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Summaries of Papers in this Issue

Further Investigations of the Athavale Method for the Determination of Silver with Silver - Molybdenum Electrodes

A wave at about −0·55 V in the current - potential graph of aqueous solutions containing silver nitrate and sulphosalicylic acid obtained by using silver and molybdenum electrodes has been shown to be due to complex molybdenum ions produced by a first-order exchange reaction at the molybdenum electrode and not to silver ions as reported by previous workers.

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Department of Science, Southend College of Technology, Southend-on-Sea, Essex.

Analyst, 1972, **97**, 409–411.

An Analytical and Kinetic Study of the Periodate Oxidation of Vanadium(IV) in Acidic Medium

The kinetics of the reaction between sodium periodate and vanadium(IV) have been studied in 5 M perchloric acid and at 5·54 M ionic strength. Under these conditions, the reaction was found to obey the rate equation

$$-\frac{d[V(IV)]}{dt} = \frac{k_0 K_1 [V(IV)] [IO_4^-]^2}{1 + K_1 [IO_4^-]}$$

Values of k_0 of 0·27 l mol^{-1} s^{-1} and K_1 of 2·24 l mol^{-1} were obtained at 16 °C and the activation energy was 18·4 ± 1 kcal mol^{-1}. The reaction rate is also inversely proportional to the hydrogen-ion concentration and a possible mechanism is proposed that satisfies the experimental results.

A procedure for the determination of vanadium(IV) by spectrophotometric titration with periodate is described and is compared with other oxidimetric methods for the determination of vanadium(IV).

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Analyst, 1972, **97**, 412–419.

An Investigation of the Optimum Composition of Poly(vinyl chloride) Matrix Membranes Used for Selective Calcium-sensitive Electrodes

Studies of the composition parameters of the poly(vinyl chloride) matrix membrane selective calcium-sensitive electrode showed that phosphate sensor and phosphonate mediator are interdependent constituents. The most practical electrodes examined were obtained from membranes containing 28·8 per cent. w/w of poly(vinyl chloride) *plus* 71·2 per cent. w/w of dioctyl phenylphosphonate and monocalcium dihydrogen tetra(didecylphosphate) in a 10 : 1 weight ratio. Membranes containing only didecylphosphoric acid sensor gave sluggish electrodes with short linear response ranges. Monocalcium di(didecylphosphate) sensor produced better electrode characteristics than didecylphosphoric acid, but the membranes showed evidence of the presence of finely divided material.

In complementary work, no practically useful electrodes were obtained with any of cellulose acetate, ethylcellulose, collodion and pyroxylin as alternative matrix materials to poly(vinyl chloride).

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Chemistry Department, University of Wales Institute of Science and Technology, Cardiff, CF1 3NU, Wales.

Analyst, 1972, **97**, 420–427.

A Gas-chromatographic Method for the Determination of Increased Bromide Concentrations in Blood

A method is described for the determination of bromide in blood by gas chromatography. The bromide, in a protein-free filtrate, is oxidised to bromine by potassium permanganate in acidic solution and extracted into cyclohexane containing cyclohexene to give 1,2-dibromocyclohexane, which is determined by gas chromatography using 1,6-dibromohexane as an internal standard. About 70 per cent. of the bromide present is converted into 1,2-dibromocyclohexane; chlorine, from chloride, reacts to give 1,2-dichlorocyclohexane. Oxalate, fluoride or heparin, which may be present as a preservative or an anti-coagulant, do not interfere and 1,2-dibromocyclohexane is not obtained from carbromal or Bromvaletone under the conditions described. The method is applicable in the range from 10 to 100 mg of bromide per 100 ml of blood (0·1 to 1·0 mg ml^{-1}) and to higher levels after dilution of the sample.

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Analyst, 1972, **97**, 428–432.

Determination of Benzoic Acid in Meat and Meat Products by Gas Chromatography

A gas-chromatographic method for the determination of benzoic acid in meat and meat products is described with particular reference to the open-pack type of minced meat sold for domestic pets. The sample is steam distilled, the acidified distillate extracted with diethyl ether, the extract evaporated to dryness and the residue dissolved in methanol and subjected to gas chromatography on 3 per cent. SE-30 on 80 to 100-mesh Chromosorb W at 170 °C with dimethyl phthalate as the internal standard. The method gives satisfactory results in the range 0·1 to 0·5 per cent. of benzoic acid in the meat.

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Analyst, 1972, **97**, 433–436.

A Combined Column-chromatographic and Infrared Spectrophotometric Determination of Diosgenin and Yamogenin in Fenugreek Seed

A routine procedure is described for the determination of diosgenin and yamogenin in fenugreek seed. A crude extract is obtained by acidic hydrolysis of the seed, neutralisation and extraction of the insoluble matter with light petroleum. By means of column chromatography this extract is freed from fixed oil and other substances, thus yielding a mixture of diosgenin and yamogenin suitable for infrared spectrophotometric analysis. The results calculated for duplicate analyses of 2·5-g samples of commercial Moroccan seed, expressed as a 95 per cent. confidence interval of the mean sapogenin value, are 0·96 $\pm$ 0·017 per cent. for diosgenin *plus* yamogenin, 0·58 $\pm$ 0·008 per cent. for diosgenin and 0·38 $\pm$ 0·016 per cent. for yamogenin. The procedure has been found to be satisfactory for column loadings of up to 75 mg of diosgenin *plus* yamogenin in the presence of up to 600 mg of fixed oil, which is approximately three times the weight of extractive from 2·5 g of Moroccan seed.

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Analyst, 1972, **97**, 437–441.

THE ANALYST

Further Investigations of the Athavale Method for the Determination of Silver with Silver - Molybdenum Electrodes

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A wave at about −0·55 V in the current - potential graph of aqueous solutions containing silver nitrate and sulphosalicylic acid obtained by using silver and molybdenum electrodes has been shown to be due to complex molybdenum ions produced by a first-order exchange reaction at the molybdenum electrode and not to silver ions as reported by previous workers.

VARIOUS methods for the determination of silver ions in aqueous and non-aqueous solutions and in the presence of complexing agents have been investigated.[1] One method used was the polarographic analysis of aqueous solutions containing silver nitrate and sulphosalicylic acid by using a silver cathode and a molybdenum anode.

EXPERIMENTAL AND RESULTS

The electrodes were made from silver and molybdenum wires of diameters 0·37 and 0·71 mm, respectively. An epoxy resin (Araldite, C.I.B.A. Ltd.) was cast in the form of a rod around the silver-wire electrode leaving a length of 2 mm exposed, which was cleaned with dilute (2 M) nitric acid and washed with distilled water. The molybdenum wire was cleaned by abrasion with fine emery paper and washed with distilled water; a 20-mm length of molybdenum wire was immersed in each experiment.

CURRENT - POTENTIAL GRAPHS—

Sulphosalicylic acid was used as a complexing agent and also as a supporting electrolyte. Current - potential graphs were obtained by using a Heathkit d.c. polarograph at 20 °C. Fig. 1, curve I, shows a typical current - potential graph obtained by using a silver cathode and molybdenum anode in 10^{-1} M sulphosalicylic acid after de-aeration with nitrogen. The potential was expressed with respect to the molybdenum electrode and the rate of increase in potential was 2 V min^{-1}. In sulphosalicylic acid solution, hydrogen ions were discharged at about −0·65 V.

The addition of silver nitrate resulted in a discharge of silver ions at about +0·2 V. The diffusion current was found to be proportional to the concentration of silver over the range $2{\cdot}5 \times 10^{-4}$ to 2×10^{-3} M, which is in agreement with the results obtained by Kolthoff and Lingane.[2]

TIME-DEPENDENT EFFECTS—

It was observed that when the electrodes were left in contact with the solution a polarographic wave developed at about −0·55 V, and the magnitude of this wave increased with time. Fig. 1, curve II, shows the current - potential graph for a freshly prepared 10^{-3} M solution of silver nitrate in 10^{-1} M sulphosalicylic acid solution, and curve III shows the graph for the same mixed solution obtained 30 minutes later. When the electrodes were then transferred to a fresh solution, a normal graph was obtained.

It was found that when 10^{-2} M silver nitrate in 10^{-1} M sulphosalicylic acid solution was used, the solution slowly became yellow in colour, and this yellow solution was shown by analysis to contain molybdenum; spectroscopic analysis indicated a $\lambda_{max.}$ at 345 nm. Electron-probe microanalysis of the molybdenum electrodes showed the presence of silver on the electrode surfaces.

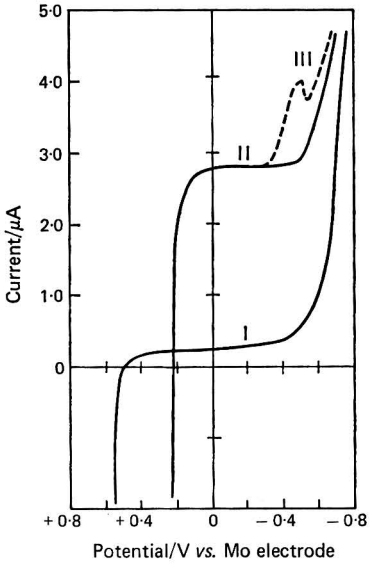

Fig. 1. Current *versus* potential graphs obtained by using a silver cathode and molybdenum anode in 10^{-1} M sulphosalicylic acid after de-aeration with nitrogen. I, Sulphosalicylic acid alone; II, with 10^{-3} M silver nitrate added; and III, with 10^{-3} M silver nitrate added, after 30 minutes

SPECTROSCOPIC DETERMINATION OF EXCHANGE KINETICS—

Spectroscopic analysis with a Pye Unicam SP800B spectrophotometer was carried out to investigate the exchange reaction between the molybdenum electrode and silver ions in solution. A special cell (Fig. 2) was used to monitor continuously the change in absorbance at 345 nm of a solution of silver nitrate in 10^{-1} M sulphosalicylic acid in contact with molybdenum, while maintaining de-aeration and stirring at 17 °C.

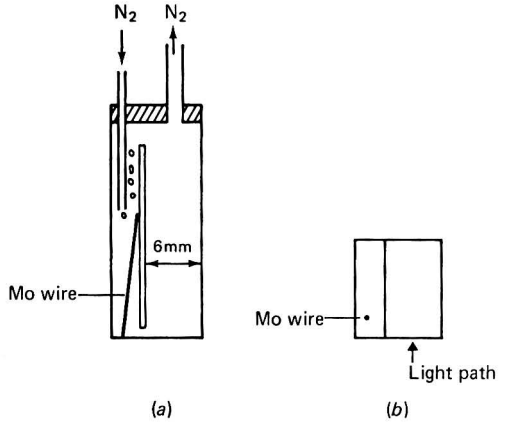

Fig. 2. Two-compartment cell. (*a*) Side view; and (*b*) plan view

The results showed that the absorbance at 345 nm increased with time, and the exchange was shown to be first order with respect to the silver-ion concentration. The specific rate constant was found to be $8{\cdot}7 \times 10^{-4}$ s^{-1} by using a cell with a molybdenum electrode having an area of 0·85 cm^2. A further experiment showed that the value of the rate constant was directly proportional to the area of the molybdenum electrode.

Discussion and conclusions

It is concluded that the wave at about −0·55 V, previously reported by Athavale, Dhaneshwar and Dhaneshwar[3] to be due to the discharge of silver ions, is due to complex molybdenum ions produced by a first-order exchange reaction at the molybdenum electrode. It is likely that the results obtained by the previous workers were misinterpreted because of a chance correlation between the height of the wave and the silver-ion concentration. As the detection level of the differential cathode-ray polarograph that they used is lower and its rate of recording is faster than that of the d.c. polarograph, the concentration of the complex molybdenum ion formed would be sufficient to show a wave almost as soon as the electrodes were immersed in the solution. Furthermore, the amount of the complex molybdenum ion formed per unit time before equilibration is a function of the initial silver concentration, so that the apparent correlation between silver concentration and peak height is accounted for provided that approximately the same time elapses between the immersion of the electrodes and the polarography of each of the solutions, which is, in fact, very likely.

Both types of instruments will produce waves that are proportional to the silver-ion concentration, but the actual "mechanics" of the recording will take slightly longer when the d.c. instrument is used.

