[2]

Solvent mixtures—

The stationary phase consisted of a mixture of methanol and saturated aqueous formaldehyde (1 + 1). Tailing occurred at lower concentrations of methanol and higher concentrations lead to monophasic conditions. The mobile phase consisted of light petroleum and benzene mixtures. As the ΔR_m values for replacement of light petroleum by benzene were proportional to the benzene concentration, as reported for the Zaffaroni systems[4] and Bush systems,[3] it is not necessary to define precise solvent mixtures. R_{mo} for the family is defined as the R_m value determined for the mobile phase of light petroleum alone. For precision, observations were made within the limits +0·6 to −0·6 R_m units. It was found that

R_{mo} = observed R_m + 0·026 (benzene concentration, per cent. v/v, when preparing).

In the Bush systems[3] the benzene concentration factor was 0·0195 R_m units.

Results

The R_{mo} values obtained by chromatography of ninety-eight steroids are summarised in Tables I and II.

Table I

Key steroid R_{mo} values

Steroid*	Trivial name	R_{mo}†
P^4-3:20-one	Progesterone	−0·45
5β P-3α-ol-20-one	Pregnanolone	−0·37
P^5-3β-ol-20-one	Pregnenolone	−0·18
5α A-3α-ol-17-one	Androsterone	+0·02
5β A-3α-ol-17-one	Aetiocholanolone	+0·27
A^5-3β-ol-17-one	Dehydro-epi-androsterone	+0·37
5β P-3α:17α:20α-ol	Pregnanetriol	+1·34
P^4-3:11:20-one-17α:21-ol	Cortisone	+2·25
P^4-3:20-one-11β:17α:21-ol	Cortisol	+2·54

* Bush[1] abbreviated nomenclature.

† $R_{mo} = R_m$ of substance in methanol - aqueous formaldehyde - light petroleum mixture (1+1+2), obtained by calculation if necessary; see text.

Table II

Mean ΔR_m values in formaldehyde systems

Substituent	ΔR_m	Number of examples	±Standard deviation
1α-ol	1·40	3	0·15
1β-ol	1·20	1	—
2α-ol	0·81	1	—
6α-ol	1·81	1	—
6β-ol	1·44	2	—
7-one	1·19	1	—
7α-ol	1·84	1	—
11-one	0·91	8	0·17
11α-ol	1·52	3	0·16
11β-ol	1·09	12	0·15
12α-ol	1·49	1	—
16β-ol	2·03	3	—
17-one	1·65	1	—
17α-ol (P)	1·03	9	0·18
20-one	0·89	1	—
21-ol	1·04	6	0·24
CH_3	0·22	4	—
5α → 5β	0·12	9	0·08
3α-ol → 3β-ol	0·29	2	—
3-one → 3β-ol	0·58	2	—
4-ene-3-one → 3α-ol-5β	0·24	10	0·07
11-one → 11β-ol	0·26	7	0·07
20-one → 20β-ol	0·33	5	0·14
20-one → 20α-ol	0·57	5	0·15

Discussion

On plotting the steroid R_{mo} values obtained with and without formaldehyde, the points were found to group about three parallel lines. Each group shared a common number of functional groups, being di-, tri- and tetra-, or more, oxygenated.

An alternative method of plotting is summarised in Fig. 1, where the plot of ΔR_m value caused by the formaldehyde is plotted against the number of polar functional groups. In this figure the observations are plotted as the mean value of each group and the standard error. It is clear that the observed values cluster in a manner useful in characterising steroids from natural sources into one of the following groups: tetra-, or more, oxygenated; tri-oxygenated, with indications at the upper and lower ends of androstane or pregnane skeletons; di-oxygenated androstane or pregnane derivatives, with no ambiguity.

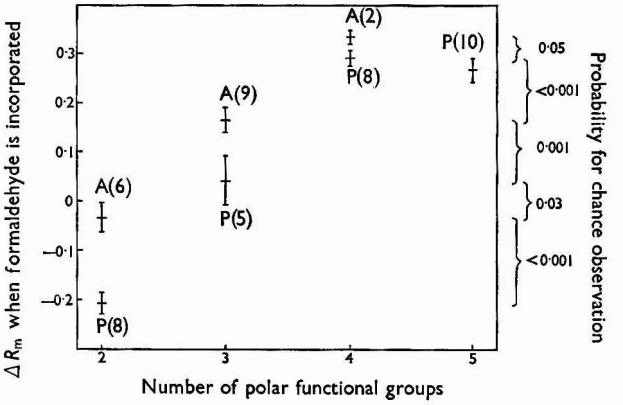

Fig. 1. Correlation of ΔR_m when formaldehyde is incorporated with several polar functional groups. The mean, number of steroids in each group, standard error and the probabilities of significance of differences are indicated. Groups marked A are androstane derivatives and P, pregnane

The above characterisation of steroids by number of functional groups was carried out initially on simple hydroxylic and ketonic steroids without vicinal effects. On extension to vicinally substituted steroids no marked divergence was found for 2,3-, 16,17-, 17,20- and 20,21-di-substitution, provided that the substances were not penta-substituted. Double bonds in isolation, or in conjugation with carbonyl groups, had no effect, and the acetoxy group behaved as di-functional.

A further examination of the results was carried out separately in the androstane and pregnane groups by multiple regression to distinguish between the effects of hydroxyl groups and ketones and assess each standard error. The pairs of values were not significantly different, providing justification for the empirical procedure used above, in which the ΔR_m effects of hydroxyl and ketone groups were assumed to be equal.

The present procedure has been applied in the characterisation of substances from the urine of a new-born child[5] and indicated that certain oxidation products were tri-oxygenated androstane derivatives. This was in agreement with other observations on these substances.

Although the present study is limited to steroids it may be presumed that similar results would be obtained with other groups of substances that do not react chemically with formaldehyde.

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The Use of Depleted Cells as Inocula in Vitamin Assays

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Preparation of assay inocula in such a way that the cells contain a reduced level of the vitamin to be assayed gives low background growth in plates, and the greater contrast enhances zone definition. Steps required to be taken to find the optimum conditions for preparation of inocula are described. The assays of nicotinamide, pantothenic acid, folic acid and vitamin B_6 can be improved in this way.

A FAMILIAR problem in microbiological assays is high "blank" growth. In our early attempts to assay folic acid by a plate (agar-diffusion) method, in which *Lactobacillus helveticus* was used, there was insufficient contrast between background growth and response zone. The inoculum was prepared by growing cells in "enriched culture medium"[1] for 18 hours and then washing twice with 0·85 per cent. saline. (The levels of the relevant B vitamins in the "complete" medium used were established by microbiological assay and are shown in Table I.) Further washing of the cells with 0·85 per cent. saline reduced the background growth, but only slowly and incompletely, as if the cells could store folic acid.

In our first attempt at depletion, the cells were washed, re-suspended and incubated in a volume of folic acid free medium equal to that of folic acid rich medium ("enriched culture medium") in which they were grown; this treatment gave no reduction in background growth. When cells grown in folic acid rich medium were diluted and incubated in a volume of folic acid free medium such that considerable increase in cell numbers could take place, background growth in plates was reduced, and the contrast was sufficiently great for zones to be well defined. The depleted cells are prepared by growing in "enriched culture medium" at 37° C for 18 hours, and a small volume is diluted fifty times in folic acid free medium, incubated for 7 hours and used without washing.

It appears that cells growing in vitamin-rich media can accumulate vitamins to such an extent that, on being diluted and incubated in a medium deficient of one vitamin, they can divide until they are depleted of the vitamin absent in the medium.

Because this treatment of *L. helveticus* cells in folic acid assay improved zone quality, attempts were made to improve other assays in the same way. The plate assays of vitamin B_6 with *Saccharomyces carlsbergensis* (with an inoculum grown initially on malt extract - agar), and pantothenic acid and nicotinamide with *Lactobacillus arabinosus* (with an inoculum grown initially on "enriched culture medium"), have been improved in this way (see Table I). The suspensions can be stored in the refrigerator (about +5° C) for at least 1 week and are suitable for use in tube assays.

The details of techniques for producing depleted inocula would probably differ between organisms, strains, media and laboratories. The steps required to be taken to find the optimum conditions for depletion are as follows.

PROCEDURE—

(1) Dilute a small volume of culture grown in a vitamin-rich medium with a medium free from the appropriate vitamin, incubate and determine, by taking viable counts, at intervals, when multiplication has ceased. This is the depletion time.

(2) Dilute graded volumes of culture grown in a vitamin-rich medium with a medium free from the appropriate vitamin, incubate for the length of time indicated in (1) and measure the extent of multiplication by taking viable counts. This will indicate the minimum volume of culture which, on dilution and incubation, gives the maximum number of cells. These cells will be fully depleted.

(3) Determine the optimum concentration of depleted cells for use in an assay plate.

TABLE I

VITAMIN LEVELS IN MEDIA FOR INOCULA

	Amounts per ml of medium			
	Pantothenic acid	Nicotinamide	Folic acid	B_6
"Enriched culture medium"	0·27 μg	1·5 μg	2·3 ng	—
Malt extract - agar* ..	—	—	—	0·14 μg

* Difco malt extract 4·5 per cent.; agar 1·5 per cent.

That micro-organisms can accumulate vitamins and then multiply in the absence of one of them has been known for some time,[2,3,4] and the incubation of cells in media low in, or free from, vitamin has been advocated for assay inocula. Gibbons and Engle,[5] for example, to demonstrate menadione qualitatively, prepared menadione-deficient *Bacteroides melaninogenicus* cells by incubating the organism in broth free from menadione "where limited growth occurred." Simpson[6] recommends growing *L. helveticus* for 16 to 18 hours in 100 ml of single-strength assay medium containing riboflavin, washing and re-suspending the cells in the same volume of single-strength assay medium, without riboflavin, and incubating for 16 to 18 hours. In our experience, the cells must be able to multiply in order to deplete, and to effect this the cells should be diluted before they are incubated in the vitamin-free medium. Strohecker and Henning[7] put forward the idea of depletion, but in no instance do they incubate in the absence of the vitamin to be assayed, and they describe no plate assays.

The mechanism of the accumulation and depletion of vitamins is not yet known in detail or fully understood. Work has begun towards a better understanding of this phenomenon, and it is hoped to publish the results in due course.

I thank Mr. S. A. Price for helpful criticism of the text.

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Species Identification of Cooked Fish by Disc Electrophoresis

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The present objective methods of identifying fish species are based on the species-specific protein-separation patterns obtained on electrophoresis of the water-soluble sarcoplasmic proteins of fish muscle. As the proteins must be in their native undenatured state, electrophoretic identification of fish species has, so far, been restricted to raw fish.

An extension of the electrophoretic method to the identification of cooked fish is described. The protein fragments extractable in 6 M urea from the denatured proteins of cooked muscle can also be separated by electrophoresis into species' characteristic patterns that could be used for species identification. The separation patterns obtained on polyacrylamide gel for the urea extracts of cooked herring, halibut, plaice, salmon, cod and haddock are presented. In its present form the method does not apply to canned fish.

The identity of a fish species is usually readily determined from the physical appearance of the whole fish, and it is only when the distinguishing features normally used for identification have been removed, as in a fillet, that the identity of the species can be in doubt. After further processing, such as cooking, and subsequent incorporation into fish cakes and fish pastes, identification by sensory means is often impossible. There is, therefore, a need for a reliable non-sensory method of identifying the species in fish products whenever there can be doubt as to its authenticity. This is of importance commercially because of the possibility of substituting cheaper for more expensive varieties of fish.

An objective method of species identification is based on the species characteristic protein separation patterns obtained after electrophoresis of the water-soluble sarcoplasmic proteins of the muscle.[1] Zone electrophoresis on starch gel[2] has been used by Thompson[3] as a routine analytical method of species identification, and, more recently, disc electrophoresis on polyacrylamide[4] has been used by Payne,[5] Mancuso,[6] Torry Research Station[7] and Thompson.[8] These methods of identifying species can only be applied to raw undenatured muscle as the separation patterns are obtained from the proteins in their native, undenatured state. If the fish is cooked or dried the proteins become denatured by heating and coagulate to form precipitates, which can no longer be examined by this method. Partially cooked products such as fish fingers can, however, be examined provided there is still sufficient undenatured flesh to give a satisfactory protein separation.

This paper describes a possible extension of species identification to cooked fish products.

Methods

Preparation of cooked fish fillets—

Heat fish fillets on a steam-bath for 30 minutes in covered casseroles.