The wave at about −0·55 V is due to complex molybdenum ions and could be used for the determination of molybdenum. For example, 5×10^{-6} M ammonium molybdate in 10^{-1} M sulphosalicylic acid solution produces a significant wave.

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An Analytical and Kinetic Study of the Periodate Oxidation of Vanadium(IV) in Acidic Medium*

BY D. J. B. GALLIFORD† AND J. M. OTTAWAY

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The kinetics of the reaction between sodium periodate and vanadium(IV) have been studied in 5 M perchloric acid and at 5·54 M ionic strength. Under these conditions, the reaction was found to obey the rate equation

$$-\frac{d[V(IV)]}{dt} = \frac{k_o K_1 [V(IV)] [IO_4^-]^2}{1 + K_1 [IO_4^-]}$$

Values of k_o of 0·27 l mol^{-1} s^{-1} and K_1 of 2·24 l mol^{-1} were obtained at 16 °C and the activation energy was 18·4 ± 1 kcal mol^{-1}. The reaction rate is also inversely proportional to the hydrogen-ion concentration and a possible mechanism is proposed that satisfies the experimental results.

A procedure for the determination of vanadium(IV) by spectrophotometric titration with periodate is described and is compared with other oxidimetric methods for the determination of vanadium(IV).

APART from its use in the Malaprade reaction,[1,2] the application of periodate as an analytical reagent tends to be disregarded in favour of other more fully established oxidising agents. This is undoubtedly because of the reported instability of periodate solutions[3] and the complicated nature of the species present[4] and is also said by some workers[1] to be due to the cost of the reagent. Although there is now sufficient evidence to show that periodate solutions can be kept for long periods if stored in the absence of light,[2,5] it remains to be shown whether periodate has sufficient advantages over other reagents for it to be used in any particular analytical determination. An obvious application lies in the field of catalytic analysis, in which many normally slow reactions involving periodate can be catalysed by trace amounts of metals such as ruthenium,[6] manganese[7] and chromium.[8] In the present paper, the titrimetric determination of vanadium(IV) with sodium periodate is discussed, and in this instance periodate appears to have a definite advantage over other reagents owing to its more rapid rate of reaction with vanadium(IV).

The kinetics of the reactions of vanadium(IV) with bromate[9] and chlorate and iodate[10] have recently been reported and are of interest in that all three reactions appear to proceed by way of formation of an intermediate complex formed between the halate and vanadium(IV). This complex takes the form of a $VO^{2+}.XO_3^-$ species for chlorate and bromate but $VO^{2+}.(XO_3^-)_2$ for iodate. The kinetics of the reaction between periodate and vanadium(IV) are also described in this paper and it is demonstrated that the mechanism of this reaction is very similar to those of the other halates.

Few previous studies of the oxidation of reduced forms of vanadium by periodate have been reported.[11,12] Mazor and Erdey[11] have described the reaction of vanadium(II) with periodate and recommend the application of one of several indicators or potentiometry to locate the end-point for the oxidation of vanadium(II) to vanadium(III). However, their studies do not extend to the oxidation of vanadium(IV). Hara[12] has reported a method for the determination of periodic acid with vanadium(IV), but this method involves the reaction of periodic acid with manganese(II) and the titration with vanadium(IV) of the manganese(VII) formed. The direct reaction of vanadium(IV) with periodate does not therefore appear to have been studied previously, even though the procedure described here is considerably simpler than Hara's procedure.

EXPERIMENTAL

Analytical-reagent grade chemicals were used whenever possible and water distilled from an all-quartz still was used in the preparation of all solutions. Specific solutions were prepared and standardised as follows.

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Vanadium(IV) solution, 0·2 M—Vanadyl sulphate was dissolved in 0·05 M perchloric acid and the solution was standardised by titration with 0·1 M cerium(IV) sulphate solution.[13–15] The titration was carried out potentiometrically by using a platinum wire - S.C.E. couple and potentials were measured with an E.I.L. 23A pH meter. The titration was conducted in 0·5 M sulphuric acid medium at 70 °C. The cerium(IV) solution was separately standardised with respect to primary standard arsenite.[1]

Sodium periodate, 0·4 M in 5 M perchloric acid solution—Analytical-reagent grade sodium periodate that had been dried in air at 110 °C for 1 hour[5] was dissolved in a mixture of distilled water and the calculated volume of 60 per cent. w/w perchloric acid. After 12 days this solution started to decompose[5] and a fresh solution was therefore prepared at fortnightly intervals. For the photometric titrations, solutions of analytical-reagent grade sodium periodate in dilute sulphuric acid were used. These solutions were standardised with reference to primary standard arsenite.[16] An aliquot of the periodate solution was neutralised with sodium hydrogen carbonate, a 3-g excess of which was added. Three grams of potassium iodide were then added and the liberated iodine was titrated with standard arsenite.

The methods of preparation and, when necessary, standardisation of the other solutions used in this work, *viz.*, potassium bromate, perchloric acid, potassium chlorate and sodium perchlorate solutions, have been described previously.[9,10]

Spectrophotometric titrations—

Spectrophotometric titrations of vanadium(IV) with bromate, chlorate and periodate were carried out by using a Hitachi Perkin-Elmer photoelectric titration attachment (139–0720) positioned between the monochromator and phototube compartments of a Hitachi Perkin-Elmer 139 spectrophotometer. The reactions were followed by measuring the decrease in absorbance at 765 nm, an absorption maximum of vanadium(IV), at which wavelength no other species interferes. The titration volume was 100 ml.

Kinetic studies—

Kinetic investigations of the reaction were carried out spectrophotometrically, by measuring the decrease in absorbance of the reactant solution at 765 nm, on a Hitachi Perkin-Elmer 139 spectrophotometer to which was attached a Honeywell Electronik 15 strip-chart recorder. A two-limbed reaction vessel was used to contain the reactant solutions during the 30-minute period of thermostatic control prior to the start of the reaction. The vanadium(IV) solution was placed in one limb and the periodate solution in the other. Both solutions were kept at the same acid concentration and ionic strength, before mixing, by making suitable additions of perchloric acid and sodium perchlorate to both limbs. The total reaction volume was 20 ml. The reaction was initiated by thoroughly mixing the solutions, the recorder chart drive being switched on at the same time. After mixing the solutions, a sample was transferred to a 20-mm spectrophotometer cell that had previously been housed in the thermostatically controlled cell compartment of the spectrophotometer, and a continuous record of the change in absorbance with time was obtained.

Unlike the other similar systems reported previously,[9,10] considerable difficulties were experienced in reproducing reaction rate measurements of the vanadium(IV) - periodate reaction. With the reported evidence of the instability of periodate solutions, and in particular their photodecomposition with formation of ozone, the effects of light and oxygen on the reaction were investigated; no detectable effect on the rate or the reproducibility was observed. Investigations on the nature and stability of periodate solutions[5] showed that such solutions were stable over the period of our experiments and could not account for the observed variations. Trace impurities in the reagents were considered but were neglected as they would be expected to provide a consistent background. After some frustrating studies,[17] it was discovered that chromium(VI) "leached" from the walls of the reaction vessel was the cause of the irreproducibility. This material originated from the cleaning mixture used in the pre-treatment of glassware and, as will be reported elsewhere,[8] was found to accelerate the reaction at levels as low as 0·01 p.p.m. of chromium(VI). When a new cleaning procedure involving the use of 50 per cent. aqueous nitric acid solution was substituted in the pre-treatment of glassware, reaction rates measured under the same conditions were fully reproducible, and this procedure was used in all the work reported in this paper.

In any kinetic study, the choice of initial reactant concentrations is governed by the rate of the reaction, the experimental technique and the sensitivity of the analytical measurement. In this instance, it was found that the reaction of vanadium(IV) with periodate at hydrogen-ion concentrations of between 0·1 and 3 M was too fast to follow by using the technique involving a recorder coupled to a conventional spectrophotometer. The use of a stopped-flow apparatus was considered[17] but the equipment available did not cover the required spectral range in the region of 765 nm and could not be used. The reaction was slow enough to be studied by the above procedure at acid concentrations higher than 4 M and this technique was therefore used despite the possible disadvantages. The effects of the concentrations of vanadium(IV) in the range 0·008 to 0·024 M, of concentrations of sodium periodate in the range 0·10 to 0·35 M and of temperature over the range 17·6 to 26·4 °C were studied at a perchloric acid concentration of 5·00 M and an ionic strength of 5·54 M. The effect of perchloric acid concentration in the range 4 to 8 M was studied at an ionic strength of 8·1 M. Sodium perchlorate was used to maintain constant ionic strength.

In all experiments, the periodate was kept in large excess over the vanadium(IV), and the concentrations of periodate and hydrogen ions therefore remained effectively constant throughout the reaction. Under these conditions, pseudo first-order kinetics are maintained and are defined by equation (1)—

$$-\frac{\mathrm{d}\,[\mathrm{V(IV)}]}{\mathrm{d}t} = k\,[\mathrm{V(IV)}] \qquad (1)$$

where k is the pseudo first-order rate constant.

Results and discussion

Kinetics—

Under the conditions used, the reduction of periodate by vanadium(IV) proceeded only as far as the formation of iodate. The absorbance of a vanadium(IV) solution in the presence of a 50-fold excess of iodate showed no detectable decrease within 15 minutes and only a 13·5 per cent. decrease in 15 hours at room temperature, which is consistent with the earlier study of the vanadium(IV) - iodate reaction that had to be conducted at 50 °C.[10] The stoicheiometry of the present reaction is therefore represented by equation (2)—

$$IO_4^- + 2VO^{2+} + H_2O \rightarrow IO_3^- + 2VO_2^+ + 2H^+ \qquad (2)$$

Graphs of log $[VO^{2+}]$ *versus* time were straight lines in all kinetic experiments and were therefore consistent with equation (1), and values of the pseudo first-order rate constant, k, shown in Tables I and II were obtained from the slopes of these graphs. In order to minimise the reaction rates, the reactions were performed at the lowest possible temperature that could be used and stabilised to within 0·1 °C. As this temperature was to some extent

Table I

Effects of temperature and vanadium(IV) and periodate concentrations on the pseudo first-order rate constant of the vanadium(IV) - periodate reaction in 5 M perchloric acid and at 5·54 M ionic strength

Initial [V(IV)]/M	[$NaIO_4$]/M	Temperature/°C	k/s^{-1}	k_o/l mol^{-1} s^{-1}
0·008	0·15	18·9	0·0179	0·474
0·012	0·15	18·9	0·0191	0·506
0·016	0·15	18·9	0·0177	0·469
0·020	0·15	18·9	0·0193	0·511
0·024	0·15	18·9	0·0189	0·501
0·010	0·10	16·0	0·0054	0·292
0·010	0·15	16·0	0·0102	0·270
0·010	0·20	16·0	0·0167	0·270
0·010	0·25	16·0	0·0239	0·266
0·010	0·30	16·0	0·0328	0·272
0·010	0·35	16·0	0·0417	0·271
0·010	0·05	17·6	0·0021	0·418
0·010	0·05	19·4	0·0025	0·492
0·010	0·05	21·6	0·0041	0·807
0·010	0·05	24·2	0·0051	1·005
0·010	0·05	26·4	0·0057	1·124

TABLE II

EFFECT OF HYDROGEN-ION CONCENTRATION ON THE PSEUDO FIRST-ORDER RATE CONSTANT OF THE VANADIUM(IV) - PERIODATE REACTION

Initial [V(IV)], 0·005 M; [$NaIO_4$], 0·075M; temperature, 20·0 °C; and ionic strength, 8·1 M

$[H^+]$/M	k/s^{-1}
4·0	0·0125
5·0	0·0094
6·0	0·0077
7·0	0·0068
8·0	0·0059

dependent on the ambient temperature, kinetic experiments carried out at different times were necessarily performed at different temperatures.

The constancy of values of k with variation in vanadium(IV) concentration (Table I) confirms the first-order dependence on vanadium(IV). Reaction rates were found to be independent of the concentrations of the products, *viz.*, vanadium(V) and iodate, but values of k show a definite dependence on periodate, for which the order is 1·6. This type of fractional order was found earlier for the oxidation of vanadium(IV) by iodate,[10] but the experimental rate equation derived in this instance was not satisfactory for the present results. The observed periodate dependence of the reaction is in agreement with the rate law

$$-\frac{d[V(IV)]}{dt} = \frac{k_1[V(IV)][IO_4^-]^2}{1 + k_2[IO_4^-]} \quad .. \quad .. \quad .. \quad (3)$$

which can be demonstrated as follows. By comparison of equation (3) with equation (1) it can be seen that

$$k = \frac{k_1[IO_4^-]^2}{1 + k_2[IO_4^-]} \quad .. \quad .. \quad .. \quad .. \quad (4)$$

or

$$\frac{[IO_4^-]}{k} = \frac{1}{k_1[IO_4^-]} + \frac{k_2}{k_1} \quad .. \quad .. \quad .. \quad .. \quad (5)$$

A graph of $[IO_4^-]/k$ *versus* $1/[IO_4^-]$ was found to be a straight line (Fig. 1) with a definite positive intercept, confirming the significance of the k_2 term. From Fig. 1 values of k_1 and k_2 of 0·61 l^2 mol^{-2} s^{-1} and 2·24 l mol^{-1}, respectively, are obtained for a temperature of 16 °C, an ionic strength of 5·54 M and a hydrogen-ion concentration of 5 M.

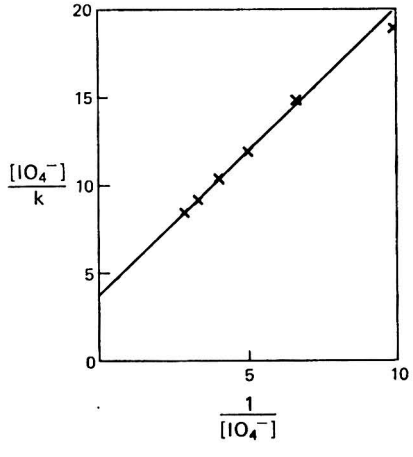

Fig. 1. Graph of $[IO_4^-]/k$ *versus* $1/[IO_4^-]$ for the data on the variation of k with $[IO_4^-]$ given in Table I

By analogy with other similar systems,[9,10] this form of rate law suggests that an equilibrium step occurs prior to the rate-determining step. A possible reaction path is

$$IO_4^- + VO^{2+} \overset{K_1}{\rightleftharpoons} IO_4^-.VO^{2+} \text{ (fast)} \quad .. \quad .. \quad .. \quad (6)$$

$$IO_4^-.VO^{2+} + IO_4^- \xrightarrow{k_0} \text{products (slow)} \quad .. \quad .. \quad .. \quad (7)$$

The rate-determining step is then the reaction of the intermediate complex species, $IO_4^-.VO^{2+}$, with a further IO_4^- ion and gives rise to the rate equation

$$\text{Rate} = -\frac{d[V(IV)]}{dt} = k_0 [IO_4^-] [IO_4^-.VO^{2+}] \quad .. \quad .. \quad (8)$$

As

$$K_1 = \frac{[IO_4^-.VO^{2+}]}{[IO_4^-] [VO^{2+}]} \quad .. \quad .. \quad .. \quad .. \quad (9)$$

$$\text{Rate} = k_0 K_1 [IO_4^-]^2 [VO^{2+}] \quad .. \quad .. \quad .. \quad .. \quad (10)$$

If $[VO^{2+}]_T$ and $[IO_4^-]_T$ represent the total unreacted concentrations of these species present at any time, then as periodate is present in considerable excess

$$[IO_4^-] \approx [IO_4^-]_T$$

but

$$[VO^{2+}]_T = [VO^{2+}] + [VO^{2+}.IO_4^-] \quad .. \quad .. \quad .. \quad (11)$$

Substitution for $[VO^{2+}.IO_4^-]$ from equation (9) into equation (11), and substitution of the resulting value of $[VO^{2+}]$ into equation (10), gives

$$\text{Rate} = \frac{k_0 K_1 [IO_4^-]^2_T [VO^{2+}]_T}{1 + K_1 [IO_4^-]_T} \quad .. \quad .. \quad .. \quad .. \quad (12)$$

This equation is identical in form with the experimental rate equation and, by comparison, values of 2·24 l mol^{-1} and 0·27 l mol^{-1} s^{-1} were obtained for K_1 and k_0, respectively, for the conditions outlined above. The temperature dependence of this rate constant gave an average value of the activation energy of 18·4 ± 1 kcal mol^{-1} over the range 17·6 to 26·4 °C.

The pseudo first-order rate constant was found to be inversely proportional to the hydrogen-ion concentration in the range 4 to 8 M, as shown in Table II. The most important periodate species present in solutions of this acid concentration are H_5IO_6 and IO_4^-,[5] and the most probable explanation of the hydrogen-ion dependence is that the species IO_4^- is the active oxidising species and that the concentration of this species is controlled by the equilibrium

$$H_5IO_6 \rightleftharpoons IO_4^- + 2H_2O + H^+ \quad .. \quad .. \quad .. \quad .. \quad (13)$$

The precise elucidation of the hydrogen-ion effect is very difficult because not only is the value of the equilibrium constant for reaction (13) unknown at this ionic strength but in equation (12) the values of both $[IO_4^-]$ and K_1 will depend on the hydrogen-ion concentration. However, that reaction (13) is the controlling reaction seems reasonable and the full mechanism will be represented by reactions (13), (6) and (7). The hydrogen-ion dependence is, however, very important for the analytical application of this reaction. Comparison of the rates of the reactions suggests that at 1 M hydrogen-ion concentration, the rates of the periodate and bromate oxidations of vanadium(IV) will be similar, but that at lower acid concentrations, the rate of the periodate reaction will become very much faster and more useful analytically, and this is discussed in the next section.

SPECTROPHOTOMETRIC TITRATION OF VANADIUM(IV) WITH SODIUM PERIODATE—

The visual or potentiometric titration of vanadium(IV) with periodate is obviously unlikely to be very successful because of the evidence of the slow reactions of the vanadium(V) - vanadium(IV) couple with common visual indicators and at electrodes.[15] Spectrophotometric determination of end-points, which depends on the availability of a species with the required absorptivity and a sufficiently high rate in the main titration reaction, was suitable in this instance. Titrations were carried out at ambient temperatures that varied between 15 and 20 °C by adding 0·2-ml increments of sodium periodate titrant solution from a 2-ml burette to the vanadium(IV) solution that had been diluted to 100 ml. As expected, the time required

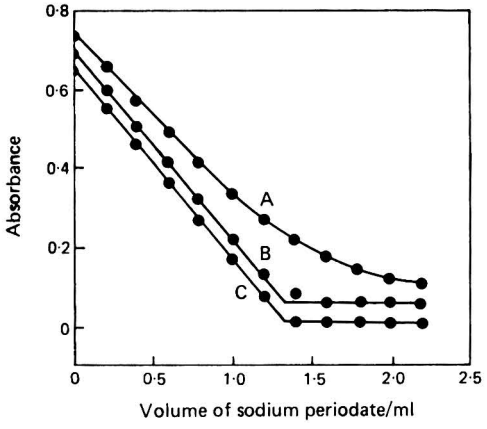

Fig. 2. Spectrophotometric titrations of 3·50 ml of 0·198 M vanadium(IV) in A, 0·5 M, B, 0·05 M and C, 0·01 M sulphuric acid with 0·2598 M sodium periodate. Absorbance readings taken 1 minute after each addition of titrant

for steady absorbance readings to be obtained varied with the acid concentration used. At 0·5 M sulphuric acid concentration, 10 to 11 minutes were necessary even in the presence of a large excess of vanadium(IV) at the beginning of the titration, but at 0·05 M acid concentration readings were steady in 1 minute and at 0·01 M acid concentration 30 s were sufficient. Similar results are obtained up to pH 4, above which interference from the precipitation and aerial oxidation of vanadium(IV) occurs. A comparison of titrations carried out in 0·5, 0·05 and 0·01 M sulphuric acid, in which absorbance readings were taken 1 minute after the addition of titrant, is shown in Fig. 2 and the beneficial effect of the lower acid concentration is clearly seen. As only three or four readings are required on each side of the end-point in order to locate the end-point precisely by extrapolation, it follows that a titration can be completed in approximately 5 minutes at 0·01 M sulphuric acid concentration. Perchloric acid behaved in a similar manner to sulphuric acid. Chromium(VI) is known to catalyse the reaction of periodate with vanadium(IV)[8] but is of no advantage in the present procedure because in the presence of vanadium(IV) it would be reduced to chromium(III), which is not effective as a catalyst. It would be useful, however, if the

TABLE III

THEORETICAL AND OBSERVED TITRATION VALUES FOR THE SPECTROPHOTOMETRIC TITRATION OF 3·50 ml OF 0·198 M VANADIUM(IV) SOLUTION WITH SODIUM PERIODATE

Titration volume 100 ml

Conditions	Theoretical titre/ml	Observed titre/ml
0·01 M H_2SO_4	1·334	1·336
0·05 M H_2SO_4	1·334	1·332
0·05 M H_2SO_4	1·334	1·336
0·05 M H_2SO_4	1·334	1·330
0·05 M H_2SO_4	1·334	1·336
0·05 M H_2SO_4	1·334	1·330
*0·5 M H_2SO_4	1·334	1·330
0·05 M H_2SO_4	1·289	1·286
0·05 M H_2SO_4	1·289	1·287
0·01 M H_2SO_4	1·334	1·335
0·1 M $HClO_4$	1·322	1·323
*1 M $HClO_4$	1·322	1·324

All absorbance readings were taken 1 minute after addition of titrant, except for those titrations marked * in which up to 30 minutes had to be allowed.

titration was conducted in the reverse direction, *i.e.*, by addition of vanadium(IV) to periodate. However, in 0·01 M sulphuric acid solution the reaction is sufficiently rapid for the addition of a catalyst to be unnecessary. The accuracy of this procedure for the determination of 3·50 ml of 0·198 M vanadium(IV) solution by using approximately 0·25 M sodium periodate solution is demonstrated by the results in Table III and was about $\pm$0·3 per cent. Accurate results are obtained at all acid concentrations but titrations at higher concentrations took a proportionately longer time to carry out. The theoretical titre was calculated from the weight of sodium periodate taken and the standardisation of the vanadium(IV) solution by potentiometric titration with cerium(IV) solution, which was itself standardised against primary standard sodium arsenite.

The determination of vanadium(IV) by spectrophotometric titration with periodate is obviously sufficiently rapid and accurate for most practical purposes. Comparison of this technique with the spectrophotometric titration involving the use of bromate or chlorate as titrant is shown in Fig. 3 and much sharper end-points are obtained with periodate. The critical factor is the dependence of the rate of the periodate reaction on the hydrogen-ion concentration. No such dependence is observed with the bromate or chlorate reactions[9,10] and the titration curves are the most satisfactory that can be obtained without either raising the temperature or considerably lengthening the time of the titration.

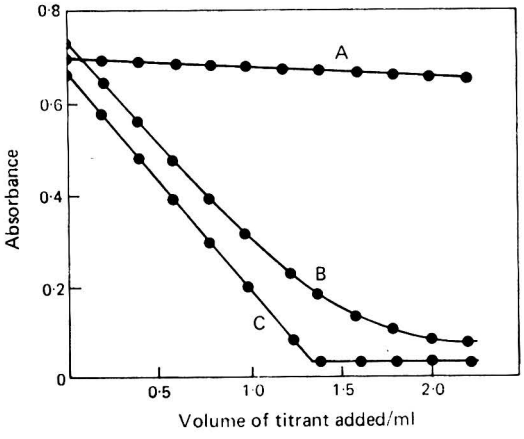

Fig. 3. Spectrophotometric titrations of 3·50 ml of 0·198 M vanadium(IV) with A, 0·1509 M sodium chlorate (in 0·2 M sulphuric acid), B, 0·0903 M potassium bromate (in 0·5 M sulphuric acid) and C, 0·2598 M sodium periodate (in 0·01 M sulphuric acid). Absorbance readings taken 1 minute after each addition of titrant

The more commonly used titrants for vanadium(IV) are cerium(IV) and manganese(VII), and both of these reagents are reported to require elevated temperatures of 60 to 80 °C for the titrations to be carried out successfully.[1,13,14,18,19] Rechnitz and Rao[15] have, however, recommended the spectrophotometric titration of vanadium(IV) with cerium(IV) in sulphuric acid medium. The second-order rate constant for this reaction is 1350 l mol^{-1} s^{-1} in 1 M sulphuric acid but no advantage from selection of acid concentration appears to be available in the cerium(IV) titration as the rate is only slightly affected by the hydrogen-ion concentration.[15] In our experience, the use of periodate is at least as satisfactory as cerium(IV) and is preferable to that of many other common oxidising agents, the advantages of the proposed procedure being the speed and accuracy of the titration coupled with the proved stability of periodate solutions.