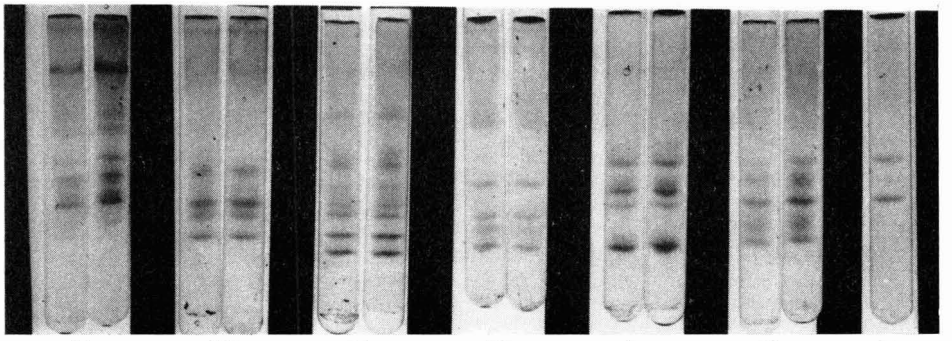

Fig. 1. Electrophoretic patterns of urea extracts of cooked fish (a to f) of (a) herring, (b) halibut, (c) plaice, (d) salmon, (e) haddock and (f) cod; (g) is the pattern for the sarcoplasmic proteins of cod in 6 M urea. Acrylamide gel concentration is 7·5 per cent. w/v

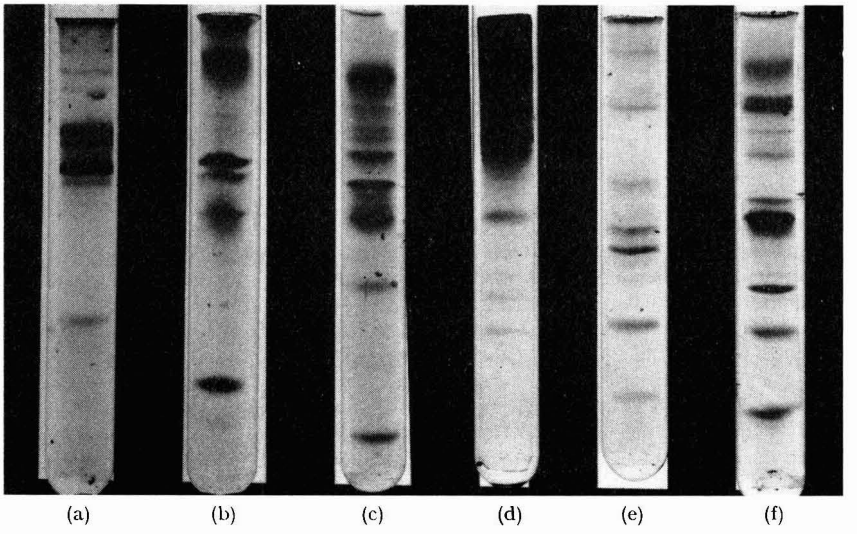

Fig. 2. Electrophoretic patterns of water extracts (the sarcoplasmic proteins) of raw fish of (a) herring, (b) halibut, (c) plaice, (d) salmon, (e) haddock and (f) cod. Acrylamide gel concentration is 6·0 per cent. w/v

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UREA EXTRACT OF FISH—

Break up a 25-g portion of fish fillet or fish product and suspend it in 50 ml of 10 M urea solution. After allowing it to stand overnight at room temperature, remove the insoluble fish residue by centrifuging for 20 minutes at 6000 × *g*. Use the supernatant solution directly for electrophoresis.

DISC ELECTROPHORESIS—

Tris - glycine buffer solution—Weigh 28·8 g of glycine and 6·0 g of tris(hydroxymethyl) aminomethane and dissolve it in water. Make the solution up to 1 litre and adjust the pH to 8·6. Dilute 1 to 10 for use as the solvent for the gel reagents and as the electrolyte solution for electrophoresis.

Acrylamide gel rods—To prepare a 7·5 per cent. w/v acrylamide gel, dissolve 3·0 g of "Cyanogum 41"* in 20 ml of tris - glycine buffer solution. Add 10 ml of 1·60 per cent. w/v β-dimethylaminopropionitrile[9] and 10 ml of 0·20 per cent. w/v ammonium persulphate. Transfer the solution quickly to the gelling tubes (7·5 × 0·5 cm), fill to a depth of 6·5 cm, overlayer with water according to the procedure of Ornstein and Davis,[4] and set aside to polymerise at room temperature for about 20 minutes. Prepare a 6 per cent. gel by reducing the amount of "Cyanogum 41" to 2·40 g.

Method of electrophoresis—After polymerisation is complete transfer the tubes to a disc electrophoresis apparatus† in a chilled room at 1° C. Carry out a pre-run for 20 minutes at 200 volts to remove any discontinuities in the gel. Apply 30 to 60 μl of the urea extracts of the fish directly to the tops of the gels by dipping a syringe through the upper electrolyte compartment. Carry out the electrophoresis for 55 minutes at 280 volts.

Staining and developing the gels—When the run has been completed remove the gels from the tubes[4] and stain them for half an hour in a 0·1 per cent. solution of Amido black in 7 per cent. acetic acid solution. Wash out the excess of dye with the acetic acid solution and allow to stand overnight in a methanol - acetic acid - water solvent (21 + 3 + 96). Examine the developed gels the following morning.

RESULTS AND DISCUSSION

In Fig. 1 are given the electrophoretic separation patterns of the protein residues extracted into 6 M urea from cooked herring, halibut, plaice, salmon, cod and haddock. For comparison the patterns of the corresponding water extracts from raw fish (the sarcoplasmic proteins) are given in Fig. 2. In general, the urea-extracted protein residues have fewer slow moving components and there is a greater over-all similarity of the pattern that makes differentiation more difficult. For example, the patterns for the closely related species cod and haddock, are similar and are more likely to be confused than are the corresponding sarcoplasmic protein patterns. Nonetheless, the patterns obtained for all six species are sufficiently different to allow an unequivocal identification of the species to be made.

Preliminary examination of some cooked fish products was carried out. When the urea extracts from canned herring were examined by this method there were no well identifiable protein zones as are obtained from herring steam-cooked at atmospheric pressure. A fish "chip" preparation made from a homogenised mixture of cod flesh, potato powder and starch (5 + 7 + 1), which had been cooked in vegetable oil at 260° C, gave a pattern identifiable as that of cod. There was no contribution to the pattern from the small amount of vegetable protein present. A commercial white fish cake of unspecified fish content gave a pattern easily recognisable as that of cod.

Urea is well known as an agent for splitting hydrogen and hydrophobic bonds in native proteins[10] and, as such bonds are believed to form when proteins are denatured by heating, it is not surprising that fragments of these denatured proteins are extracted with strong urea solutions. In fact, when raw fish were examined by this method the separation patterns of the urea extracts were the same as the corresponding ones from urea extracts of cooked fish, suggesting that non-covalent bonds of this type are formed on cooking and that they are subsequently broken by urea. As shown in Fig. 1 (f and g), the sarcoplasmic proteins contribute to only part of the pattern of the urea extract of the fish flesh as a whole. The remainder of the zones must derive from the myofibrillar and connective tissue proteins.

* "Cyanogum 41" is obtainable from British Drug Houses Ltd. It contains 95 per cent. of acrylamid monomer and 5 per cent. of bisacrylamide.

† Supplied by the Shandon Scientific Company Ltd., London.

Conclusion

Electrophoretic examination of the protein fragments in urea extracts of fish appears to be a promising method for the identification of species in cooked products. A more extensive survey would, however, be necessary before it could be used as an objective method.

In its present form it is not applicable to the identification of canned products. It is restricted to fish cooked under atmospheric pressure.

Mr. B. W. Thomson assisted in the experiments described in this paper. The work described was carried out as part of the programme of the Ministry of Technology.

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Observations on the Distribution and Determination of Fluorine Compounds in Biological Materials, Including Soils

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The distribution of fluorine compounds found in biological materials, including soils, is outlined. Several improvements are proposed for the sampling and ashing of animal and plant tissues, and soil, including the use of finely ground suspensions in dilute agar solution. Some modifications are suggested in the separation of fluorine by diffusion as hydrofluoric acid and its subsequent determination with alizarin complexan. The effect of silica in the analysis of plants and soils for fluorine is discussed.

In recent years, the determination of small amounts of fluorine in biological materials has been facilitated by the increasing adoption of diffusion procedures for the separation of fluoride from interfering substances,[1,2,3,4] and by the introduction of alizarin complexan for the direct determination of the fluoride ion.[3,5,6] In applying these methods to the determination of fluorine in a variety of materials, including plant tissues and soils, difficulties have been experienced, and it is thought that the following observations may be useful to other workers interested in this subject.

The distribution of fluorine compounds in biological materials—

It has now been established that, in addition to the main fluoro minerals, cryolite ($3NaF.AlF_3$), fluorspar (CaF_2), fluorapatite [$CaF_2.3Ca_3(PO_4)_2$] and sellaite (MgF_2), which are present in soils and geological structures, and, in respect of fluorapatite, in animal tissues such as bone and teeth, fluorine is present in several tropical plants as ω-mono-fluorinated carbon compounds. Marais[7] was the first to report that the toxic principle of *Dichapetalum cymosum* (a poisonous South African plant) is a fluoroacetate, which has now been isolated from two Australian plants, *Acacia georginae*[8] and *Gastrolobium grandiflorum*.[9] Longer-chain fluorocarboxylic acids have also been identified in a few other *Dichapetalum* species.[10,11] There is an extensive literature on the rôle of fluorine in the structure of bone, teeth and soft animal tissues and, with the advent of drugs and pesticides containing organofluorine compounds, there is increasing interest in the distribution of the forms of fluorine in soil and other biological specimens. In Table I, an attempt is made to group, in a somewhat arbitrary manner, the fluorine-containing compounds found in biological materials.

Table I

Distribution of fluorine in biological materials

	Animal tissues and body fluids		Plants	Soils
Inorganic	Ionised fluorine in blood, urine, and soft tissues. Fluorapatite in bone and teeth	Mainly acid-labile F^-	Water and dilute acid-extractable F^-, probably simple fluorides. Perchloric acid diffusible F^-, possibly fluorophosphates	Water and dilute acid-extractable F^-. Perchloric acid diffusible F^-. Fluoro minerals with aluminium, calcium, magnesium, silicon and phosphorus; diffusible after fusion with potassium hydroxide
Organic	Fluorine-containing drugs in blood and urine. Protein-bound F^-, probably acid-labile		Alkali-labile, short and long-chain carbon - fluorine compounds soluble in organic solvents	None so far identified

In these studies, the water-extractable fluorine in soils is considered to be that which is extracted after shaking 10 g of finely ground soil with 50 ml of distilled water for 30 minutes at room temperature on a reciprocating shaker. The fluoride that is liberated by treatment with 47 per cent. w/w perchloric acid at 60° C for at least 24 hours has been called the acid-labile or diffusible fluoride and, in soils, it is suggested that it can be regarded as the fluoride potentially available to the plant. It will include fluorapatite, as well as simpler fluorides, but, in all probability, will exclude organically combined fluorine and complex minerals containing fluorine and silica. To determine total fluorine in soils the material must be fused with strong alkali.

With most plant tissues, observations in this laboratory indicate that the total fluorine is also the diffusible or acid-labile fluorine, but in some tropical species,[11] in addition to inorganic fluoride, there are two groups of organically bound fluorine that are not degraded by strong perchloric acid. One group contains alkali-labile compounds, and the other, fluorine derivatives soluble in organic solvents, such as light petroleum and carbon tetrachloride. The alkali-labile group consists of short-chain compounds, such as fluoroacetates, from which the fluorine can readily be removed by treatment with strong alkali.[10] It is possible that some of the fluorine present in these plants is conjugated with protein.

Little seems to be known about the inorganic fluorine compounds in plant tissues. That some plants contain high levels suggests that the fluorine is in a comparatively inactive form, perhaps combined with calcium or magnesium, or even as fluorophosphates, and stored in various tissue cells of the plant. While several techniques, such as infrared spectroscopy and gas chromatography, need to be used for the actual identification of specific components, the final evaluation of results may well depend on the ability to determine accurately extremely small amounts of fluorine.

Analytical procedures

Preparation of samples—

Plant specimens—Surface contamination is best removed by placing the fresh material in a polythene or polypropylene domestic colander, and immersing it in distilled water. The sample should then be dried between sheets of clean white filter-paper. Small amounts should be air-dried at about 25 to 30° C by spreading the sample on filter-paper and placing it in a warm room or cupboard; oven-drying at 60° C overnight is also suitable. It is unwise to dry at 100° C, because of the possibility of loss of volatile fluorine compounds. Conventional determinations for dry-matter content can be carried out on suitable sub-samples at 105° C.

Soils—About 500 g of soil, spread evenly in a tray, 10 × 8 inches, should be air-dried at 80° to 90° F for 48 hours.

Soft animal tissues—These should be chopped into small pieces and dried at 60° C for 48 hours.

Bone—The marrow should be removed, the bone sawn into small pieces, then finely ground in a hammer-mill and de-fatted by extraction with light petroleum. It is customary to express the fluorine content as a percentage of the bone ash or de-fatted bone.

Grinding of samples—

Soft animal and plant tissues—After first grinding the dried tissue in a hammer-mill, further grinding in a metal ball-mill for 18 to 24 hours will usually reduce the material to a suitably fine powder. In this laboratory, a special steel ball-mill is used, the interior of which is chromium-plated, with beaters of chromium-plated steel rod. A Casella grain-mill has also proved useful for the preliminary grinding of small or difficult plant samples. The mechanism of this apparatus consists of four stainless-steel knives that rotate at several thousand r.p.m. against four similar, but stationary, knives within a stainless-steel chamber, the fine plant particles falling through a sieve into a glass container. Conventional hammer-mills do not always reduce the dried tissue to a sufficiently fine powder. After grinding, the material is passed successively through stainless-steel or nylon sieves of 8-inch diameter, and of B.S.S. 100 and 300 mesh, which have mesh apertures of 150 and 53 μ, respectively; these are supplied by Messrs. Endecotts Ltd. If the material is ground long enough, it is possible to pass all of it through the 300-mesh sieve, thus producing particles that are even smaller than those of the standard kale described by Bowen.[12]

Soils—Grinding in the ball-mill to pass the 300-mesh sieve has been found to be the most satisfactory procedure.