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An Investigation of the Optimum Composition of Poly(vinyl chloride) Matrix Membranes Used for Selective Calcium-sensitive Electrodes

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Studies of the composition parameters of the poly(vinyl chloride) matrix membrane selective calcium-sensitive electrode showed that phosphate sensor and phosphonate mediator are interdependent constituents. The most practical electrodes examined were obtained from membranes containing 28·8 per cent. w/w of poly(vinyl chloride) *plus* 71·2 per cent. w/w of dioctyl phenylphosphonate and monocalcium dihydrogen tetra(didecylphosphate) in a 10 : 1 weight ratio. Membranes containing only didecylphosphoric acid sensor gave sluggish electrodes with short linear response ranges. Monocalcium di(didecylphosphate) sensor produced better electrode characteristics than didecylphosphoric acid, but the membranes showed evidence of the presence of finely divided material.

In complementary work, no practically useful electrodes were obtained with any of cellulose acetate, ethylcellulose, collodion and pyroxylin as alternative matrix materials to poly(vinyl chloride).

A previous paper[1] described the construction and performance of a selective calcium-sensitive membrane electrode based on a membrane with a liquid ion exchanger incorporated in a poly(vinyl chloride) matrix. A continuing investigation has now shown that the operation of the procedure for preparing the calcium form of the ion exchanger, namely, shaking didecylphosphoric acid *plus* dioctyl phenylphosphonate with a 1 M solution of calcium chloride and drying the product over calcium chloride, is critical and can sometimes lead to an opaque membrane that exudes a small amount of concentrated calcium chloride solution. The optical photomicrographs of such a membrane on a visible-light microscope show evidence of pores, at least some of which are open to the surface (Fig. 1). Membrane electrodes prepared from membranes with these exudations lack the characteristic behaviour previously described[1] for the calcium-selective poly(vinyl chloride) electrode. On the other hand, poly(vinyl chloride) matrix membranes prepared from the Orion 92–20–02 liquid ion exchanger were transparent with no microscopic heterogeneities and gave no exudates, while electrodes prepared from these membranes responded in the expected[1] manner.

The work described in this paper involved a study of the effect of varying the poly(vinyl chloride) membrane parameters, and the step involving shaking didecylphosphoric acid - dioctyl phenylphosphonate with calcium chloride solution for preparing the calcium sensor has been eliminated so as to avoid the occurrence of exudates.

Complementary work has also been carried out with alternative matrix materials, namely, cellulose acetate with an acetyl group content of 52·5 to 53·5 per cent. and ethylcellulose with an ethoxy group content of about 45 per cent. (both gifts from Courtaulds Ltd.), and the cellulose nitrate preparations collodion, prepared from acetone and having a total solids content of 0·077 g ml^{-1}, and pyroxylin (both obtained from British Drug Houses Ltd.). These materials gave membranes with a poor electrode quality and the details are therefore not described here. However, the behaviour of the membrane electrodes produced, including their frequent inability to distinguish between different calcium levels, suggests that hydrophilicity is undesirable in a matrix material.

Experimental

Preparation of calcium electrodes—

Preparation of materials—Didecylphosphoric acid was prepared[2] by the reaction of phosphoryl chloride with decanol, followed by alkaline hydrolysis, acidification and extraction stages. The calcium salts were prepared[2] by the reaction of an aqueous ethanolic solution of didecylphosphoric acid with calcium hydroxide.

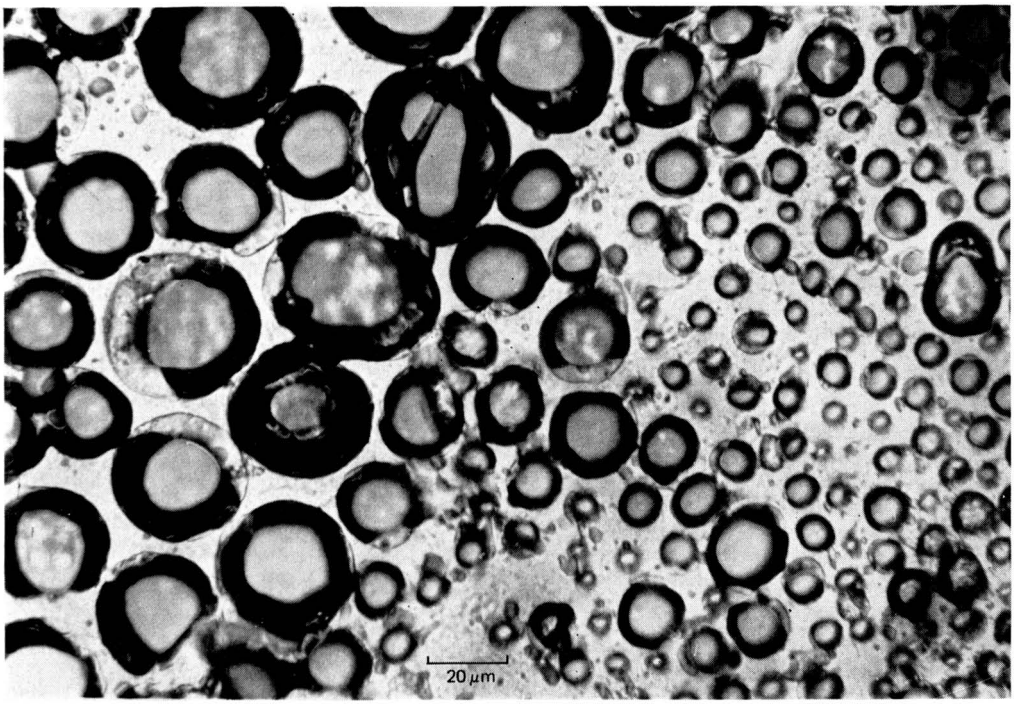


Fig. 1. Photomicrograph on visible-light microscope showing pores (dark circles) in poly(vinyl chloride) matrix membrane incorporating didecylphosphoric acid - dioctyl phenylphosphonate shaken with a molar solution of calcium chloride

[To face page 420

Dioctyl phenylphosphonate was prepared[2] from phenylphosphonic dichloride and octanol. After distillation to remove the excess of octanol, the product was purified by gel-permeation chromatography.

Membrane preparation—To obviate the type of problem sometimes caused by shaking ion-exchange materials with aqueous calcium chloride, the appropriate ion-sensitive material,[2] namely, didecylphosphoric acid (HX), monocalcium di(didecylphosphate) (CaX_2) or monocalcium dihydrogen tetra(didecylphosphate) (CaH_2X_4), was introduced with dioctyl phenylphosphonate[2] and poly(vinyl chloride) (Breon S110/10, formerly known as Breon 113; B.P. Chemicals International Ltd.), each in the proportions shown in Table I. The mixture was dissolved in 5 ml of analytical-reagent grade tetrahydrofuran, then the solution was poured into a 33 mm i.d. glass ring resting on a glass plate and the membrane (0·2 mm thick) cast as previously described.[1]

Electrode assembly—A 7 mm diameter disc of the membrane was affixed to a recessed end of a piece of 9·5 mm o.d. poly(vinyl chloride) tubing with a solution of poly(vinyl chloride) in tetrahydrofuran as adhesive. The other end of the poly(vinyl chloride) tubing was threaded on its inside surface such that the tube formed a de-mountable sensor, which was screwed on to an externally threaded piece of rigid poly(vinyl chloride) tubing attached to a glass tube provided with a ground-glass socket. The other parts of the electrode were assembled as previously described.[1]

EVALUATION OF ELECTRODE PERFORMANCE—

Preparation of calcium chloride solutions—Calcium chloride was prepared by dissolving analytical-reagent grade calcium carbonate in analytical-reagent grade concentrated hydrochloric acid (35·4 per cent. w/v) diluted 1 + 1 with water and evaporating until crystallisation occurred. The crystals were dissolved in water to give a 1 M stock solution, which was used to prepare all other solutions by serial dilution. De-ionised water distilled from alkaline potassium permanganate was used throughout.

Determination of calcium-ion response—Measurements were taken for the electrochemical cell calcium electrode | test solution || reference electrode, by using a Beckman Research Model pH meter accurate to within ±0·05 mV. The reference electrode was an Electronic Instruments Limited calomel electrode, Type RJ23, with a remote micro-scale liquid junction. The potential of this cell is a function of calcium-ion activity with a linear response over the activity range shown in Table II. The calcium-ion activities were based on those of Bates, Staples and Robinson[3] using the following form of the Debye - Hückel equation at 25 °C—

$$-\log f = \frac{0{\cdot}5115z^2\sqrt{I}}{(1 + 0{\cdot}3291d\sqrt{I})} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where f is the activity coefficient, z is the ionic charge, I is the ionic strength and d is an ionic size parameter, which for calcium chloride[3] is 0·473 nm.

Effect of foreign cations on electrode response—The above cell assembly was used for determining selectivity ratios and the comparative potential responses of the various poly(vinyl chloride) calcium-sensitive electrodes were determined in mixed calcium chloride - foreign cation chloride solutions by taking a constant background of interferent, j, and varying the calcium activity. The selectivity ratio, K_{Caj}, was then calculated in the manner previously described[1,4] from the relation

$$K_{Caj} = \frac{a_{Ca}}{(a_j)^{2/y}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

The term a_{Ca} is defined by the intercept of the horizontal interferent response line with the calcium calibration line and y is the valency of the interfering ion. Interferent activities were calculated from equation (1) by using the appropriate value[3] for d.

Time responses—Dynamic responses were determined by rapidly changing the calcium-ion activity in solutions containing calcium ions alone and in mixed solutions containing calcium and interferent cations. The responses represent the times required to achieve a steady potential (±0·2 mV) and were noted by recording the output from the pH meter on a Bryans, Model 27000, chart recorder at a chart speed of 1 cm s^{-1}. The calcium-ion activity was rapidly altered by introducing a more concentrated calcium chloride solution either with a syringe or by pouring it from a beaker.

TABLE I

COMPOSITION AND PHYSICAL CHARACTERISTICS OF POLY(VINYL CHLORIDE) MATRIX MEMBRANES

Membrane number	Phosphonate-to-salt ratio	Weight composition of membrane: Poly(vinyl chloride)/g	Dioctyl phenylphosphonate/g	Didecylphosphoric acid/g	Salt CaX_2/g	Salt CaH_2X_4/g	Physical properties of membrane
1		0·13	0·371	0·029	—	—	Transparent, colourless, soft and flabby
2		0·08	0·186	0·017	—	—	Transparent, colourless, soft and flabby
3	10 : 1	0·08	0·18	—	0·018	—	Faintly opalescent, white, soft and flabby. Amorphous, finely divided material visible under the microscope. Phosphonate: $CaX_2 = 10$
4	10 : 1	0·08	0·18*	—	0·018	—	Faintly opalescent, white, soft and flabby. Amorphous, finely divided material visible under the microscope
5	10 : 1	0·08	0·18	—	—	0·018	Transparent, colourless, soft and rubbery but with no inclusions
6	10 : 1	0·08	0·18	—	—	0·018†	Transparent, colourless, soft and rubbery but with no inclusions. Phosphonate: $CaH_2X_4 = 10$. Poly-(vinyl chloride) content = 28·8 per cent. w/w
7	3 : 1	0·08	0·15	—	0·050	—	Faintly opalescent, white, soft and rubbery. Amorphous, finely divided material visible under the microscope. Phosphonate: $CaX_2 = 3$
8	1·6 : 1	0·08	0·13	—	0·080	—	Almost opaque, white, soft and rubbery. Amorphous, finely divided material visible under the microscope. Phosphonate: $CaX_2 = 1·6$
9	21 : 1	0·08	0·189	—	—	0·009†	Transparent, colourless, soft and rubbery with no inclusions. Phosphonate: $CaH_2X_4 = 21$
10	4·5 : 1	0·08	0·162	—	—	0·036†	Transparent, colourless, soft and rubbery with no inclusions. Phosphonate: $CaH_2X_4 = 4·5$
11	10 : 1	0·06	0·198	—	—	0·0198†	Transparent, colourless, soft and rubbery but very fragile. No inclusions. Phosphonate: $CaH_2X_4 = 10$. Poly(vinyl chloride) content = 21·6 per cent. w/w
12	10 : 1	0·10	0·162	—	—	0·0162†	Transparent, colourless, soft and rubbery with no inclusions. Phosphonate: $CaH_2X_4 = 10$. Poly-(vinyl chloride) content = 35·9 per cent. w/w
13		0·08	0·20 g of Orion 92–20–02 exchanger				Transparent, soft and rubbery with no inclusions

* Dioctyl phenylphosphonate, a gift from Orion Research Inc., was used instead of the prepared material.
† Prepared by taking equivalent proportions of didecylphosphoric acid and monocalcium di(didecylphosphate).

TABLE II

RESPONSE AND SELECTIVITY DATA FOR CALCIUM-SENSITIVE POLY(VINYL CHLORIDE) MATRIX MEMBRANE ELECTRODES

Parent membrane number	Potential response in $CaCl_2$ solution (S.C.E. reference): Region of linear response: Upper activity limit/M	Lower activity limit/M	Slope per decade/mV	Average static response time*/minutes: ±0·2 mV	±0·5 mV	Selectivity ratio, $K_{Ca,j}$ (figures in brackets refer to static response times* in minutes to ±0·2 mV): j = Mg: 10^{-1} M	10^{-3} M	j = Na: 10^{0} M	10^{-1} M	10^{-2} M	j = K: 10^{0} M	10^{-1} M
1	$2·6 \times 10^{-2}$	$1·3 \times 10^{-4}$	38	51†	35	—	—	—	—	—	—	—
2	$6·0 \times 10^{-3}$	$5·5 \times 10^{-4}$	42	27	15	—	—	$3·1 \times 10^{-3}$	—	—	—	—
3	$3·6 \times 10^{-3}$	$5·5 \times 10^{-5}$	44	16 32†	14 23†	$5·4 \times 10^{-2}$ (26)	$2·0 \times 10^{-2}$ (23)	—	$2·2 \times 10^{-1}$ (15)	1·1 (17)	$4·7 \times 10^{-3}$ (20)	$6·2 \times 10^{-2}$ (27)
4	$2·6 \times 10^{-2}$	$2·4 \times 10^{-5}$	32	50	38	$2·4 \times 10^{-2}$	‡	—	$5·9 \times 10^{-1}$	4·8	$2·1 \times 10^{-2}$	$1·5 \times 10^{-1}$
5	$2·6 \times 10^{-2}$	$2·8 \times 10^{-4}$	31	7	6	$2·4 \times 10^{-2}$	‡	1×10^{-3}	—	—	—	—
6	$2·6 \times 10^{-2}$	$6·0 \times 10^{-5}$	30	11 19†	3 12†	$2·4 \times 10^{-2}$ (23)	‡	$5·0 \times 10^{-4}$ (18)	$2·8 \times 10^{-3}$	‡	$1·6 \times 10^{-4}$	‡
7	$5·0 \times 10^{-3}$	$8·0 \times 10^{-5}$	48	23	10	$4·7 \times 10^{-2}$ (27)	$1·6 \times 10^{-2}$ (13)	—	$5·2 \times 10^{-1}$ (24)	$5·6 \times 10^{-1}$ (35)	$2·6 \times 10^{-2}$ (23)	$1·7 \times 10^{-1}$ (23)
8	$1·2 \times 10^{-2}$	$4·0 \times 10^{-4}$	31	38 40†	27 32†	$2·1 \times 10^{-2}$ (32)	‡ (37)	—	$4·2 \times 10^{-2}$ (39)	$5·9 \times 10^{-1}$ (46)	$6·2 \times 10^{-4}$ (40)	$7·4 \times 10^{-3}$ (27)
9	$5·5 \times 10^{-3}$	$7·0 \times 10^{-5}$	41	24 20†	12 17†	$3·6 \times 10^{-3}$ (52)	‡	$1·2 \times 10^{-3}$ (10)	—	—	—	—
10	$2·6 \times 10^{-2}$	$1·0 \times 10^{-4}$	31	20 16†	13 6†	$7·4 \times 10^{-2}$ (7)	‡	$6·6 \times 10^{-4}$ (8)	‡	‡	—	—
11	$6·0 \times 10^{-3}$	$1·0 \times 10^{-5}$	36	20 7†	11 5†	$2·9 \times 10^{-2}$ (21)	‡	$1·8 \times 10^{-3}$ (5)	—	—	—	—
12	$2·6 \times 10^{-2}$	$3·0 \times 10^{-4}$	32	9 16†	4 4†	$8·9 \times 10^{-2}$ (26)	‡	$1·5 \times 10^{-4}$ (55)	‡	‡	—	—
13	3×10^{-2}	$3·5 \times 10^{-5}$	31	16	9	$1·4 \times 10^{-2}$	—	$2·7 \times 10^{-4}$	—	—	$6·2 \times 10^{-4}$	—

* Time to attain steady potential (to within ±0·2 mV unless otherwise stated) after transferring the electrode to a solution ten times more dilute and averaged over the range of 10^{-1} to 10^{-6} M solutions.

† Time to attain steady potential (to within ±0·2 mV unless otherwise stated) after transferring the electrode to a solution ten times more concentrated and averaged over the range of 10^{-1} to 10^{-6} M solutions.

‡ No inteference, response curve characteristic of solutions containing only calcium.

Static time responses were taken as the times required for the cell incorporating the membrane electrode to attain a steady potential after transferring the electrode to a solution having a ten-fold difference in concentration. The electrode was wiped with paper tissue after removing it from the initial solution and before introducing it into the other solution. The times recorded represent the beginning of the 10-minute period during which the potential was constant to within $\pm 0{\cdot}2$ mV. In addition, the beginning of the 10-minute period during which the potential varies by not more than 0·5 mV was noted.

Results and discussion

Determination of optimum membrane composition—

Table I summarises the composition and physical character of various membranes, and Table II summarises the results for the potential response times and selectivities of the corresponding calcium electrodes prepared from the membranes. Results for membranes from which either phosphonate mediator or sensor phosphate was absent are not included because in no instance did these give functional electrodes, that is, dioctyl phenylphosphonate alone in a membrane did not give an electrode with a stable potential response when examined in calcium chloride solutions while phosphate alone gave opaque membranes with solid inclusions whose electrodes were erratic in response.

Effect of sensor on the quality of calcium membrane electrodes—The results for membranes 1 to 6 show the effect of different sensing constituents in phosphonate-mediated poly(vinyl chloride), namely, didecylphosphoric acid (HX) and its salts, CaX_2 and CaH_2X_4. A notable feature of the preparation of the membrane and its physical character was the ease with which didecylphosphoric acid dissolved in the membrane composition to give transparent membranes (1 and 2) compared with the existence of finely divided material in those membranes (3 and 4) prepared from the salt, CaX_2. The acid salt, CaH_2X_4, even though it is not readily soluble in the membrane composition, gave a clear, rippled membrane (5), while the membrane composition incorporating the acid salt, CaH_2X_4, prepared *in situ* by taking equimolar proportions of didecylphosphoric acid and the salt CaX_2, readily took up the components to produce a clear membrane (6) of regular thickness.

With regard to response, electrodes from membranes 1 and 2 containing didecylphosphoric acid were relatively sluggish and gave short linear response regions and hyper-Nernstian slopes in calcium chloride solution. These electrodes were classified as being inferior in performance, as were electrodes from membranes 3 and 4 containing the salt CaX_2, although in this instance the linear response region was more extensive. Electrodes from membranes 5 and 6 containing the acid salt, CaH_2X_4, showed a performance comparable with that of the poly(vinyl chloride) matrix membrane calcium-sensitive electrode previously described[1] and also with that of the poly(vinyl chloride) electrode 13 prepared in this investigation whose membrane incorporated Orion 92–20–02 liquid ion exchanger. In this respect, electrodes from membrane 6 possessed a long near-Nernstian range and superior calcium selectivity in the presence of sodium and potassium ions compared with electrodes from membranes with CaX_2 sensor. The electrodes showed characteristic responses[1] to calcium with variation in pH, while those based on CaX_2 membranes showed an abrupt break at pH 5 with an almost Nernstian response to hydrogen ions below pH 3 (Fig. 2).

Even though static response times for the electrode of membrane 6 were of the order of minutes, the dynamic response time on changing from 5×10^{-3} to $2{\cdot}7 \times 10^{-2}$ M calcium chloride solutions was 7 s.

Effect of phosphonate-to-calcium salt (CaX_2) ratio on electrode quality—Despite the fact that membranes 3 and 4 with CaX_2 gave electrodes that were not as efficient as those prepared from membranes with the acid salt, CaH_2X_4, additional membranes (7 and 8) were made containing CaX_2 in an increased proportion. The electrodes (from membranes 7 and 8) gave linear responses (Table II) over a restricted range of calcium levels, although apart from this result and sluggish response, membrane 8 gave electrodes with certain redeeming features, such as near-Nernstian slope and good selectivity for calcium ions over magnesium, sodium and potassium ions.

The electrode from membrane 3 generally showed superior time-response behaviour, both statically (Table II) and dynamically. In this latter respect, the electrode gave a dynamic response of 7 s on changing from 5×10^{-3} to $6{\cdot}9 \times 10^{-2}$ M calcium chloride solution,

both in the absence of other salts and in the presence of 10^{-1} M magnesium chloride solution; the corresponding time for the electrode from membrane 8 was 1 minute.

Effect of phosphonate-to-acid salt (CaH_2X_4) ratio on electrode quality—Membrane 6 clearly possesses the most suitable calcium electrode properties. This was confirmed by comparing its complementary electrode with electrodes prepared from membranes containing both a lower proportion (membrane 9) and a higher proportion (membrane 10) of the acid salt (Table II).

Electrodes from membranes 6 and 9 gave similar dynamic responses and responded fully in 6 to 8 s to changes such as those from 5×10^{-4} to $6{\cdot}6 \times 10^{-3}$ M and from $6{\cdot}6 \times 10^{-3}$ to $4{\cdot}7 \times 10^{-2}$ M calcium chloride solutions in the absence of other salts. In the presence of 10^{-1} M magnesium chloride solution, the electrode from membrane 6 responds fully to changes in calcium levels in 10 to 12 s, while that from membrane 9 takes as long as 50 minutes to settle down. The electrode from membrane 10, with the lowest phosphonate-to-acid salt ratio, required more than 10 minutes to respond to dynamic changes in calcium level in the absence of other salts and 40 minutes in the presence of 10^{-1} M magnesium chloride solution.

Effect on electrode behaviour of varying the poly(vinyl chloride) contents of membranes—As membrane 6, with a phosphonate-to-acid salt ratio of 10 : 1, gave electrodes with the best practical characteristics, this ratio was retained for determining the optimum membrane poly(vinyl chloride) content. Membrane 11 represents the smallest amount of poly(vinyl chloride) that can be incorporated, consistent with satisfactory mechanical character, although the membrane bulged considerably if the head of liquid exceeded 2 cm.

Of the three membranes (6, 11 and 12) examined, the electrode from membrane 6 gave the greatest extent of near-Nernstian response (Table II). The electrodes gave comparable response times except that the one from membrane 12, containing the most poly(vinyl chloride), was frequently the most sluggish; for example, it took 12 s and 35 s to give a stable dynamic response on changing the calcium level from 5×10^{-4} to $6{\cdot}6 \times 10^{-3}$ M and from $6{\cdot}6 \times 10^{-3}$ to $4{\cdot}7 \times 10^{-2}$ M, respectively.