SAMPLING FOR ASHING OR DIRECT DIFFUSION OF ACID-LABILE FLUORIDE—

Considerable difficulty was experienced in obtaining good replication of results when 10 to 100 mg of finely ground preparations were weighed directly into the diffusion bottles or platinum crucibles. In the diffusion procedure, there was a tendency for plant powder to form into small lumps that would not disintegrate, even after contact with strong perchloric acid for 24 hours. The problem was overcome by preparing suspensions of the finely ground specimens in 0·1 per cent. agar, which remain stable for at least 24 hours.

To test the reproducibility of sampling, ten replicate 1-ml volumes of two plant and three soil suspensions containing 10 to 50 mg of sample per ml were transferred, by pipette, into small aluminium dishes and dried at 100° C for 18 hours. The weights of the dried aliquots are shown below.

Plant suspension, mg per ml	Soil suspension, mg per ml
(*a*) 51·0 to 51·9; mean 51·4 ± 0·5	(*a*) 48·9 to 49·7; mean 49·4 ± 0·5
(*b*) 22·6 to 23·0; mean 22·8 ± 0·2	(*b*) 20·6 to 22·2; mean 21·3 ± 0·9
	(*c*) 11·6 to 12·0; mean 11·8 ± 0·2

The results indicate that the weight error involved in measuring such suspensions by volume is little more than that of a balance with a sensitivity of ±0·2 mg. Even with soil (*b*), which had a high sand content, the error did not exceed ±4 per cent.

Preparation of suspensions in agar—Prepare a 0·11 per cent. w/v solution of good quality agar (Difco agar or Oxoid agar No. 3 is suitable, but not Oxoid Ionagar No. 2). Preserve it with a crystal of thymol. The solution keeps for 3 to 4 weeks.

Plant tissues—Weigh 1·0 g of finely ground plant powder into a 50-ml calibrated flask, add 5 ml of M lithium hydroxide and 2 drops of s-octyl alcohol to prevent the formation of froth. Shake it until the particles are dispersed; the plant material is partially soluble. Make up to volume with agar solution. Mix thoroughly.

Soils—Weigh suitable amounts to give 20 mg of soil per ml and suspend them in the agar solution alone.

In practice, suspensions of 20 mg of sample per ml have given the most reproducible results, but suspensions containing 50 mg of sample per ml can be used. When the agar is freshly prepared, the suspensions remain stable for a long period but, after a few days, the ability of the agar solution to hold the soil in suspension tends to deteriorate.

Ashing—Experience during the past few years has shown that some muffle furnaces cause serious contamination of ashed specimens.[3,13] Attempts to overcome this extremely difficult problem have included the use of tightly closed containers within the muffle furnace for the platinum crucibles, a silica-lined muffle furnace, a Simon-Meüller crucible furnace and high temperature hot-plates. In every instance, serious and variable extraneous contamination resulted when the temperature rose above 400° C. As it is generally considered necessary to ash some materials, *e.g.*, soils, at temperatures of about 600° C, it was essential to examine carefully the ashing procedures. Simple ashing over a small, glass spirit burner was found to eliminate contamination and give the most reliable results; it is not as tedious as may be supposed.

METHOD OF ASHING FOR SOILS AND TISSUES

REAGENTS—

Lithium hydroxide, M—This was prepared by the method of Hall.[3]

Magnesium succinate, 0·2 M—This was prepared by the method of Hall.[3]

Cellulose suspension in 0·1 per cent. agar—Suspend 50 g of Whatman cellulose powder in 500 ml of hot 0·2 M hydrochloric acid and leave it to stand for 30 minutes. Filter on a Buchner or sintered-glass funnel, and wash it with hot water until free from acid. Dry at 100° C. Prepare a suspension of 20 mg per ml in 0·1 per cent. agar alone, as for soil. Preserve it with a crystal of thymol.

PROCEDURE—

With a pipette, introduce 1 ml of the sample suspension into a platinum crucible of 15-ml capacity. Add 0·3 ml of M lithium hydroxide and 0·6 ml of 0·2 M magnesium succinate,

and mix with a small nickel spatula, washing the spatula with a few drops of distilled water and collecting them in the crucible. For soil, add 1 ml of cellulose suspension in addition. Evaporate to dryness in a stainless-steel-lined oven at 100° C. By using a pair of platinum-tipped tongs and an ordinary glass laboratory spirit burner with a brass or porcelain wick holder, and filled with methanol, carefully heat the upper half of the crucible until the sample has completely carbonised. Although no evidence has been obtained to show that losses of fluorides occur in the smoke, it seems advisable to avoid heating the sample directly at first because of the formation of smoke; continue the ashing by holding the crucible in the conventional manner over the flame. When most of the carbonaceous material has been burnt off, leaving a grey ash, allow the crucible to cool, and add a few drops of distilled water. With a micro spatula, break up the ash and gently rub any carbon particles down the wall of the crucible. Wash the spatula with a little water, collecting this in the crucible, and replace the crucible in the oven to dry. When dry, re-heat the crucible carefully over the spirit burner, starting by heating the wall, as before. During this second stage the remaining traces of carbon are removed, thus leaving a clean ash. The whole procedure for each sample should take only 2 to 4 minutes.

Fusion of siliceous material—

Soils and plant samples rich in silica need to be fused with alkali to convert fluorosilicates into fluorides. Several workers have reported on the relative merits of sodium and potassium hydroxide when used for this purpose.[14] In this work, various reagents were tried, but only with potassium hydroxide were quantitative recoveries obtained. The results of these tests are shown in Table II.

Table II

Recovery of fluoride from soil after fusion with alkalis

Test No.	Fusion reagent*	Fluoride determined, μg: Original soil	Fluoride determined, μg: + 1 μg of F^-	Difference, as percentage of added fluoride
1	Lithium hydroxide	3·60	3·60	Nil
2	Potassium hydroxide	4·56	5·47	91
3	Sodium carbonate	2·30	2·50	20
		—	2·50	20
		—	3·00	70
4	Sodium hydroxide	1·56	2·28	72
5	Sodium peroxide	1·06	1·27	21
			+ 2 μg of F^-	
6	Potassium hydroxide	1·98	3·95	98·5
7	Potassium hydroxide	4·27	6·24	98·5

* 0·5 g of solid reagent was added in each test.

Tests 1 to 5 were done with 20 mg of the same soil. Except for sodium carbonate, the reproducibility of triplicate fusions was good. The sodium carbonate fusions needed to be heated over a micro bunsen burner at a temperature of about 800° C for melting to occur. Two different soil suspensions were used for tests 6 and 7 when 2 μg of fluoride were added. In each instance the fluoride was added with the soil suspension at the beginning of the analytical procedure.

Method of fusion—

After ashing in the manner described above, add 0·5 g of solid potassium hydroxide and heat very gently, with continual swirling of the crucible over the spirit burner, until the potassium hydroxide has liquefied and all of the water has evaporated off. Continue heating until as much of the ash as possible has dissolved in the molten potassium hydroxide; this takes about 1 minute. Do not heat to such an extent as to cause the potassium hydroxide to volatilise. Some soils, particularly those of a sandy nature, do not completely dissolve. Cool, add 1 ml of water and transfer the mixture quantitatively into a 10-ml graduated tube or flask containing 3 or 4 ml of water. Wash out the crucible with four successive 1-ml volumes of 10 per cent. v/v sulphuric acid using a dropping pipette with a rubber teat. Make the volume up to 10 ml with water. The resulting solution, which is usually colourless but

contains a small amount of residue according to the type of sample, can now be used for the direct diffusion of hydrofluoric acid. Blanks on 20 mg of cellulose, with all of the reagents, must be taken through the whole ashing and fusion procedures. The fusions of soil samples and other fluorine-rich materials can be conveniently made up to 10 ml of solution, or appropriately greater volume, from which 1 ml is a maximum aliquot to remove for the diffusion of hydrofluoric acid. Samples of especially low fluorine content may be transferred directly into the diffusion bottle; in this event, dissolve the ash in 0·5 ml of 10 per cent. v/v sulphuric acid, transfer the solution into the diffusion bottle and wash out the crucible with two 0·5-ml volumes of ice-cold 70 per cent. w/w perchloric acid containing silver sulphate.[3] This procedure, however, is not suitable for ashes fused with potassium hydroxide.

DIFFUSION OF HYDROFLUORIC ACID—

Some minor modifications have been introduced into the method originally published.[3]

The papers absorbing the diffused hydrofluoric acid are now treated with 1 drop of 0·5 M sodium hydroxide containing 50 per cent. v/v of propylene glycol; this reagent has greater absorbing efficiency than 0·2 M magnesium succinate. The propylene glycol delays drying out of the papers.

It is essential to wipe the inside of the neck of the bottle with a filter-paper to ensure that no traces of acid are left that could interfere with the absorption of hydrofluoric acid and the subsequent reaction with the alizarin complexan.

Carnauba wax, which is hard and effects a better seal, is now used alone instead of the original mixed wax for sealing the caps. The cap is sealed by holding the bottle horizontally with the neck and cap over the container of hot wax and then applying the wax from a dropping pipette. The volume of liquid in the diffusion bottle should not exceed 3 ml, and maximum diffusion of fluoride is achieved by using 0·5 ml of plant or soil suspension with 1 ml of the silver - perchloric acid reagent.

THE DETERMINATION OF FLUORIDE WITH ALIZARIN COMPLEXAN—

The adoption of alizarin complexan[5,6] as a direct colour reagent for inorganic fluoride has become widespread and, in the author's experience, has removed much of the uncertainty inherent in the use of reagents that are bleached by the fluoride ion. For concentrations above 0·2 p.p.m. of fluoride in the reaction mixture, the blue fluorochelate can be measured directly at 615 nm with little error, but for lower levels, and for precise values, extraction into isobutyl alcohol containing an amine is necessary. Here again, experience has shown the need for several small changes to be made in the original method.[3]

1. The alizarin complexan, as now supplied by Messrs. Hopkin and Williams Ltd., does not need to be purified.

2. Some batches of t-butyl alcohol have been found to contain acidic impurities that either prevent the formation of the lanthanum - alizarin chelate or interfere with the extraction of the fluorochelate into isobutyl alcohol; it is therefore advisable to re-distil the reagent.

3. The composite lanthanum - alizarin reagent can be prepared more conveniently as follows.

Transfer 0·4724 g of succinic acid into a calibrated flask of 100-ml capacity, add 3·2 ml of M sodium hydroxide and sufficient water almost to dissolve the succinic acid (about 10 ml). Add 10 ml of lanthanum nitrate solution (173 mg of $La(NO_3)_3.6H_2O$ in 100 ml of water) and make up to 100 ml with water. Check the pH potentiometrically and adjust it to 4·6 with M sodium hydroxide, if necessary; the small increase in volume caused by this adjustment is of no consequence.

Now add, by pipette, to the buffered lanthanum solution an equal volume of 0·0003 M solution of alizarin complexan (23·1 mg of the complexan dissolved in 0·2 ml of M sodium acetate and made up to 200 ml with water) and mix by constant swirling, then add re-distilled t-butyl alcohol so that its final concentration is 20 per cent. v/v. The buffered lanthanum solution and the mixed reagent should be freshly prepared for each batch of determinations.

4. It has been found necessary to rinse the reaction tubes with the alizarin complexan reagent before the determinations to remove traces of contaminating substances. This can readily be done by filling the first of a set of 5-ml stoppered tubes with the solution and

transferring the rinsings from tube to tube. After allowing them to drain for a few minutes, remove the remaining reagent from the tubes with a dropping pipette drawn out to a long capillary.

5. Extraction of the fluorochelate with hydroxylammonium chloride - isobutyl alcohol is convenient with an initial volume of 1·5 ml of solvent, followed by two volumes of 1 ml, the final volume being made up to 4·0 ml. Centrifuging between extractions is unnecessary, but the final washed and chilled extract needs to be centrifuged for a few moments to clarify it.

6. Originally it was suggested that the extract of the fluorochelate in hydroxylammonium chloride - isobutyl alcohol should be washed with water. It has since been found that a more efficient removal of excess of lanthanum chelate is achieved by washing the extract with 1 ml of the succinate buffer (pH 4·6), diluted twenty times with water.

Discussion

Several modifications are suggested above, which are thought to improve the published method[3] for the determination of microgram amounts of fluorine in biological materials. Of the problems associated with the determination of this element, that of contamination during ashing was found to be the most serious. The author has recently found that some German workers use a muffle furnace lined with stainless steel to avoid the difficulties encountered with conventional equipment. The degree of contamination was found to be progressively more serious with conventional apparatus at temperatures above 400° C, and with increasing time. Ashing for long periods at 400° C may cause as much contamination as ashing for 30 minutes at 600° C. In this laboratory, a Simon-Meuller crucible furnace, with a porcelain interior, was used successfully for many determinations but eventually caused contamination. Even hot-plates capable of reaching a surface temperature of 500° to 600° C were found to be unsuitable. It is possible that the insulating materials enclosing the elements could be the source of the fluoride contamination. The use of a small spirit burner, although unsophisticated, has undoubtedly overcome the problem, even for the difficult determination of total fluorine in soils involving fusion with potassium hydroxide. Temperatures in the lower half of the crucibles were found to vary between 410° and 440° C (mean 420° C). The temperature of the floor of the crucible would almost certainly be higher, possibly about 550° C. These measurements were made with a mercury thermometer calibrated to 550° C and, although they can be regarded as only approximate, they indicate that a temperature high enough for efficient ashing was achieved, and at the same time losses caused by undesirably high temperatures were avoided.