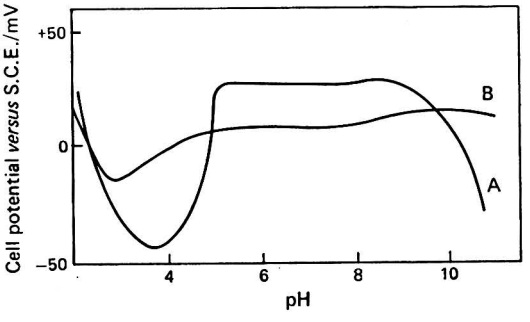
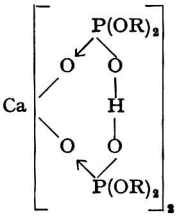


Fig. 2. The effect of pH on the response to 10^{-2} M calcium chloride solution of electrodes prepared from membranes containing CaX_2 (curve A, membrane 3) and CaH_2X_4 (curve B, membrane 6)

DISCUSSION ON ELECTRODE WITH OPTIMUM MEMBRANE COMPOSITION—

Of the various membrane electrodes examined, that prepared from the membrane containing 28·8 per cent. w/w of poly(vinyl chloride) and 71·2 per cent. w/w of dioctyl phenylphosphonate and monocalcium dihydrogen tetra(didecylphosphate) in the weight ratio of 10: 1 is the most suitable in practice. Apart from giving a near-Nernstian response (30 mV per pCa unit) when incorporated in the cell calcium electrode | calcium chloride test solution || reference electrode, it gave near-theoretical behaviour in the cell calcium electrode | calcium chloride test solution | silver(I) chloride (solid) - silver, when the response change per ten-fold change in mean calcium chloride activity was 88·2 mV over the range from 3×10^{-2} to 10^{-4} M with 84·6 mV over the extended range from 10^{-1} to 5×10^{-5} M. These values compare with the value 88·7 mV obtained by using the term $3 \times 2{\cdot}303\ RT/2F$ at 25 °C.

That this electrode should be superior to those prepared from membranes containing monocalcium di(didecylphosphate) is interesting and suggests that the availability of ion-exchange sites in the liquid ion exchanger might be a prerequisite for the most satisfactory electrodes. The availability of such sites can provide the means for specificity by mobility differences of counter-ions as for solids and not so much by the selectivity arising from equilibrium ion-exchange suggested by Eisenman[5] for liquid-membrane electrodes. However, the availability of ion-exchange sites cannot be regarded as being categorical, as the hydrogen atoms that would make this possible might themselves be involved in a "hydrogen-bonded" ring structure as has been proposed[6] for extraction complexes that involve dialkylphosphoric acids—



In this respect, it is interesting that CaH_2X_4 shows evidence of P–O–H hydrogen-bonded infrared absorption at 1630 cm^{-1} (medium, broad) in a potassium bromide disc and at 1610 cm^{-1} (weak, broad) in a Nujol mull. However, there is no indication of the more characteristic P–O–H hydrogen-bonded infrared absorption at about 2300 to 2200 cm^{-1}, while that at 2650 cm^{-1} (in a Nujol mull only) is a very weak, broad band.

Regardless of its operating mechanism, the electrode is highly functional and from the practical standpoint is selectively responsive to calcium ions over ions such as magnesium, sodium and potassium with which calcium ions are frequently found in admixture (Table III).

Even though pH does not affect the electrode behaviour over a wide range, the electrode should not be exposed for unduly long periods to solutions with pH less than about 3·5. This follows from experience with an electrode prepared from a membrane (6) of the optimum composition, which was immersed for 20 hours in 10^{-2} M calcium chloride solution adjusted to pH 2·4 with hydrochloric acid. After immersion, the electrode had the same extensive linear calibration graph, but the original near-Nernstian slope had altered to 48 mV per decade and was reduced only to 40 mV per decade despite soaking the electrode for 4 days in 10^{-1} M calcium chloride solution. The selectivity ratio, $K_{Ca,Na}$, was also affected and the value of 5×10^{-4} in 1 M sodium chloride solution before immersion increased to $3{\cdot}7 \times 10^{-3}$ after immersion. The change that occurs in such acidic conditions suggests that calcium is leached from the ion exchanger,[7] thus producing an irreversible change in the nature of the ion-exchange system. Indeed, the slope of the calibration graph for the electrode after such treatment is more characteristic of electrodes that have didecylphosphoric acid sensor membranes (Tables I and II). It is reassuring to note that control electrodes similarly immersed in 10^{-2} M calcium chloride solution at pH 3·6 and 5·6 showed no change in character.

TABLE III

SELECTIVITY RATIOS, K_{Caj}, DETERMINED BY INTERCEPT METHOD AT 25 °C IN SOLUTIONS CONTAINING CALCIUM AND INTERFERENT

Interfering ion, j	Concentration of interfering ion		
	10^{0} M	10^{-1} M	10^{-2} M
Li^+	—	$2{\cdot}0 \times 10^{-1}$	1·2
Na^+	$5{\cdot}0 \times 10^{-4}$	$2{\cdot}8 \times 10^{-3}$	*
K^+	$1{\cdot}6 \times 10^{-4}$	*	—
Rb^+	$2{\cdot}3 \times 10^{-4}$	*	—
Cs^+	$8{\cdot}8 \times 10^{-4}$	*	—
NH_4^+	$1{\cdot}1 \times 10^{-2}$	$1{\cdot}3 \times 10^{-2}$	—
Mg^{2+}	—	$2{\cdot}4 \times 10^{-2}$	*

* No interference, response curve characteristic of solution containing only calcium.

As with poly(vinyl chloride) - nitrate electrodes,[8] the calcium electrode with a membrane of optimum composition (6) is stable to gamma-radiation and possesses essentially the same calibration slope, activity range and response times after exposure to a total dose of cobalt-60 gamma-radiation of $6{\cdot}5 \times 10^2$ rad over 16 hours.

Conclusion

Phosphate sensor and phosphonate mediator are interdependent constituents of selective calcium-sensitive poly(vinyl chloride) matrix membrane electrodes, with monocalcium dihydrogen tetra(didecylphosphate) being mandatory in an appropriate amount for the best electrodes.

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A Gas-chromatographic Method for the Determination of Increased Bromide Concentrations in Blood

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A method is described for the determination of bromide in blood by gas chromatography. The bromide, in a protein-free filtrate, is oxidised to bromine by potassium permanganate in acidic solution and extracted into cyclohexane containing cyclohexene to give 1,2-dibromocyclohexane, which is determined by gas chromatography using 1,6-dibromohexane as an internal standard. About 70 per cent. of the bromide present is converted into 1,2-dibromocyclohexane; chlorine, from chloride, reacts to give 1,2-dichlorocyclohexane. Oxalate, fluoride or heparin, which may be present as a preservative or an anti-coagulant, do not interfere and 1,2-dibromocyclohexane is not obtained from carbromal or Bromvaletone under the conditions described. The method is applicable in the range from 10 to 100 mg of bromide per 100 ml of blood (0·1 to 1·0 mg ml^{-1}) and to higher levels after dilution of the sample.

Sedatives of the bromoureide type such as carbromal [*N*-(1-bromo-1-ethylbutyryl)urea] and Bromvaletone [*N*-(2-bromo-3-methylbutyryl)urea] are almost completely metabolised in the body[1] and evidence for their ingestion is usually found in an increased level of bromide in the blood. Treatment with pharmaceutical products containing potassium bromide or exposure to methyl bromide vapour may also result in a raised blood bromide level. The determination of the concentration of bromide in blood is therefore important in both toxicological and clinical investigations.

The total bromine in blood can be determined by ashing the blood sample, usually with alkali, and determining the bromine in the ash by titrimetric[2] or colorimetric[3] methods. These are lengthy procedures, particularly when large numbers of samples are to be analysed, and the amount of bromine lost on ashing appears to be variable.[3,4] Alternatively, bromine in blood can be determined directly by physical methods such as neutron-activation analysis[5] or X-ray fluorescence spectrometry.[6,7] Ion-selective electrodes have been used for the direct determination of bromide and iodide in serum[8]; blood samples, however, require the removal of protein, and the consequent dilution of the sample reduces the bromide-ion concentration to a level at which, in the presence of chloride, the electrode response is no longer linear,[8,9] the electrode becoming relatively insensitive to changes in bromide-ion concentration.

Colorimetric methods for the determination of bromide in blood require a protein-free solution, which is usually obtained by precipitating the protein with trichloroacetic acid. The bromide concentration in the filtrate is then determined from the colour intensity of the yellow - orange complex formed between the bromide ion and chloroauric acid. Measurements can be made in the visible light region[10] or, with increased sensitivity, in the ultraviolet region.[11] The procedure is simple to carry out and has been widely used,[12,13] although the method lacks specificity.[8,13] However, it cannot be used for all *post-mortem* blood samples because of the formation of a yellow - orange precipitate, which leaves an almost colourless liquid layer.

In 1960 Street[14] described an alternative colorimetric procedure in which the bromide, in a protein-free filtrate, was oxidised to bromine, extracted into cyclohexane and determined colorimetrically. When this method was used the moist cyclohexane solution of bromine was unstable and considerable fading occurred, particularly in daylight; no improvement in stability was found when the reaction was carried out in subdued light and the solutions were kept in the dark before measurement. Street recommended an ether extraction of the protein-free filtrate before oxidation and extraction with cyclohexane and he surmised that this ether extraction removed unsaturated compounds that would otherwise react with the

extracted bromine. This suggested the deliberate addition, to the cyclohexane, of a suitable alkene that would react with the bromine to form a volatile bromo-compound suitable for determination by gas chromatography. The technique was made quantitative by the inclusion of a suitable internal reference material, and further investigation led to the method described below.

The detection of bromide and other halides by gas chromatography has been reported by Macgee and Allen[15]; the halides were converted into the tetraalkylammonium salts by ion exchange and decomposed by injection at 360 °C to give the corresponding alkyl halides and trialkylamine. The method as described is relatively insensitive, requiring a 0·25 M concentration of halide.

Method

Apparatus—

A Hewlett-Packard 7620A gas chromatograph was used, equipped with a flame-ionisation detector. The stainless-steel column, of length 2·0 m and internal diameter 2 mm, was packed with 2·5 per cent. w/w OV-17* on Gas Chrom Q, 80 to 100 mesh (both from Applied Science Laboratories Inc., P.O. Box 440, State College, Pennsylvania, U.S.A.). Nitrogen was used as the carrier gas with a flow-rate of 20 ml min^{-1}. The temperature of the injection block and the flame-ionisation detector block was 200 °C and the oven temperature was programmed from 70 to 170 °C at 10 °C min^{-1}. The output from the flame-ionisation detector was connected to a 1-mV recorder. The amplifier attenuation was normally set at 8×10^2 to give peaks of adequate size. The following typical retention times were found: 1,2-dichlorocyclohexane, 3·5 minutes; 1,2-dibromocyclohexane, 4·75 minutes; and 1,6-dibromohexane, 6·8 minutes.

Ground-glass stoppered test-tubes, B14, with a capacity of 15 ml, were used (Quickfit, Catalogue No. MF24/1/5).

Reagents—

All reagents were of analytical-reagent grade (Univar, Ajax Chemicals Ltd., Auburn, New South Wales 2141) except where stated. 1,2-Dibromohexane[16] and 1,2-dibromocyclohexane[17] were prepared by published procedures.

Trichloroacetic acid solution, 20 per cent. w/v.

Sulphuric acid, approximately 5 N—Dilute 35 ml of concentrated sulphuric acid to 250 ml with water.

Potassium permanganate.

Sodium sulphite, anhydrous.

Cyclohexene reagent—Dissolve 70 mg of 1,6-dibromohexane (pure grade, Catalogue No. 3073, Koch-Light Laboratories Ltd., Colnbrook, Bucks.), and 2 ml of cyclohexene (laboratory-reagent grade) in cyclohexane (spectroscopic grade) and dilute the solution to 100 ml with cyclohexane.

Standard bromide solutions—Dissolve 1·489 g of potassium bromide in water and dilute the solution with water to 100 ml. Transfer 2·5, 5·0, 7·5 and 10·0 ml of this solution to a series of 100-ml calibrated flasks, each flask containing 0·5 g of sodium chloride, and dilute each solution with water to the mark to give solutions containing 25, 50, 75 and 100 mg of bromide per 100 ml, respectively, in 0·5 per cent. w/v sodium chloride solution.

Procedure—

Calibration—Add 10 ml of trichloroacetic acid solution to a mixture of 2 ml of a standard bromide solution and 2 ml of water, mix, and transfer 7 ml of this solution to a stoppered test-tube. Add 2 ml of 5 N sulphuric acid, 50 to 60 mg of potassium permanganate and 2 ml of cyclohexene reagent. Stopper the tube and shake it vigorously for 30 s, then add 100 to 120 mg of sodium sulphite to the reaction mixture and mix gently until the contents are decolorised. Allow the layers to separate and take 2-μl aliquots of the upper layer for gas chromatography. Measure the peak heights and calculate the ratio of peak heights for 1,2-dibromocyclohexane and 1,6-dibromohexane. Repeat the procedure for the remaining standard bromide solutions and plot a graph of peak height ratios against the concentrations of bromide.