Stabilising colloids have been used to advantage for many years in analytical chemistry. Recently Hall, Gray and Flynn[15] found gelatin to be effective in protecting the Titan yellow complex in the determination of magnesium in soil extracts, and Martin and Stephen[16] have re-examined gum ghatti in their work on the nephelometric determination of the nitrate ion. With the diffusion procedure for the separation of hydrofluoric acid, it was found necessary to grind samples to a very small particle size, and a wide variety of colloids was investigated for the preparation of stable suspensions. Poly(vinyl alcohol) and gelatin in these circumstances would not hold up suspensions of sandy soils; methylcellulose, carboxymethylcellulose and some of the natural gums, including gum ghatti, were more effective, but agar was easily the best for both soil and plant preparations. Triethanolamine was useful for plant material but presented difficulties during ashing. No trouble has been experienced in transferring the suspensions by pipette, provided the concentration does not exceed 50 mg of sample per ml of suspension; the optimum concentration for a stable suspension was 20 mg per ml.

Although several workers have successfully used other apparatus (including Perspex Conway-type diffusion units[17] sealed with silicone grease) for the separation of the fluoride as hydrofluoric acid, the author has not found these alternatives satisfactory for the type of material mentioned above. A small polythene bottle sealed with a hard wax proved to be the simplest and most effective diffusion unit, but occasionally losses of hydrofluoric acid were measured when the original softer wax was used.

The method for the spectrophotometric determination of the fluoride ion absorbed on to succinate-treated papers was devised for sub-microgram amounts.[3] For larger amounts, when several micrograms of hydrofluoric acid may diffuse from an aliquot of the sample,

the absorbing paper may need to be extracted with a much larger volume of lanthanum - alizarin complexan reagent; 2 ml of this extract can then be used for the extraction of the fluorochelate. Alternatively, the sample can be suitably diluted with 0·1 per cent. agar and re-diffused.

In the differentiation of organic from inorganic fluorine, the effects of silica in plant tissues and soils may confuse the results. Fluorosilicates form part of the fluorine in soils, and it is well established that silica is present in the roots, stems and leaves of many plants, although the amounts vary considerably. In their studies of moorland plants, Thomas and Trinder[18] found 0·09 per cent. of silica (SiO_2) (on dry weight) in the blaeberry (*Vaccinium myrtillus*) and 6·64 per cent. in white bent (*Nardus stricta*). Remmert, Parks, Lawrence and McBurney[19] found that silica seriously interfered with the determination of fluorine in plant specimens. They determined lower levels of fluorine in ashed grass than from direct distillation, and attributed the difference to occlusion by silica.

In the present work, an analogous situation was experienced when the diffusible fluoride levels of some herbages were invariably higher than the apparent total fluorine, after ashing. However, when the ash of the material was fused with potassium hydroxide the total fluorine then correlated closely with the diffusible fluoride. In those tropical plants containing fluorinated carbon compounds (which are stable in the presence of 47 per cent. w/w perchloric acid), the assumption that the difference between the diffusible fluoride and the total fluorine, determined after ashing or fusion, represents only the organically combined fluorine is vitiated in those samples in which the amount and form of silica interferes with the total fluorine determination. It is notable, however, that Peters and Shorthouse,[20] in their studies with *Acacia georginae* and other plants, did not experience interference by silicates in the determination of fluorine and, in this laboratory, all of the fluorine of sodium fluorosilicate was quantitatively determined after diffusion with perchloric acid; the problem posed by silica is, therefore, somewhat complex. Not all of the difficulties that silica introduces into the analytical partition of fluorine in plants have been resolved, and work on this facet is still in progress.

The superiority of potassium hydroxide over the other alkalis used to degrade the minerals of soil is clearly shown in Table II. Amounts of potassium hydroxide larger than 0·5 g, with prolonged heating under the conditions described, did not increase the fluorine values, so it is probable that with the good replication and recovery of added fluoride the figures given are reliable. Shell and Craig,[21] with much larger amounts of sample, used a mixture of zinc oxide and sodium carbonate at 1100° C to degrade fluorosilicates in minerals. In the microgram range, serious loss of fluorine could be expected at such a high temperature.

Since this paper was first submitted for publication, Evans and Sergeant[22] have described the determination of fluorine in soils and minerals, after fusion of 200 mg of sample with sodium carbonate. Dr. Evans kindly provided the author with three of his analysed minerals, which have now been examined for fluorine by the method described above. The results are shown in Table III, and it is evident that the two methods give similar values.

TABLE III

FLUORINE VALUES OF MINERALS

Mineral analysed	Fluorine found, p.p.m.	
	Evans and Sergeant's method	Present method
Tonalite T–1	450, 460	469, 479
Biotitic green schist	700 to 760	708, 725
Porphyritic basalt	1200	1180, 1220

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Spectrophotometric Determination of Micro Amounts of Aluminium in Plant Material with 8-Hydroxyquinoline

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The 8-hydroxyquinoline method, previously found to be more satisfactory than the aluminon method for the analysis of soil extracts, has been examined for its suitability for determining aluminium in plant tissues. An initial extraction with chloroform of the diethyldithiocarbamate complexes of the large amounts of heavy metals found in plant material has been devised. The resulting method was tested on synthetic solutions and compared with emission-spectrochemical procedures in the analysis of plant materials.

The lower limit for reliable determination by the proposed method corresponds to 4 p.p.m. of aluminium in the dried plant tissue. At this lower limit, the following interfering elements in the tissue at the percentage concentrations indicated are tolerated in the method: copper, 0·005; zinc, 0·030; iron, 0·030; manganese, 0·10; phosphorus, 0·20; and calcium, 4·0. For tissue containing 20 p.p.m. of aluminium, or more, at least five times these amounts are tolerated.

The determination of small amounts of aluminium in plant tissue in the presence of high concentrations of interfering ions presents a considerable analytical challenge. Recently, several methods have been proposed; two are based on the triammonium aurin tricarboxylate (aluminon) lake reaction,[1,2] while two others involve solvent extraction of the complex formed between aluminium and 8-hydroxyquinoline.[3,4]

In our laboratory, aluminon methods have failed to yield reproducible standard curves, while the 8-hydroxyquinoline method has proved extremely reproducible.[5] Further, for analyses of plant digests, one of the aluminon procedures[1] requires a preliminary separation by elution through an ion-exchange column; the other[2] involves separation by precipitation with ammonia solution and centrifugation, followed by adjustment of sample acidity with a pH meter. For routine analyses, these procedures are time consuming and require the analyst's attention for each individual sample. In addition, the concentrations of interfering ions tested[2] were much lower than those observed in plant tissue in subsequent studies.[1]

Rubins and Hagstrom[4] have described a sensitive and accurate method based on the fluorescence of the extracted aluminium 8-hydroxyquinolinate complex. In addition to a fluorimeter being required, the high concentrations of interfering ions observed in some plant tissue do not appear to be tolerated with their method. Further, for the initial extraction of iron it involves the use of bathophenanthroline, which is a rather expensive reagent unless iron is to be determined.[4]

Extraction of the aluminium 8-hydroxyquinolinate complex into chloroform was used by Middleton[3] to determine aluminium in leaves of the rubber tree, but no precautions to remove interfering ions were described. It is clear from previous work[5] that several ions likely to be present in plant digests could cause severe interference in the 8-hydroxyquinoline method. Thus, to provide a spectrophotometric procedure in which high concentrations of interfering ions would be tolerated, without the need for tedious precipitation or ion-exchange procedures, we undertook the investigations described below. The resulting procedure, which involves an initial extraction of the diethyldithiocarbamate complexes of heavy metals with chloroform, permits spectrophotometric determination of aluminium in 18 samples in about 2 hours.

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For tissue containing as little as 4 p.p.m. of aluminium, the following elements, with their percentage concentrations in the tissue, are tolerated: copper, 0·005; zinc, 0·030; iron, 0·030; manganese, 0·10; phosphorus, 0·20; and calcium 4·0. These are at least 10 times the relative concentrations of interfering ions tested for other methods.[2,4]

Experimental

Reagents—

Concentrated hydrochloric acid, glacial acetic acid and concentrated ammonia solution were distilled in a glass still. As the resulting ammonia solution was about 10 N, it was not used in the concentrated buffer described below. Concentrated nitric, sulphuric and perchloric acids were not purified because their distillation is somewhat hazardous. Purification of the chloroform used was not found to be necessary. All aqueous reagent solutions were prepared with de-ionised distilled water.

Buffer solution—Mix 275 ml of glacial acetic acid and 310 ml of concentrated ammonia solution, cool, and dilute with water to 500 ml. The final pH should be adjusted to 6·2, if necessary.

8-*Hydroxyquinoline solution*—Dissolve 20 g of 8-hydroxyquinoline in 1 litre of chloroform and store in a dark glass bottle. This reagent is rather variable and some batches are highly coloured. We found the "Baker Analyzed" reagent to be satisfactory.

Diethyldithiocarbamate solution—Dissolve 3 g of sodium diethyldithiocarbamate in 100 ml of water, filter through Whatman No. 41 paper, and store in the cold. Prepare freshly every few days.

Thymol blue indicator—Dissolve 0·1 g of thymol blue in 10 ml of ethanol and dilute to 50 ml with water.

Aluminium standards—Prepare a stock solution containing 50 μg of aluminium per ml in 0·1 N hydrochloric acid, either as previously described,[5] or, as in the present study, from recrystallised aluminium chloride standardised independently by precipitation of aluminium 8-hydroxyquinolinate. Prepare a working standard containing 5 μg of aluminium per ml by appropriate dilution of the stock solution with 0·1 N hydrochloric acid.

Digestion of plant material—

Three different plant tissues were analysed: tomato tops, tobacco leaves and apple leaves. The materials were dried at 60° C in a forced-draught oven and ground in a Wiley mill to pass a 40-mesh sieve. Then, 1·0-g samples were treated with 5 ml of concentrated nitric acid and digested with 5 ml of a mixture of nitric, sulphuric and perchloric acids (10 + 1 + 3), following the procedure of Johnson and Ulrich.[6] After digestion, the salts were dissolved by boiling with water,[6] and the silica residue was washed once by centrifugation with 30 to 40 ml of 6 N hydrochloric acid. The acid digest was then diluted to 100 ml with water. For tissue containing less than 8 p.p.m. of aluminium, the final volume should not be more than 50 ml.

Determination of aluminium—

Transfer, by pipette, an appropriate aliquot (up to 25 ml) of the plant digest or the standard solution, containing 2 to 20 μg of aluminium, into a 120-ml Squibb pear-shaped separating funnel calibrated at about 50 ml. Add 1 ml of N acetic acid, 2 to 4 drops of thymol blue indicator, and neutralise carefully with 6 N ammonia solution until the red colour disappears. Add 5·0 ml of buffer solution, dilute to 50 ml with water, and mix well. The resulting solution should have a pH of between 5·1 and 5·2. Add 2 ml of the diethyldithiocarbamate solution, and rapidly make three consecutive extractions, each with 5-ml portions of chloroform, shaking for 5 minutes each time and discarding the chloroform phase. Details of the extraction method have been previously described.[5] After the third extraction, allow the solution to stand for 15 minutes to decompose any remaining diethyldithiocarbamate. Add 5·0 ml of the 8-hydroxyquinoline solution in chloroform, and extract for 5 minutes. Filter the chloroform phase through a cotton pledget into a 1-cm cell and measure the absorbance at 385 mμ.

The extracted chloroform phase from the reagent blank should be used as the reference solution to minimise the effect of an absorption peak at 372 mμ, as discussed below. Throughout the procedure care must be exercised to prevent contamination of the glassware, and also to avoid losses of aluminium by adsorption.[5]

EMISSION-SPECTROCHEMICAL ANALYSIS—

Plant materials were also analysed in two independent laboratories by emission-spectrochemical procedures. The methods used by laboratory No. 1 have been previously described.[7] In laboratory No. 2, the samples were dry ashed in quartz crucibles and dehydrated with concentrated hydrochloric acid. The residue was extracted with N hydrochloric acid containing 0·5 per cent. w/v of lithium as a radiation buffer, and 0·02 per cent. w/v of nickel as an internal standard. Analyses were performed by a rotating-disc solution technique, with parameters described by Baker and Greweling.[8]

DEVELOPMENT OF METHOD

The larger amounts of copper, zinc, iron and manganese found in plant materials require extensive modifications of procedures that have been developed for the determination of aluminium in soil extracts.[5] Complexing agents, which either prevent extraction of these metals or permit their extraction before the extraction of aluminium, have been used. We sought a single agent that would extract large amounts of all of the interfering ions and tested several, including dithizone, 8-hydroxyquinaldine and diethyldithiocarbamate, with chloroform or carbon tetrachloride as solvents, and adjusting the pH to various levels. The most satisfactory combination appeared to be extraction of the diethyldithiocarbamate complexes with chloroform at pH 6, but, for the reasons described below, extraction at pH 5 was found to be preferable. Extraction of the diethyldithiocarbamate complexes is usually accomplished in alkaline solution but, as magnesium is extracted from alkaline solution by 8-hydroxyquinoline, it must be acidified before extraction of aluminium. This latter procedure gave low recoveries of aluminium, apparently because of the precipitation of aluminium hydroxide, which failed to dissolve on acidification.[9] Hence, extraction of the diethyldithiocarbamate complexes from acidic solutions was required.

Tests on aluminium standards, however, indicated that the extraction of aluminium was still incomplete after extraction of the diethyldithiocarbamate complexes with chloroform at pH 6. Aluminium was not extracted by diethyldithiocarbamate, contrary to the suggestion of Goldstein, Manning and Menis,[10] because a second extraction with 8-hydroxyquinoline removed the remaining aluminium from the aqueous phase. Further tests established that diethyldithiocarbamate was not a factor; merely by shaking the aqueous phase with either chloroform or carbon tetrachloride before extraction of aluminium 8-hydroxyquinolinate in this method, or that of Frink and Peech,[5] low recoveries of aluminium were caused. Lowering of the pH gave higher recoveries[10]; similarly, an increase in the acetate concentration at pH 6 gave higher recoveries. No ready explanation of this phenomenon is apparent. Okura, Goto and Yotuyanagi[11] reported that only monomeric aluminium ions are extracted by 8-hydroxyquinoline. However, as these solutions can be shown to be supersaturated with respect to crystalline aluminium hydroxide (gibbsite) at pH 6, it seems likely that by shaking such solutions with chloroform or carbon tetrachloride the precipitation of the hydroxide is initiated or promoted.

Further studies were required of this modified method at pH 5 in the presence of the concentrated acetate buffer. Despite the relatively short half-life of diethyldithiocarbamate in acidic solutions,[9] we found that the heavy metals could be successfully extracted at pH 5: the time of contact of diethyldithiocarbamate with the aqueous phase, however, should be kept to a minimum. In addition, at pH 5, considerable amounts of acetate enter the chloroform phase, thus causing a change in the dissociation of 8-hydroxyquinoline, and creating an absorbance peak at 372 mμ.[12] The effect of this peak is to increase considerably the absorbance of the chloroform phase extracted from the reagent blank. Therefore, pH and acetate concentration must be carefully controlled. To avoid the tedious adjustment of acidity with a pH meter, which many procedures require at this point, several indicator and buffer combinations were tested. We found that the acidity could be conveniently and reliably adjusted to between pH 5·1 and 5·2 by the recommended procedure. The resulting yellow colour of the indicator at this pH does not interfere because it is removed by the initial extraction with chloroform. Other procedural details, such as time of shaking, the drying of the chloroform phase and stability of the aluminium 8-hydroxyquinolinate complex were previously investigated[5]; the lowering of the pH from 6 to 5 for extraction should not affect these findings and, therefore, will not be discussed here.

Results and discussion

Several interfering ions were tested, both singly and in various combinations, as shown in Table I. The amounts chosen represent the upper limits of these elements likely to be found in 1 g of plant material grown in acidic soils. As the aliquot necessary for the determination of aluminium at concentrations in the tissue of 20 p.p.m., or more, represents only 0·1 g of plant material, it is obvious that, in most instances, ten times the expected amounts of the individual interfering ions can be tolerated with this method. In the event of iron and manganese interference, the tolerance is about five times the anticipated amount. The synthetic plant digest (Table I) contains five times the expected total amount of all interfering ions, and in no instance was the amount of aluminium recovered significantly different from that taken. In addition, recovery tests were made with 2 and 4-ml aliquots of actual plant digests from five different samples. An analysis of variance showed no significant differences between aliquot sizes, thus giving no evidence for the presence of negative errors.

Table I

Determination of aluminium by the proposed method in the presence of various interfering ions

Interfering ion added, μg						Amount of aluminium taken, μg: 0	10	20
						Aluminium found, μg		
Copper	Zinc	Iron	Manganese	Phosphorus	Calcium			
—	—	—	—	—	—	0·0	10·0	20·2
50	—	—	—	—	—	0·2	10·5	20·7
—	300	—	—	—	—	0·2	10·3	21·5
—	—	150	—	—	—	0·4	10·0	19·9
—	—	300	—	—	—	2·0	11·5	21·3
—	—	—	500	—	—	0·6	10·4	20·2
—	—	—	1000	—	—	1·2	10·3	22·0
50	300	150	500	—	—	0·8	10·8	20·2
50	300	300	1000	—	—	3·5	13·8	23·5
—	—	—	—	2000	—	0·0	9·8	19·9
—	—	—	—	—	40,000	0·2	8·7	20·6
—	—	—	—	2000	40,000	0·2	9·8	19·7
Synthetic plant digest*						0·4	10·1	20·0

* Contains one half of the maximum amounts of each of the interferences tested singly above.

In general, the interferences observed were in agreement with those previously reported.[9] Copper, iron and manganese are all extracted as the 8-hydroxyquinolinate complexes and, if not removed by the proposed diethyldithiocarbamate extraction, will lead to apparently high results. Although Rubins and Hagstrom[4] cite previous work that indicates that zinc 8-hydroxyquinolinate is not extracted, we found that zinc forms a white precipitate in the aqueous phase, which subsequently dissolves in the chloroform phase and causes apparently high results. As Rubins and Hagstrom[4] found that zinc did not interfere in their procedure, we assume that zinc 8-hydroxyquinolinate does not fluoresce. As previously reported,[5] both calcium and phosphorus (as orthophosphate) may lead to apparently low results, presumably because of the formation of insoluble aluminium phosphates in the one instance and of a calcium 8-hydroxyquinolinate complex, insoluble in chloroform, in the other. Magnesium was previously reported[5] to interfere in a similar manner; however, the interference was less severe than with calcium. As the amounts of magnesium in plant digests are generally less than the amounts of calcium, this source of interference was not investigated further.

From these tests, we can conclude that the proposed method should be free from interferences. However, we also wished to test the method in the actual analysis of plant material. Because published procedures[1,2,3,4] have lower tolerances for interfering ions, or lower sensitivity, or both, it seemed of little consequence to use these methods as a basis for comparison. Therefore, we chose emission-spectrochemical analysis as an independent analytical procedure. As this method may lack precision and accuracy for tissue containing low concentrations of aluminium, we selected tissue containing higher amounts, when both spectrochemical and spectrophotometric methods could be used.

Results of the comparative analyses of 18 samples are shown in Table II, and indicate considerable variability between the two spectrochemical methods, as might be expected. The results obtained by laboratory No. 1 are generally lower than those obtained by the proposed method at low concentrations of aluminium in the tissue, and the same or higher at high concentrations. The results obtained by laboratory No. 2 are generally the reverse. Some of these discrepancies are undoubtedly caused by different methods of extracting aluminium from the plant ash. However, the agreement between the proposed method and the average spectrochemical analyses is considered quite good; indeed, the correlation coefficient between the two is 0·986. As the differences shown in Table II reflect sampling and ashing errors, as well as analytical errors, it seems unlikely that the results suggest any consistent bias in aluminium analyses by the proposed procedure.

The precision of the proposed method was tested on standard solutions and plant digests. The mean coefficient of variation was 5·44 per cent. for six replicate analyses of five different standard solutions containing from 0 to 20 μg of aluminium. This is higher than the coefficient of variation of 2·89 per cent. observed for thirty similar analyses conducted during initial studies of the method when aluminium was extracted at pH 6. The greater variability at pH 5 apparently arises from the increased absorbance caused by the larger amounts of acetate in the chloroform phase, as previously discussed. Duplicate analyses were made of the digests of the 18 samples shown in Table II. An analysis of variance of the results for each kind of tissue gave a mean coefficient of variation of 6·63 per cent., which is only slightly larger than that observed for standard solutions. It should be emphasised that these estimates of the precision of the proposed method were not obtained from special replicate analyses run side by side, but rather from analyses run over a considerable period of time under ordinary laboratory conditions. Thus, they are somewhat larger than those frequently reported, but reflect more accurately the reliability of the method in routine analysis.

TABLE II

DETERMINATION OF ALUMINIUM IN PLANT TISSUE BY THE PROPOSED METHOD AND BY EMISSION-SPECTROCHEMICAL METHODS

Sample		Aluminium found, p.p.m.			
			Emission-spectrochemical method		
		Proposed method	Laboratory 1	Laboratory 2	Mean
Tomato tops	1	67	40	79	60
	2	68	30	79	54
	3	68	40	74	57
	4	87	60	84	72
	5	88	50	87	68
	6	90	60	100	80
	7	100	60	124	92
	8	114	80	124	102
Tobacco leaves	1	156	102	157	130
	2	162	137	176	156
	3	217	172	209	190
	4	228	195	221	208
	5	314	280	258	269
Apple leaves	1	249	230	200	215
	2	289	320	264	292
	3	448	440	441	440
	4	546	610	546	578
	5	562	550	500	525

CONCLUSIONS

A survey of existing spectrophotometric methods for the determination of aluminium in plant material indicated that none was entirely satisfactory for tissue containing small amounts of aluminium and large amounts of interfering ions. The 8-hydroxyquinoline method, previously found to be more reliable than the aluminon method for the analysis of soil extracts, was therefore chosen for further study. An initial extraction with chloroform of the diethyldithiocarbamate complexes of the large amounts of heavy metals found in plant

material was devised. The resulting method was tested on synthetic solutions and then compared with emission-spectrochemical procedures. These tests showed that the proposed method will enable aluminium to be determined in the presence of a 5-fold excess of the interfering ions expected in plant digests, has good precision and high sensitivity, and is particularly suitable for plant material containing less than 100 p.p.m. of aluminium.

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Direct Determination of Manganese in Soil Extracts by Atomic-absorption Spectroscopy

By M. NADIRSHAW and A. H. CORNFIELD
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Several extracting reagents are available for assessing either particular fractions of manganese in soils or the potential availability of soil manganese to plants.[1,2,3] The traditional colorimetric methods used for determining manganese in such extracts require a tedious preliminary treatment to eliminate the effects of acids or salts, and to destroy organic matter extracted from the soil as well as that present in some of the extracts. It appeared that atomic-absorption spectroscopy would be a suitable method for determining manganese directly in these extracts, as the temperature of the flame would be high enough to destroy organic matter. A study was therefore made, in which direct atomic-absorption spectroscopy was compared with a chemical method for extracts of several soils.

The extracting reagents tested were—

(*i*) N ammonium acetate (pH 7·0),[1] which extracts exchangeable and water-soluble manganese(II);

(*ii*) N ammonium acetate (pH 7·0) containing 0·2 per cent. of hydroquinone,[2] which extracts "active" manganese, probably manganese(III), in addition to exchangeable and water-soluble manganese(II), and has been shown to be a better indicator of the manganese-supplying power of soils to plants than reagent (*i*);

(*iii*) Morgan's reagent[3] (0·5 N acetic acid - 0·75 N sodium acetate, pH 4·8), which has been widely used for assessing the status of major and trace elements in soils;

and (*iv*) 0·5 N acetic acid, which was tested as a possible replacement of Morgan's reagent, as the latter tended to clog the burner slit by deposition of sodium carbonate after about 20 samples had been aspirated.

Five soils with textures ranging from sand to clay, and at pH 4·8 to 7·0, were extracted (after air-drying and grinding to pass a 2-mm sieve) by shaking 25-g portions of soil with 100 ml of each extracting reagent for 15 minutes and then filtering through a Whatman No. 1 filter-paper, with dry apparatus. The filtrates were aspirated directly into a Unicam SP90 atomic-absorption spectrophotometer, with a manganese hollow-cathode lamp and the following operating conditions: wavelength 279·5 nm, lamp current 12 mA, slit width 0·1 mm, burner height 1 cm, and 5 litres of air and 1·2 litres of acetylene per minute.

Standard graphs for each extractant were prepared to cover a narrow range (high gain) and a broader range (lower gain) of manganese concentrations. When a particular extract contained a high concentration of manganese it was diluted with the appropriate extractant, so that the determination could be made at maximum sensitivity to reduce the effects of possible interfering factors.

The colorimetric periodate oxidation method, in the presence of orthophosphoric acid following evaporation of a suitable aliquot of each extract and destruction of organic matter with aqua regia, was used as the chemical method for comparison.

For each extractant the results obtained by direct atomic absorption (preceded only by dilution when necessary) agreed to within 4 parts per 100, or better, with those obtained by the chemical method. The coefficient of variation ranged from 0·5 to 6·7 per cent. in the atomic-absorption method and from 1·0 to 6·7 per cent. in the colorimetric method. In addition, recovery tests were made of known amounts of manganese added to each type of extract of each soil. The average recoveries ranged from 97·7 to 99·0 per cent.

The authors thank the Agricultural Research Council for the loan of a Unicam SP90 atomic-absorption spectrophotometer.