* An alternative column material to OV-17 is FS1265 (QF-1), a fluorosilicone.

Samples—Add 10 ml of trichloroacetic acid solution to a mixture of 2 ml of a blood sample and 2 ml of water and mix well. Filter the mixture through a Whatman No. 541 (or equivalent) filter-paper, transfer 7 ml of the filtrate to a stoppered test-tube and follow the procedure described above under *Calibration*. Convert the peak height ratio found into bromide concentration in milligrams per 100 ml by using the calibration graph. If the concentration of bromide found is greater than 100 mg per 100 ml, repeat the determination but with 1 ml of blood, 3 ml of water and 10 ml of trichloroacetic acid solution.

Discussion and results

The choice of alkene was governed by a number of factors: it must not be too volatile, so as to avoid losses when operating at room temperature, and it must react rapidly with the low concentrations of bromine involved to form only one derivative. Initial experiments were carried out with hex-1-ene (boiling-point 64 °C). Potassium permanganate in dilute sulphuric acid solution was used as the oxidant and the bromine was extracted into cyclohexane as recommended by Street[14]; blood proteins were precipitated with trichloroacetic acid, which did not interfere with the subsequent gas chromatography. Precipitated manganese compounds and excess of permanganate ions were removed by the addition of sodium sulphite, which addition also resulted in better separation of the cyclohexane layer. The expected reaction product was 1,2-dibromohexane (boiling-point 210 °C) and the isomeric 1,6-dibromohexane (boiling-point 240 °C) was chosen as the internal standard. In the presence of bromide appreciable amounts of two other compounds were formed in addition to 1,2-dibromohexane and the yield of these compounds increased with increasing bromide concentration to give a non-linear calibration graph.

Table I

Yield of 1,2-dibromocyclohexane from various bromide concentrations

Bromide ion* concentration/mg per 100 ml	Peak height ratio† (found)	Mean	Peak height ratio† (calculated for 100 mg of bromide per 100 ml)
25	0·181, 0·175, 0·191	0·182	0·728
50	0·354, 0·360, 0·362	0·358	0·716
75	0·558, 0·542, 0·539	0·546	0·729
100	0·731, 0·736, 0·751	0·739	0·739
		Mean	0·728

* Added as potassium bromide.
† Relative to 1,6-dibromohexane.

Further experiments were carried out with cyclohexene (boiling-point 83 °C), which is reported[18] to give only *trans*-1,2-dibromocyclohexane (boiling-point 220 °C) on addition of bromine. 1,6-Dibromohexane and 1,2-dibromocyclohexane were adequately resolved by using the conditions described under Method and the former compound was retained as an internal standard. The acidic permanganate solution, when treated with cyclohexene in cyclohexane, did not give any peaks with retention times similar to those of 1,2-dibromocyclohexane and 1,6-dibromohexane. In the presence of bromide the major peak produced corresponded to 1,2-dibromocyclohexane and was accompanied by negligible amounts of two other compounds, which appeared between the major peak and the internal standard. The yield of 1,2-dibromocyclohexane from aqueous potassium bromide solutions was about 70 per cent., estimated from the relative peak heights of known amounts of 1,2-dibromocyclohexane and 1,6-dibromohexane, and was reasonably constant for varying bromide concentrations (Table I). The use of solid potassium permanganate gave consistently higher yields of 1,2-dibromocyclohexane, calculated from peak height ratios, than did the use of an equal amount of potassium permanganate added in solution. In the presence of sodium chloride solution (5 mg ml^{-1}) an additional peak appeared just before the 1,2-dibromocyclohexane peak; this was attributed to *trans*-1,2-dichlorocyclohexane.[19] Standard bromide solutions were prepared, containing sodium chloride at the level normally found in blood, in addition to potassium bromide.

The method was applied to five blood samples that were known to contain less than 1 mg of total bromine per 100 ml, as determined by X-ray fluorescence spectrometry. In each instance the chromatogram consisted of a peak from the internal standard, together with a peak corresponding to 1,2-dichlorocyclohexane and a negligible peak (less than 1 mm in height) corresponding to 1,2-dibromocyclohexane. Recovery experiments were carried out with blood samples to which had been added various amounts of potassium bromide; the results are shown in Table II. An over-all recovery of 100·8 per cent. was obtained in the range from 10 to 100 mg of bromide per 100 ml. The presence of potassium oxalate (0·25 per cent. w/v), sodium fluoride (1·0 per cent. w/v) or heparin (0·02 per cent. w/v) did not affect the recovery of bromide added to blood at the 100 mg per 100 ml level.

TABLE II

RECOVERY OF BROMIDE ADDED TO BLOOD*

Bromide concentration/mg per 100 ml			Number of determinations	Mean recovery, per cent.
Added†	Found	Mean		
0	Not detected	—	5	—
10	9·2 to 11·5	10·8	6	108
25	23·0 to 26·7	24·2	6	96·8
50	47·3 to 51·4	49·6	6	99·2
75	72·3 to 75·9	73·9	6	98·5
100	97·8 to 104·3	101·6	12	101·6
			Mean	100·8

* Containing less than 1 mg of total bromine per 100ml.
† Added as potassium bromide.

Further blood samples, from cases of overdosage with bromoureides, which were expected to contain increased concentrations of bromide, were also examined. The same samples were also analysed to determine total bromine content by X-ray fluorescence spectrometry.[20] The results are shown in Table III. The differences found between total bromine and bromide-ion concentration in the presence of bromoureide suggested that organically combined bromine was not oxidised to free bromine under the conditions used. Saturated aqueous solutions of carbromal and Bromvaletone were prepared and 4-ml aliquots of each treated as described under Procedure omitting the addition of 2 ml of water. Gas chromatography of aliquots from each cyclohexane extract showed negligible 1,2-dibromocyclohexane (peak height less than 1 mm), although each 4-ml aliquot was equivalent to 2 ml of blood containing 20 and 140 mg of bromide, respectively, per 100 ml. Under the conditions used, only bromide ions are oxidised and converted into 1,2-dibromocyclohexane.

TABLE III

BROMIDE CONCENTRATIONS FOUND IN *post-mortem* BLOOD BY GAS CHROMATOGRAPHY COMPARED WITH THE TOTAL BROMINE CONCENTRATION FOUND BY X-RAY FLUORESCENCE SPECTROMETRY

Sample number	Bromide concentration found by gas - liquid chromatographic method		Total bromine concentration found by X-ray method/ mg per 100 ml
	Replicate determinations/ mg per 100 ml	Mean/ mg per 100 ml	
1	84·2, 89·3, 85·2	86·2	84
2	58·7, 58·3, 59·5	58·8	61
3	35·5, 33·1, 34·4	34·3	34
4	12·0, 12·9, 12·6	12·5	16*
5	75·3, 73·4, 70·2	72·9	74†
6	72·1, 72·5, 73·5	72·7	71
7	7·7, 7·3, 8·5	7·8	10‡
8	10·0, 9·7, 9·0	9·6	11§

* Carbromal detected by thin-layer chromatography.
† Sample contained 0·42 per cent. w/v of ethanol found by gas - liquid chromatography.
‡ Carbromal and Bromvaletone detected by thin-layer chromatography.
§ Decomposed sample.

Conclusion

A simple, specific method for the determination of increased concentrations of bromide in blood by gas chromatography has been developed. Chloride, at the normal level found in blood, does not interfere, and combined bromine, as in carbromal and Bromvaletone, gives a negligible response.

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Determination of Benzoic Acid in Meat and Meat Products by Gas Chromatography

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A gas-chromatographic method for the determination of benzoic acid in meat and meat products is described with particular reference to the open-pack type of minced meat sold for domestic pets. The sample is steam distilled, the acidified distillate extracted with diethyl ether, the extract evaporated to dryness and the residue dissolved in methanol and subjected to gas chromatography on 3 per cent. SE-30 on 80 to 100-mesh Chromosorb W at 170 °C with dimethyl phthalate as the internal standard. The method gives satisfactory results in the range 0·1 to 0·5 per cent. of benzoic acid in the meat.

BENZOIC acid has low toxicity and is widely used as a preservative in certain foodstuffs. Recent work by Bedford and Clarke,[1,2] arising from reports in the veterinary literature of an unusual syndrome in cats,[3,4] has shown that benzoic acid is particularly poisonous to this species. A 0·5 per cent. concentration of benzoic acid in the diet may be lethal, although 0·2 per cent. can be fed indefinitely without ill effects. As the source of the benzoic acid in the instances reported proved to be minced meat sold by pet shops, the need arose for a rapid and simple method for the determination of benzoic acid in this material.

Classically, benzoic acid has been determined by steam distillation and ether extraction[5] followed by a colorimetric method.[6,7] More recently, use has been made of ultraviolet spectrophotometry,[8] which may be preceded by paper[9] or thin-layer chromatography,[10,11] and of gas - liquid chromatography.[12–14] None of the published methods, however, seemed suitable for our purpose; they were either not applicable to meat products or were too time consuming. Ultraviolet spectrophotometry, although sometimes successful, was often unsatisfactory owing to the presence of a volatile dye in the meat, while it proved difficult to elute benzoic acid quantitatively from spots on paper or thin-layer chromatograms. We found that the best results could be obtained by the use of the established steam-distillation and ether-extraction technique followed by gas - liquid chromatography.

METHOD

APPARATUS—

Distillation and extraction—The apparatus was constructed from standard Quickfit components.

Gas - liquid chromatography—The instrument used was a Varian Aerograph 1400, with a flame-ionisation detector. The column consisted of a 5-foot length of 3 mm internal diameter stainless-steel tubing, packed with 3 per cent. SE-30 on 80 to 100-mesh Chromosorb W. The operating conditions were: column temperature, 170 °C; detector and oven temperatures, 220 °C; and carrier gas (nitrogen) flow-rate, 15 ml min^{-1}.

REAGENTS—

The reagents used were of general-purpose reagent grade unless otherwise stated.

Dimethyl phthalate.

Diethyl ether.

Methanol—Analytical-reagent grade.

Orthophosphoric acid, sp. gr. 1·75.

Sodium chloride.

Sodium hydroxide solution, 1 M—Analytical-reagent grade.

Sulphuric acid, 2 M—Analytical-reagent grade.

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DISTILLATION—

Into a 200 × 29-mm test-tube are introduced a sample of meat (2 g), sodium chloride (4 g), water (10 ml) and orthophosphoric acid (2 ml). The test-tube is connected to a condenser by means of a distillation head carrying an inlet tube, which is closed by a small rubber bung at the commencement of the distillation. The contents of the test-tube are heated until the sodium chloride begins to crystallise out, when the rubber bung is removed and the steam supply connected. The distillate passes from the condenser through a long bent glass tube, which dips into sodium hydroxide solution (5 ml) in a 50-ml measuring cylinder. Distillation is continued until 45 ml of distillate have been collected. It is essential that the volume of liquid in the distillation tube does not become large enough to re-dissolve the sodium chloride.

EXTRACTION AND CONCENTRATION—

The distillate is acidified with 2 M sulphuric acid (10 ml) and extracted with three 25-ml volumes of diethyl ether. The combined ethereal extracts are passed through a 100 × 20-mm column of anhydrous sodium sulphate into a 250 × 40-mm test-tube with the aid of a slight vacuum. The column is washed with diethyl ether (25 ml) and the combined extracts and washings are evaporated to dryness under reduced pressure, the test-tube being immersed in a water-bath maintained at 40 °C so as to prevent condensation of water in the tube.

GAS - LIQUID CHROMATOGRAPHY—

The residue left after evaporation of the diethyl ether is dissolved in 2 ml of a 0·1 per cent. w/v methanolic solution of dimethyl phthalate and 1 μl of this solution is injected into the chromatograph. The concentration of benzoic acid in the sample is determined from the ratio of the height of the benzoic acid peak to that of the dimethyl phthalate peak by using the calibration graph prepared as described below.

PREPARATION OF CALIBRATION GRAPH—

Standard solutions containing 0·05, 0·1, 0·15, 0·2, 0·3, 0·4 and 0·5 per cent. w/v of benzoic acid in a 0·1 per cent. w/v methanolic solution of dimethyl phthalate are prepared. Aliquots (1 μl) of these standard solutions are subjected to gas chromatography, and a graph is drawn of the ratio of the peak height of benzoic acid to the peak height of dimethyl phthalate against the concentration of benzoic acid. The ratio of the peak heights in the sample is referred to the graph, which gives the percentage concentration of benzoic acid in the sample directly.