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Amended *April 24th*, 1968

Analytical Methods Committee

REPORT PREPARED BY THE MEAT PRODUCTS SUB-COMMITTEE

Nitrogen Factor for Barley

THE Analytical Methods Committee has received the following Report from its Meat Products Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

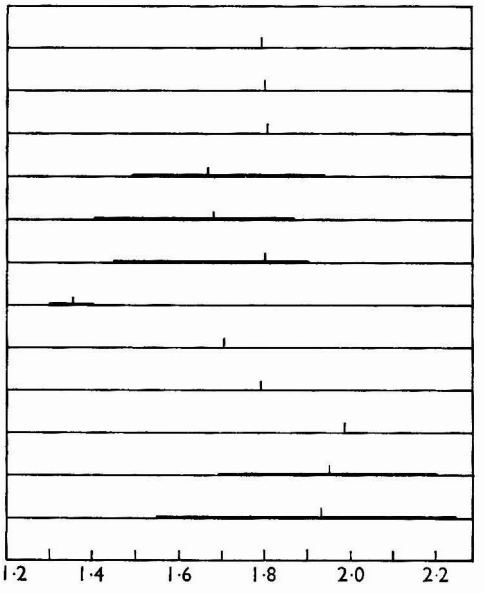
REPORT

The Meat Products Sub-Committee of the Analytical Methods Committee responsible for the preparation of this Report was constituted as follows: Dr. S. M. Herschdoerfer, (Chairman), Mr. S. Back, Mr. P. J. Cooper (resigned February, 1967), Mr. P. O. Dennis. Mr. H. C. Hornsey, Dr. A. J. Kidney, Mr. T. McLachlan, Dr. R. A. Lawrie, Dr. A. McM. Taylor, Mr. G. Walley (appointed February, 1967), Mr. R. E. Weston (appointed February, 1967) and Mr. E. F. Williams, with Mr. P. W. Shallis as Secretary.

In continuing its work on establishing nitrogen factors for use in the analysis of meat products, the Sub-Committee has found it necessary also to establish correction factors to be applied when a meat product contains a relatively high proportion of a cereal filler. Most comminuted meat products manufactured in the United Kingdom contain a percentage of cereal filler. Sausages usually are made with some rusk, and the Sub-Committee has twice reported on the nitrogen content of rusk.[1,2]

In the manufacture of blood puddings, barley is the usual filler added, and the Sub-Committee has collected analytical results for the nitrogen contents of more than 1000 samples of pearl barley. The results of these analyses are shown in Fig. 1.

No. of samples	Range (N_2 as % of carbohydrate)	Mean	
500*	–	1·79	
153*	–	1·80	
492*	–	1·81	
18	1·49—1·93	1·67	
6	1·41—1·87	1·68	
6	1·45—1·90	1·80	
9	1·30—1·40	1·36	1·77
1	–	1·71	
1	–	1·79	
1	–	1·98	
5	1·69—2·21	1·95	
5	1·55—2·25	1·93	



*These results were obtained from commercial organisations and no figures for the ranges of results were available

Fig. 1. Nitrogen contents of barley expressed as a content of carbohydrate. Horizontal lines represent the range of nitrogen contents, short vertical lines indicate the average values.

Recommendation

The Sub-Committee recommends that 1·8 per cent. should be used as the correction for the nitrogen content of pearl barley.

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1. Analytical Methods Committee, *Analyst*, 1961, **86**, 560.
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Analytical Methods Committee

REPORT PREPARED BY THE MEAT PRODUCTS SUB-COMMITTEE

Nitrogen Factor for Blood

THE Analytical Methods Committee has received the following Report from its Meat Products Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The Meat Products Sub-Committee of the Analytical Methods Committee responsible for the preparation of this Report was constituted as follows: Dr. S. M. Herschdoerfer (Chairman), Mr. S. Back, Mr. P. J. Cooper (resigned February, 1967), Mr. P. O. Dennis, Mr. H. C. Hornsey, Dr. A. J. Kidney, Mr. T. McLachlan, Dr. R. A. Lawrie, Dr. A. McM. Taylor, Mr. G. Walley (appointed February, 1967), Mr. R. E. Weston (appointed February, 1967) and Mr. E. F. Williams, with Mr. P. W. Shallis as Secretary.

The Sub-Committee, in continuing its investigations on the nitrogen contents of various types of meat,[1 to 8] has examined a number of samples of blood, which is used in the manufacture of blood puddings, and the results are shown in Fig. 1.

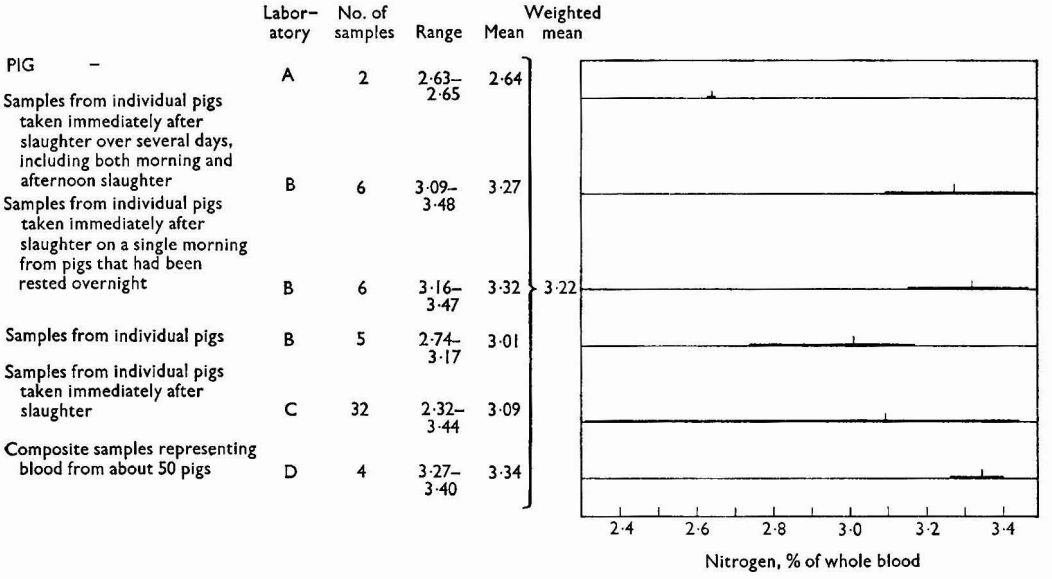

Fig. 1. Nitrogen contents of pigs' blood. Horizontal lines represent the range of nitrogen contents, short vertical lines indicate the average values.

The analyses were carried out on pigs' blood, which is the usual raw material for blood puddings.

It is interesting to note the wide range of the figures quoted by laboratory C, indicating the degree of inaccuracy necessarily attached to an analysis of black puddings.

RECOMMENDATION

The Sub-Committee recommends an average factor of 3·2 for determining the blood content of products manufactured from pigs' blood.

Acknowledgment

The Sub-Committee thanks those organisations listed below for their assistance—

C. & T. Harris (Calne) Ltd.
J. Sainsbury Ltd.
T. Wall & Sons (Meat & Handy Foods) Ltd.

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5. ——, *Ibid.*, **1965**, **90**, 256.
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8. ——, *Ibid.*, **1967**, **92**, 326.

Book Reviews

KIRK-OTHMER ENCYCLOPEDIA OF CHEMICAL TECHNOLOGY. VOLUME 13. Second Edition. MANGANESE COMPOUNDS TO NITROPHENOLS. Edited by HERMAN F. MARK, JOHN J. MCKETTA, jun., DONALD F. OTHMER and ANTHONY STANDEN. Pp. xiv + 894. New York, London and Sydney: Interscience Publishers, a division of John Wiley & Sons Inc. 1967. Price £23 10s.; price per volume for subscribers to the complete set of 18 volumes £17 10s.

This volume is Number 13 of the series, which means it starts the last third of the complete issue (see also *Analyst*, 1963, **88**, 899, *et seq.*). Metallic elements, which are the subject of what now appears to be a standard style of monograph, are manganese, mercury, molybdenum, nickel and niobium. There is a short but useful section on the analysis of niobium ores and compounds that indicates some of the difficulties involved in this type of work. This applies particularly in the presence of tantalum. The introduction of liquid - liquid and ion-exchange techniques has proved very helpful in this connection, but in many instances it is necessary to stop with a mixture of niobium and tantalum oxides and to determine the relative amounts of these from standards by the use of emission or X-ray spectrography. The monographs on the other four metals are of interest, but they break no new ground from an analytical point of view.

Food analysts will be specially interested in the monographs on Margarine, Meat and Meat Products, Milk and Milk Products and Molasses. As usual, they reflect American quality standards; for margarine there is a full section on legal aspects. These could be useful for reference purposes to workers in this country. The meat monograph comprises 17 pages, and its scope is wide, ranging through structure and composition, production, processing and by-products to the uses of animal fats, such as lard, shortening and oleo. Milk also comprises an important monograph (of 71 pages), and it also covers milk products, such as cheese, ice cream, dried milk and milk by-products. The treatment is mainly from a manufacturing point of view, and the short section on instant-dried whole milk will be of particular interest in this country, especially as grading requirements for different types of such milk are tabulated. Milk by-products dealt with include lactose, casein, and even alcohol and yeast produced by fermentation, as well as vitamins and whey vinegar. Strangely, there is no mention of the important use of casein in paper coating, although other uses are mentioned. Nor does the monograph on molasses mention their use in admixture with ground bagasse pith as a cattle food, although, here again, other uses of molasses of this nature are mentioned. In fairness it should be added that the coverage of this work is so very comprehensive that there is a tendency for the reviewer to seize on omissions and give them prominence perhaps beyond their real importance, merely because the over-all standard is so high.

Under the heading of what may be described as operational techniques, there are several interesting monographs, including Mass Spectrometry, Mechanical Testing, Metal Surface Treatments, Microencapsulation, Microscopy—Chemical, and Mixing and Blending. The monograph on microencapsulation (21 pages) deserves to be singled out for special mention because this is a highly technical and topical subject about which relatively little is known. The fact that so much has been printed on a subject so thick with trade secrets may be regarded as something of a "scoop" for this encyclopedia. The applications of encapsulation are much wider than are generally realised. Examples are the production of non-tacky adhesives, an apparent contradiction in terms, but of obvious significance when referring to the adhesive before use; improved forms of agricultural chemicals; food products, for instance, the encapsulation of flavour oils to allow them to be handled as a dry powder; the production of non-carbon copying papers; and detergents, paints, pharmaceuticals, fuels and rubber chemicals.

The section on Microchemistry in the same category is relatively brief, namely 9 pages, *plus* 114 references; it will certainly be useful to the general reader, but analysts are unlikely to learn very much that is new. Much the same applies to the monograph on chemical microscopy, although there is an interesting section on contemporary developments. This includes the use of ultraviolet radiation to obtain contrast when a specimen contains a group that absorbs strongly at some determined wavelength. Fluorescence microscopy is now a well accepted technique, and dispersion staining is gaining acceptance in this type of work as a method of recognising particulate substances from the colour resulting from the difference between the dispersion of refractive index of a solid specimen and that of a reference liquid. A recent useful development in this field, in which the Becke Line is used to determine the refractive index of a crystal in a

similarly refracting medium is, however, not mentioned. Also in this category there is a monograph on Microorganisms (34 pages) that describes the nature and properties of the organisms under their various headings, namely, viruses, bacteria, fungi, algae and protozoa. Structural details are dealt with, and also cultivation and special culture techniques, but microbiological analysis is not covered; there are four pages of references.

What may be described as the organic chemistry monographs include the Methacrylic Compounds, with their well known industrial applications, Naphthalene and its Derivatives and associated Naphthenic Acids, Nitration, and nitro-compounds including Nitroparaffins and Nitrophenols. The naphthenic acids are a relatively restricted class of products that is rapidly assuming increasing importance. In 1965, over 24 million pounds were produced in the U.S.A. alone. The principal uses are in lubricants and dryers, as industrial catalysts for the oxidation of hydrocarbons, as preservatives for wooden fabrics, in rust inhibitors and in emulsifiers.

Finally, there is the category of what the reviewer has come to regard as fringe and often unexpected subjects, which in the present volume include Marketing and Marketing Research, Matches and Micas. The two pages on marketing have, of course, no analytical implications, but the treatment used will certainly appeal to scientists as a matter of general interest, if only because of its method of approach. Apparently, product life cycles can be reduced to mathematical expression in terms of charts showing cost - risk - quality and price - volume - profit relationships. This, it seems, leads to the conception of "Driving Force," which answers the question why a particular product should be bought, and this also can be expressed mathematically and analysed statistically. Price - volume exclusion charts may also appeal to those with an analytical turn of mind, as they deduce what can be sold above a certain volume unless the price is below a certain figure. The more mundane subject of matches is dealt with shortly in some 7 pages, but here again these contain information that seldom gets into print, and the formulations given and comments on toxicity and safety are well worth noting for reference purposes.

It is again apparent that the usefulness and interest of this series, restricted perhaps in some instances, is well maintained in this latest volume. JULIUS GRANT

MODERN CEREAL CHEMISTRY. By D. W. KENT-JONES, Ph.D., B.Sc., F.R.I.C., and A. J. AMOS, O.B.E., Ph.D., B.Sc., F.R.I.C. Sixth Edition. Pp. x + 730. London: Food Trade Press Ltd. 1967. Price 190s.

No textbook on cereal chemistry is better known the world over than that of the authors. The new edition has been prepared with the same care as its five predecessors. The printing in Brightype litho is admirably clear, although type sizes appear to vary somewhat in the 730 pages.

The emphasis of the book is on wheaten flour, its milling, treatment, testing, baking into bread, cake and biscuits and uses for special purposes, including brewing and breakfast foods. Despite this emphasis, however, there is to be found valuable and up-to-date information on Barley, which has a chapter to itself contributed by the late Mr. H. M. A. Cherry Downes and Dr. A. Macey, together with a further chapter on Rye, Oats, Maize, Rice and the Potato. Soya is included in this chapter because of its utility in improving the protein value of cereals, and the authors are fully up to date in referring to its recent use in foods specially designed for calorie-controlled diets.

Indeed, being up to date is clearly something of which the authors can be proud, for some of their references are to within months of the publication of the book, yet they seem not to have been hastily included but carefully considered, even although the value of the Brabender Do-corder might seem to some to have been scantily dismissed. In the chapter on Animal Feedingstuffs the text was again up to date in the inclusion of a short section on the Aflatoxins, and their detection in feedstuffs is well described in the latter part of the book dealing with general analytical procedures. It was a little disappointing not to see the obvious connection made with mycotoxins generally when *Aspergillus flavus*, the causative organism of aflatoxin, was so frequently mentioned in the chapter on the fungal microflora of cereals and cereal products.

Minor criticisms of this nature should, however, in no way detract from the general excellence of the new edition, including the Index. There is a wealth of references to original research work in every aspect of cereal science and technology and, although the price has risen to nine pounds ten shillings, no serious worker in the field should deny himself the information available by failing to purchase this new volume. If, as one suspects, Dr. Kent-Jones made the revision a retirement project, one can truly say to him and his co-author, Dr. Amos, posterity will be grateful.

J. B. M. COPPOCK

METHODS IN GEOCHEMISTRY AND GEOPHYSICS. 7. ATOMIC ABSORPTION SPECTROMETRY IN GEOLOGY. By ERNEST E. ANGINO and GALE K. BILLINGS. Pp. x + 144. Amsterdam, London and New York: Elsevier Publishing Company. 1967. Price 70s.

It is sometimes salutary for a chemist to look into the work of other scientists and technologists in order to gain a fresh outlook on a familiar topic. Geochemists have long been concerned with analytical techniques, obviously taking a particularly applied interest. In this series of monographs the subjects include chromatography, X-ray emission and chemical analysis of, *e.g.*, silicates. This new title is obviously topical, but whether the contents are deserving of merit is debatable. The approach is mainly experimental, following an elementary theoretical introduction. Thus, the parts of the equipment are briefly reviewed and complete commercial instruments are listed. Interferences, general and specific, receive considerable attention but the treatment is not exhaustive. Part II of the book, 64 pages, is concerned with applications to water, ore, silicate, sediment, and isotope analyses; generally, sufficient experimental details are given for the reader to start the analyses, but it will be realised that in this approach not all elements that can be examined by atomic-absorption spectrometry will be included and references, which are liberally supplied, must be consulted for details. The treatment of data receives scant attention and more modern techniques are barely mentioned.

The book may appeal to geochemists who feel safer with a book written by authors in the same subject, but chemists will find no advantage over books already available to them.

D. A. PANTONY

A CONTRIBUTION TO THE ANALYTICAL CHEMISTRY OF SILICATE ROCKS: A SCHEME OF ANALYSIS FOR ELEVEN MAIN CONSTITUENTS BASED ON DECOMPOSITION BY HYDROFLUORIC ACID. By F. J. LANGMYHR and P. R. GRAFF. Pp. 128. Oslo: Universitetsforlaget. 1965. *Norges Geologiscke Undersokelse Number* 230.

Basically, the booklet is a medium for publishing a method for the analysis of silicate rocks involving the use of hydrofluoric acid decomposition in preference to alkali fusion. As such it reveals a novel approach and contains a number of techniques worthy of serious consideration. The details of the method and discussion of difficulties and interferences are voluminous if not comprehensive; in fact, it may be thought that some of the matters raised are unlikely to be relevant in practice.

The booklet is divided into five main sections, the first two dealing with criticisms of the traditional scheme of analysis, its early modifications and several newer "novel" schemes. The third section is a discussion of the use of hydrofluoric acid as an agent for decomposing silicate rocks, while the fourth and fifth cover details of the proposed scheme and a detailed discussion of its efficiency *vis-à-vis* other selected schemes, respectively. It is the first three sections that the reviewer, whose interest is centred on the analysis of ceramics, finds not altogether acceptable.

The authors quote Chirnside (*J. Soc. Glass Technol.*, 1959, **43**, 5T) in the course of their discussion as an argument against some of the "novel" schemes of analysis. In the same paper, Chirnside also says "I think it proper and necessary to point out—that the advocates of some of these newer schemes often unfairly compare and contrast their value with the classical techniques, not of today but of fifty years ago. They ignore, or seem to be completely unaware of, the modifications and improvements that have taken place in what might be broadly called 'classical techniques'. . . ." The authors are guilty of the same error. In their criticism of conventional and "modified" conventional schemes they have not noted a whole range of papers published as a result of co-operative work by analysts in the glass, ceramic and steel industries. In particular, the work of the Society of Glass Technology Analysis Committee, which will be found in the society's journal, and that of the British Ceramic Research Association and its co-operative committees, published in the *Transactions of the British Ceramic Society*, overcome many of the objections that the authors cite in justification of their rejection of the traditional approach. It is a great pity that the past few years have shown an increasing dichotomy of thought between the geological and the ceramic analyst, whose problems are essentially similar.

In the section on the use of hydrofluoric acid as a means of decomposition, the authors make the positive claim that fluorine can be and is eliminated completely by sulphuric acid. In this connection one finds it regrettable to read "It is unfortunate that Hillebrand did not support his wide ranging decision with experimental data" in the paragraph immediately following a reference to unpublished results of the authors themselves—quoted to disprove Hillebrand. The finding is entirely contradictory to a considerable volume of experience by British ceramic analysts

that sufficient traces of fluorine remain to interfere with the accurate determination of alumina, certainly by the EDTA method. Langmyhr has now published "evidence" (*Analytica Chim. Acta*, **1967**, **39**, 516) in which he shows that evaporation with sulphuric acid does eliminate fluorine, but without the presence of a sample.

Although the authors appear to share Chirnside's strictures on the quality of the techniques of separation, they are open to similar criticism, *e.g.*, filtering an ammonia-group precipitate through a grade 3 sintered-glass Gooch crucible and then washing the precipitate four or five times with a total volume of wash liquor of 10 ml $\pm$ 1 ml. This is surely not only unsatisfactory but impracticable.

The authors themselves are unhappy about their alumina results obtained after this separation and Wänninen and Ringbom's technique for the titration of alumina (*Analytica Chim. Acta*, **1955**, **12**, 308), and it would appear probable that the fault lies in the poor handling of the ammonia-group precipitate, or in the fact that fluorine is still present. A similar method of determination, but removing the silica by dehydration and excluding any precipitation of the ammonia-group oxides (B.S. 1902 : Parts 2A and 2B), gave a total range of 0·6 per cent. at the 88 per cent. level over eighteen determinations by nine laboratories, with a standard deviation of 0·17 per cent., as against the authors' figures of about 0·4 per cent. standard deviation for ten results by one analyst. Further possible substantiation of fluoride retention may well exist in the total iron figures, which should be better than they are.

The methods that the authors propose are worth study; they show a number of interesting points of technique. In addition, the booklet contains a wealth of information tucked away in the guise of discussion. It is a pity that the authors were not content to let the quality of their work speak for itself rather than entering a detailed discussion of the weaknesses of other methods. It is this approach that serves to demonstrate their failure to be aware of the very considerable progress that has been made in the field in the last few years and a regrettable bias against more traditional approaches.

H. BENNETT

THE FORMATION AND PROPERTIES OF PRECIPITATES. By ALAN G. WALTON. Pp. xii + 232. New York, London and Sydney: Interscience Publishers, a division of John Wiley & Sons Inc. 1967. Price 88s.

In the preface to this book, Professor Walton states ". . . the formation and properties of precipitates should be regarded as interrelated phenomena which, when separated, are more or less intelligible but together form a very complicated picture. It is only by examination of each piece that the entire picture can conceivably be integrated and understood as a whole."

The book certainly provides a well balanced and logically developed account of the present state of our knowledge, or ignorance, of the phenomenon of precipitation. Professor Walton is to be congratulated for presenting such an excellent survey, which should prove extremely valuable to those working in this area of chemical research.

The opening chapters, dealing with nucleation and the kinetics of crystal growth, lay the foundations for the discussions in the succeeding chapters of co-precipitation, surface properties and morphology. Whenever possible, the presentation is quantitative, but mathematics is not allowed to dominate the treatment of the subject. When mathematics is used, the author interprets the physical significance and provides relevant experimental results. Reading is facilitated by the division of each chapter into several sections, and four of the chapters end with useful summaries.

The final chapter, dealing with precipitation from complex precipitation systems, is contributed by Dr. Helga Füredi of the Ruđer Bošković Institute, Zagreb. Experimental methods are outlined and examples are given of the applications of graphical methods to the representation of complex systems. This treatment is, of course, somewhat empirical, and contrasts with the more fundamental approach of the remainder of the book. Nevertheless, this chapter is a useful review of published work, most of which has previously been available only in Croatian journals.

The book is copiously illustrated with graphs, photographs and drawings. A considerable quantity of experimental data is presented, and several indications are given of further lines along which fruitful research might be pursued. The importance of precipitation studies in diverse fields such as analytical and physical chemistry, physiology and geology is stressed. Each chapter ends with a formidable list of references, including papers as recent as 1966. The book is well indexed with separate author, subject, organic and inorganic compound indexes. It is well bound and printed, and appears, to the reviewer, to be virtually devoid of errors or misprints.

E. NEWMAN

ADVANCES IN ELECTROCHEMISTRY AND ELECTROCHEMICAL ENGINEERING. Edited by PAUL DELAHAY and CHARLES W. TOBIAS. Volume 6. ELECTROCHEMISTRY. Edited by PAUL DELAHAY. Pp. xii + 482. New York, London and Sydney: Interscience Publishers, a division of John Wiley & Sons Inc. 1967. Price 155s.

This, the sixth volume of an admirable series, is nearly 100 pages longer than any of its predecessors. However, quality has not suffered at the expense of quantity; all the articles are of the high standard we have come to expect, as well as covering subjects of vital interest to the modern electrochemist. Thus, the oxygen electrode and porous electrodes are essential features of the practical electrochemistry of fuel cells and electrosynthesis, while electrochemical methods provide an important method for the study of metal complexes; the structure of the electrical double layer must be understood in order to elucidate fully the mechanism of electrode reactions, and the study of such processes as insulators is an exciting new field that may yield practical results in the future.

The extra length of the present volume is caused by the massive 200-page article by Barlow and Macdonald on the theory of inner layer structure, *i.e.*, the structure of the region extending by one or two molecular diameters from the metal surface into the electrolyte. Much of this article consists of the detailed mathematical analysis of the consequences of a simple model, but although the model may be simple the mathematics is not. Most readers will be grateful that the sections on "qualitative discussion" and "discussion of results" bulk so large and include clear statements about the physical meaning of the models involved. Readers not concerned in the controversies will also welcome the leavening of lively remarks mainly about other theoretical approaches and the development of the subject. Recent reviews by Levine, Mingins and Bell, *J. Electroanal. Chem.*, 1967, **13**, 280, and by Hurwitz (in "Electrosorption," edited by E. Gileadi, Plenum Press, New York, 1967) give other points of view on this subject.

Hoare provides a comprehensive and balanced account of the oxygen electrode on noble metals. The 327 references indicate the great effort that has been devoted to solving this problem, which is so important for efficient electrochemical energy conversion. It is evident that no single mechanism for this reaction can be given and that all the factors governing the rate are not yet understood. Nevertheless, this review is a major step on the way to putting the many details together in a coherent picture.

Koryta's article on the electrochemical reactions of metal complexes is the shortest in this volume, but it is well packed with valuable information. He discusses the kinetics of direct discharge of metal complexes, as well as the effect of chemical reactions in the bulk of the solution. Adsorption and double-layer effects are illustrated largely with work from the Polarographic Institute in Prague where many of these ideas were developed.

The treatment of porous and rough electrodes by de Levie should be required reading for anyone trying to interpret results obtained with solid electrodes, however smooth these are believed to be. This article in itself could justify the marriage of theoretical and applied electrochemistry to which this series is dedicated. The fact that Riemann studied the problem of current distribution in 1855 is a reminder that this marriage was consummated more faithfully in the early years of electrochemistry.

In the final article Mehl and Hale summarise the present state of that unexpected newcomer to electrochemistry, "Insulator Electrode Reactions," a promising offspring from solid-state physics. The rapid development of the electrochemical study of insulators is mainly attributable to the work done by their group at the Cyanamid Institute in Geneva. They have provided a clear and readable account of the essential background as well as of the peculiarly electrochemical aspects.

The printing and layout of this volume are of the usual high standard that we expect from this publisher.

R. PARSONS

Errata

DECEMBER (1963) ISSUE, p. 931, equation at centre of page, last term.

For "$\frac{8}{\text{Weight of sample}}$ p.p.m." *read* "$\frac{16}{\text{Weight of sample}}$ p.p.m."

MARCH (1968) ISSUE, p. 159, 9th line under "BUFFER SYSTEM." *For* "increased" *read* "decreased."

IBID., p. 163, Table II, 4th column, last line. *For* "0·006" *read* "0·0006."

Summaries of Papers in this Issue

Amperometric Titration of Copper and Cadmium in the Presence of Zinc, Cobalt and Nickel with Sodium Diethyldithiocarbamate

The reaction of some metal ions with diethyldithiocarbamate in 0·1 M sodium hydroxide solution is investigated polarographically. Methods are presented for the amperometric titration of cadmium in the presence of zinc and cobalt, and of cadmium and copper in admixture.

A. BROOKES and A. TOWNSHEND

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

Analyst, 1968, **93**, 425–428.

The Rapid Dissolution of Plutonium Dioxide by a Sodium Peroxide - Sodium Hydroxide Fusion, Followed by Determination of the Plutonium Content by Controlled-potential Coulometry

A method is described for the dissolution of plutonium dioxide, followed by determination of the plutonium content by controlled-potential coulometry. The plutonium dioxide is brought into solution by fusion with a mixture of sodium peroxide and sodium hydroxide at 600° C for 15 minutes in an alumina crucible. The cold melt is leached with water, which is then acidified with sulphuric acid. The solution is heated for 15 minutes to decompose hydrogen peroxide and, after cooling, diluted to a suitable volume. The plutonium content of an aliquot containing about 4 mg of plutonium is determined by controlled-potential coulometry. A potential of +0·30 volt *versus* a S.C.E. is used for reduction to plutonium(III), whereas +0·70 volt *versus* a S.C.E. is used for the quantitative oxidation to the plutonium(IV) state.

Mean recoveries on 100-mg amounts of plutonium dioxide that had been ignited at 850° C were 99·95 per cent., with a coefficient of variation of 0·11 per cent. For the complete dissolution of samples previously ignited at higher temperatures (about 1600° C), an increase in the ratio of the weight of fusion mixture to sample is necessary. Mean recoveries on 50-mg amounts of plutonium dioxide that had been ignited at 1600° C were 99·85 per cent., with a coefficient of variation of 0·54 per cent.

G. W. C. MILNER and D. CROSSLEY

Analytical Sciences Division, U.K.A.E.A. Research Group, Atomic Energy Research Establishment, Harwell.

Analyst, 1968, **93**, 429–432.

A Stable, Solid-state, High Voltage Source for Electrode Polarisation

A simple, cheap, versatile, solid-state, high voltage source is proposed as a replacement for high tension battery supplies. It is capable of providing up to 70 μA at 1000 volts, with drift and noise less than 0·1 per cent., and can be used for electrode polarisation, radiation counting tube supplies and other small-current applications.

E. BISHOP

Chemistry Department, University of Exeter, Stocker Road, Exeter, Devon.

Analyst, 1968, **93**, 433–435.

A Semi-automatic Timed End-point Karl Fischer Titrator

The construction, operation and evaluation are described of a low-cost instrument for providing semi-automatic analysis of the water content of liquids and gases by means of a Karl Fischer titration.

B. COPE

"Shell" Research Limited, Carrington Plastics Laboratory, Urmston, Manchester.

Analyst, 1968, **93**, 436–440.

Summaries of Papers in this Issue

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Analyst, 1968, **93**, 436–440.

The Use of Depleted Cells as Inocula in Vitamin Assays

Preparation of assay inocula in such a way that the cells contain a reduced level of the vitamin to be assayed gives low background growth in plates, and the greater contrast enhances zone definition. Steps required to be taken to find the optimum conditions for preparation of inocula are described. The assays of nicotinamide, pantothenic acid, folic acid and vitamin B_6 can be improved in this way.

L. GARE
Beecham Research Laboratories, Vitamins Research Station, Walton Oaks, Dorking Road, Tadworth, Surrey.

Analyst, 1968, **93**, 456–457.

Species Identification of Cooked Fish by Disc Electrophoresis

The present objective methods of identifying fish species are based on the species-specific protein-separation patterns obtained on electrophoresis of the water-soluble sarcoplasmic proteins of fish muscle. As the proteins must be in their native undenatured state, electrophoretic identification of fish species has, so far, been restricted to raw fish.

An extension of the electrophoretic method to the identification of cooked fish is described. The protein fragments extractable in 6 M urea from the denatured proteins of cooked muscle can also be separated by electrophoresis into species' characteristic patterns that could be used for species identification. The separation patterns obtained on polyacrylamide gel for the urea extracts of cooked herring, halibut, plaice, salmon, cod and haddock are presented. In its present form the method does not apply to canned fish.

I. M. MACKIE
Ministry of Technology, Torry Research Station, P.O. Box 31, 135 Abbey Road, Aberdeen.

Analyst, 1968, **93**, 458–460.

Observations on the Distribution and Determination of Fluorine Compounds in Biological Materials, Including Soils

The distribution of fluorine compounds found in biological materials, including soils, is outlined. Several improvements are proposed for the sampling and ashing of animal and plant tissues, and soil, including the use of finely ground suspensions in dilute agar solution. Some modifications are suggested in the separation of fluorine by diffusion as hydrofluoric acid and its subsequent determination with alizarin complexan. The effect of silica in the analysis of plants and soils for fluorine is discussed.

R. J. HALL
Ministry of Agriculture, Fisheries and Food, National Agricultural Advisory Service, Kenton Bar, Newcastle upon Tyne NE1 2YA.

Analyst, 1968, **93**, 461–468.

Spectrophotometric Determination of Micro Amounts of Aluminium in Plant Material with 8-Hydroxyquinoline

The 8-hydroxyquinoline method, previously found to be more satisfactory than the aluminon method for the analysis of soil extracts, has been examined for its suitability for determining aluminium in plant tissues. An initial extraction with chloroform of the diethyldithiocarbamate complexes of the large amounts of heavy metals found in plant material has been devised. The resulting method was tested on synthetic solutions and compared with emission-spectrochemical procedures in the analysis of plant materials.

The lower limit for reliable determination by the proposed method corresponds to 4 p.p.m. of aluminium in the dried plant tissue. At this lower limit, the following interfering elements in the tissue at the percentage concentrations indicated are tolerated in the method: copper, 0·005; zinc, 0·030; iron, 0·030; manganese, 0·10; phosphorus, 0·20; and calcium, 4·0. For tissue containing 20 p.p.m. of aluminium, or more, at least five times these amounts are tolerated.

C. R. FRINK and D. E. PEASLEE
The Connecticut Agricultural Experiment Station, New Haven, Connecticut.

Analyst, 1968, **93**, 469–474.

Direct Determination of Manganese in Soil Extracts by Atomic-absorption Spectroscopy

The tedious preliminary treatment of soil extracts required before manganese can be determined by traditional colorimetric methods is avoided by direct aspiration of the filtered extract for atomic-absorption spectroscopy.

Results for manganese extracted into four different reagents used for assessing the manganese status of soils are in close agreement with those found by the colorimetric potassium periodate method after removal of organic matter.

M. NADIRSHAW and A. H. CORNFIELD

Chemistry Department, Imperial College of Science and Technology, London, S.W.7.

Analyst, 1968, **93**, 475.

Nitrogen Factor for Barley

Report prepared by the Meat Products Sub-Committee.

ANALYTICAL METHODS COMMITTEE

9/10 Savile Row, London, W.1.

Analyst, 1968, **93**, 476–477.

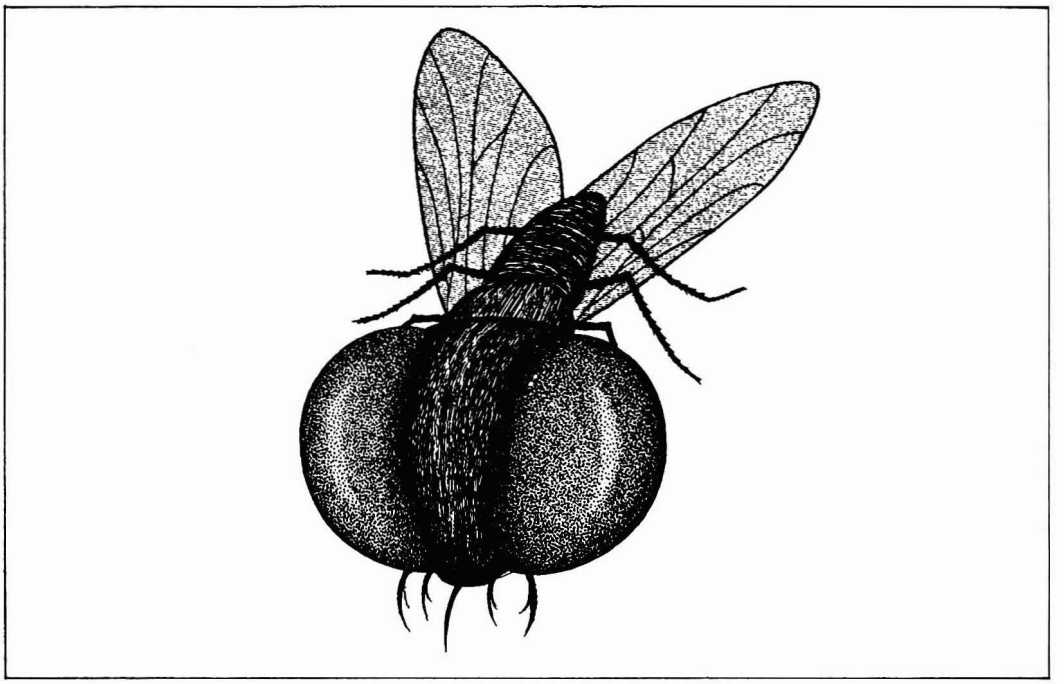
Nitrogen Factor for Blood

Report prepared by the Meat Products Sub-Committee.

ANALYTICAL METHODS COMMITTEE

9/10 Savile Row, London, W.1.

Analyst, 1968, **93**, 478–479.



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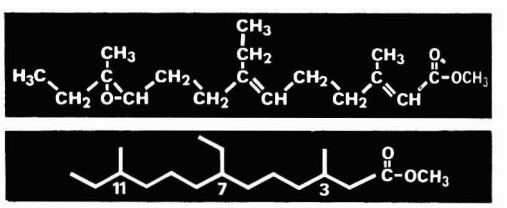

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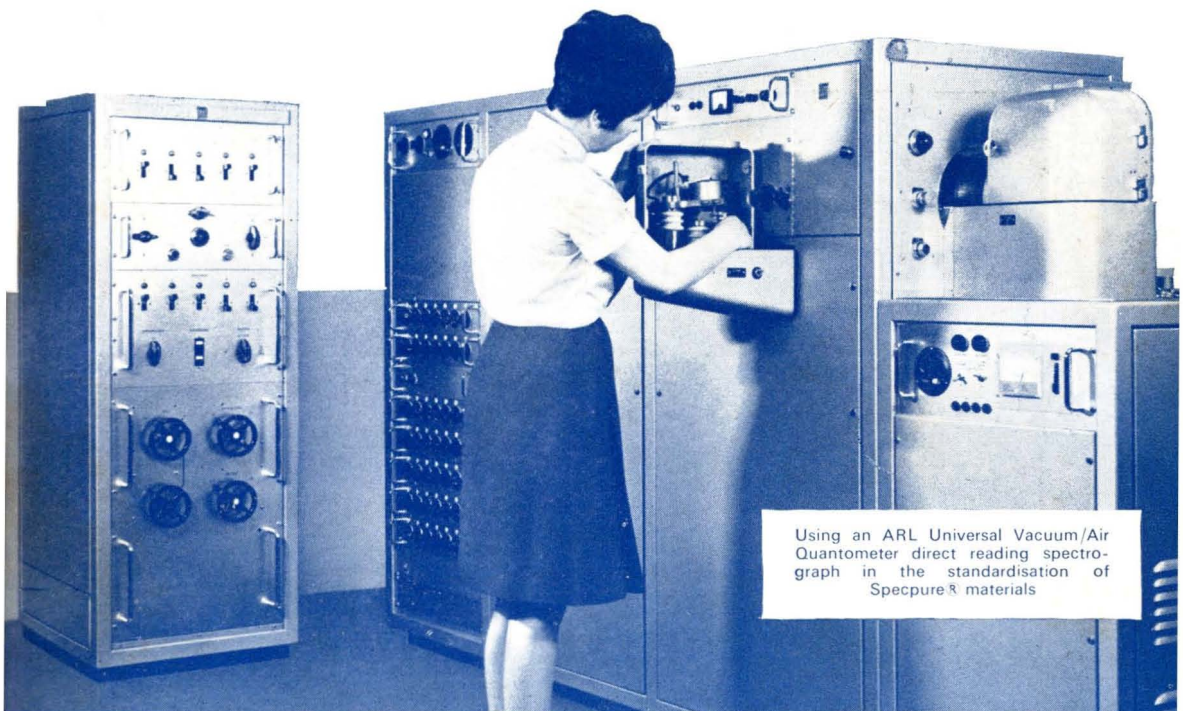

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