RESULTS

ASSESSMENT OF THE METHOD—

Volumes of 0·2, 0·4, 0·6, 0·8 and 1·0 ml of an aqueous solution of sodium benzoate, equivalent to a 1 per cent. solution of benzoic acid, were added to a series of 2-g aliquots of minced beef. The solutions represented concentrations of benzoic acid of 0·1, 0·2, 0·3, 0·4 and 0·5 per cent., respectively. A further 2-g aliquot, to which no benzoic acid had been added, served as a control. The benzoic acid was determined by the method described above, two determinations being carried out in each instance. The results are shown in Table I.

TABLE I

RECOVERY OF BENZOIC ACID ADDED TO MINCED BEEF

Sample	Benzoic acid added/mg	Recovery	
		mg	per cent.
1	2·0	2·2, 2·0	110, 100
2	4·0	3·6, 3·3	90, 83
3	6·0	4·9, 4·3	82, 72
4	8·0	7·4, 7·6	93, 95
5	10·0	8·7, 9·2	87, 92

OTHER PRESERVATIVES—

The same method was applied to minced beef to which various other preservatives had been added. Nicotinic acid was found to be non-volatile under the conditions used in the

TABLE II

RETENTION TIMES RELATIVE TO DIMETHYL PHTHALATE AT 170 °C

Compound	Retention time
Sorbic acid	0·41
Benzoic acid	0·50
o- and *p*-chlorobenzoic acid	0·78
Dimethyl phthalate	1·00
Methyl *p*-hydroxybenzoate	1·00
Ethyl *p*-hydroxybenzoate	1·25
Propyl *p*-hydroxybenzoate	1·76

experiment. Sorbic acid, *o*- and *p*-chlorobenzoic acids and the methyl, ethyl and propyl esters of *p*-hydroxybenzoic acid were distilled, extracted and subjected to gas chromatography. Their retention times relative to dimethyl phthalate are shown in Table II, and a gas chromatogram of a combined sample is shown in Fig. 1. It can be seen that at 170 °C methyl *p*-hydroxybenzoate has the same retention time as dimethyl phthalate. If, however, the column is run at 140 °C, or programmed from 120 to 160 °C, the compounds are clearly separated (Fig. 2).

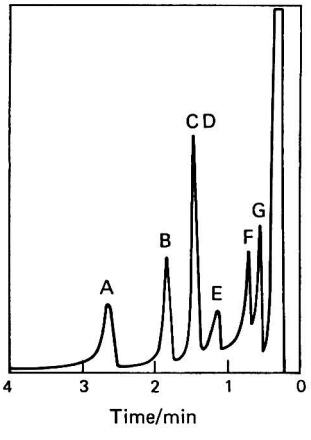

Fig. 1. Chromatogram of various preservatives run with column at 170 °C. A, propyl *p*-hydroxybenzoate; B, ethyl *p*-hydroxybenzoate; C, dimethyl phthalate; D, methyl *p*-hydroxybenzoate; E, *o*- and *p*-chlorobenzoic acid; F, benzoic acid; and G, sorbic acid

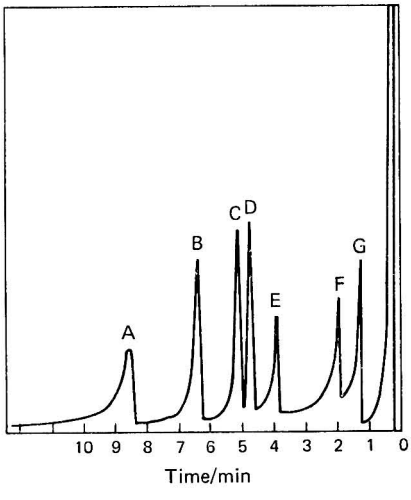

Fig. 2. Chromatogram of various preservatives with column temperature-programmed from 120 to 160 °C at a rate of 4 °C min^{-1}. A, propyl *p*-hydroxybenzoate; B, ethyl *p*-hydroxybenzoate; C, methyl *p*-hydroxybenzoate; D, dimethyl phthalate; E, *o*- and *p*-chlorobenzoic acid; F, benzoic acid; and G, sorbic acid

DISCUSSION

We have used the method successfully in a number of instances in which poisoning of cats by benzoic acid has been suspected; concentrations of benzoic acid in the meat of up to 0·8 per cent. have been found. Correlation is limited by factors unconnected with analytical chemistry, however, as one of the main causes of poisoning is the uneven mixing of the benzoic acid in the meat, and frequently the sample submitted for analysis has a different composition from that which poisoned the cat.

It should be noted that concentrations of benzoic acid that might be lethal in the cat can be fed to a dog without ill effects. The susceptibility of the cat is due to the fact that its glucuronic acid conjugation mechanism, which serves as a secondary de-toxication pathway in other species, is markedly defective.[15,16]

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A Combined Column-chromatographic and Infrared Spectrophotometric Determination of Diosgenin and Yamogenin in Fenugreek Seed

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A routine procedure is described for the determination of diosgenin and yamogenin in fenugreek seed. A crude extract is obtained by acidic hydrolysis of the seed, neutralisation and extraction of the insoluble matter with light petroleum. By means of column chromatography this extract is freed from fixed oil and other substances, thus yielding a mixture of diosgenin and yamogenin suitable for infrared spectrophotometric analysis. The results calculated for duplicate analyses of 2·5-g samples of commercial Moroccan seed, expressed as a 95 per cent. confidence interval of the mean sapogenin value, are 0·96 ± 0·017 per cent. for diosgenin *plus* yamogenin, 0·58 ± 0·008 per cent. for diosgenin and 0·38 ± 0·016 per cent. for yamogenin. The procedure has been found to be satisfactory for column loadings of up to 75 mg of diosgenin *plus* yamogenin in the presence of up to 600 mg of fixed oil, which is approximately three times the weight of extractive from 2·5 g of Moroccan seed.

Gas - liquid chromatography[1] and densitometric thin-layer chromatography[2] have been used to determine diosgenin in *Dioscorea* tuber extracts in which the yamogenin (the 25β-epimer of diosgenin) and fixed oil contents are both very low. The separation of non-spirostan steroids from oily solutions has been achieved by thin-layer chromatography[3] and by partition between hexane and 85 per cent. ethanol,[4] followed in both instances by gas - liquid chromatographic analysis. The infrared spectrophotometric procedure described[5] for the quantitative determination of the C_{25} epimeric steroidal sapogenins involved the use of diosgenin (25α) and sarsasapogenin (25β), the latter because pure yamogenin (25β) was not available at the time. The separation of pure yamogenin from diosgenin is difficult. We have achieved this separation by preparative thin-layer chromatography with continuous elution and have studied the infrared spectrophotometry required to determine the pure epimers individually in the presence of each other in all proportions.[6]

Acidic hydrolysis of Moroccan *Trigonella foenum-graecum* L. (fenugreek) seeds, and neutralisation followed by extraction of the insoluble matter with light petroleum, affords a mixture of 1 per cent. of diosgenin and yamogenin with 6 per cent. of fixed oil and small amounts of free sterol, sterol esters, spirostadienes and gitogenin (see Fig. 1). The degree of accuracy attained with the method[6] can be achieved only in the absence of unwanted components of the crude extract, and we have developed a routine procedure for their removal by column chromatography. The assay is applicable to whole seed kept under aqueous conditions for the purpose of including plant auxins or other substances as a means of increasing sapogenin yield,[7] and any changes in the diosgenin-to-yamogenin ratio occurring as a consequence of these post-harvest treatments can be detected.

Experimental

Apparatus—

Chromatographic columns—These were made with a 10-mm bore and an effective length of 300 mm and included an integral sinter (porosity 0) and tap. The columns were fitted with 100-ml separating funnels and, when large volumes of solvent caused air locks to form at the top of the columns, the air was released by inserting a length of PTFE tubing through the bore of the tap.

Desaga "S" chamber—This chamber was obtained from Camlab (Glass) Ltd., Cambridge.

Spectrophotometer—A Hilger H800 double-beam recording infrared spectrophotometer with a rock salt prism and 1-mm path length cells was used.

Vacuum oven—This was a size 1 oven, from A. Gallenkamp and Co. Ltd., London.

Vacuum rotary evaporator—A Büchi evaporator, from Orme Scientific Ltd., Manchester, was used.

REAGENTS AND MATERIALS—

Concentrated hydrochloric acid (sp. gr. 1·18).
Antimony(III) chloride.
Hexane.
Ethyl acetate.
Acetone.
Light petroleum, boiling range 40 to 60 °C.
Dilute ammonia solution—A 10 per cent. w/w dilution of ammonia solution.
Chloroform—AnalaR grade material from BDH Ltd., Poole, Dorset, was used.
Silica gel for adsorption—Woelm, from Koch-Light Laboratories Ltd., Colnbrook.
Diosgenin and yamogenin—These sapogenins were isolated by preparative thin-layer chromatography.[6]
Ampoules—The ampoules were 5-ml amber glass snapules from Adelphi (Tubes) Ltd., London.
Fenugreek seed—This was obtained from London Spice Market. Commercial Moroccan seed was sorted to remove weed seeds and soil, but not immature seed. The moisture content was ascertained by determining the loss on drying at 105 °C for 16 hours on duplicate 5-g seed samples. All infrared results were expressed on a moisture-free basis.

ROUTINE COLUMN AND INFRARED PROCEDURE FOR A 2·5-g SEED ANALYSIS—

The crude extract was prepared by refluxing 2·5 g of whole seed with 100 ml of 2 N hydrochloric acid for 2 hours. The mixture was cooled and filtered and the residue made alkaline with dilute ammonia solution before being dried overnight at 70 °C. This material, with its filter-paper, was extracted in a Soxhlet apparatus for 24 hours with light petroleum, which was then removed *in vacuo*, leaving an oily residue. The columns were packed with 6 g of silica gel for adsorption (Woelm, activity II), with hexane - ethyl acetate (9 + 1). The oily residue was then transferred to the column by using a total of 10 ml of the solvent mixture, and collection of the eluate was begun at a flow-rate of 1 ml min^{-1}. A further 90 ml of the same solvent mixture were added and 85 ml of eluate (which contained all the unwanted material) collected, *plus* three 5-ml fractions for a thin-layer chromatographic check. Then, 55 ml of a 3 + 1 mixture of the solvents were used to collect 40 ml of eluate (containing diosgenin and yamogenin together), again followed by three 5-ml fractions for the thin-layer chromatographic check. The residues following evaporation of each of the 5-ml fractions were dissolved in 1 ml of chloroform and 10 μl applied to a silica gel G plate which was placed in an "S" chamber with a hexane - ethyl acetate (4 + 1) mixture, which was allowed to run for 15 cm. After drying, the plates were sprayed with antimony(III) chloride (300 per cent. w/v in concentrated hydrochloric acid) and heated at 100 °C for 15 minutes.

All the diosgenin and yamogenin extracts from one column were bulked, evaporated to dryness and left *in vacuo* overnight at 50 °C to remove moisture and yellow pigment. The residue was dissolved in 3·92 ml of AnalaR chloroform (the same 4-ml flask was used each time) and the solution kept in sealed 5-ml amber ampoules until the start of the infrared analysis. All the solutions from one experiment were assayed in random order on the same day and checked for purity by thin-layer chromatography. The spectrum from each solution was determined three times over the frequency range from 1050 to 850 cm^{-1} by using a Hilger H800 spectrophotometer under the following conditions: 1-mm path length cell; slit width 550 μm at 900 cm^{-1}; autoslit 25; gain 7; damping 4; and scan speed 33 minutes per revolution. The calibration graphs used were those required for the ratio method[6] and were prepared from determinations on pure isolated epimers. The diosgenin content was obtained from a graph of diosgenin contents at a wavenumber of 900 cm^{-1} prepared in the presence of yamogenin at the same diosgenin-to-yamogenin concentration ratio (6 : 4) as was present in the seed used. The yamogenin content was calculated from the diosgenin value by simple proportion, as the exact diosgenin-to-yamogenin concentration ratio was provided by the ratio graph that was obtained by plotting the diosgenin-to-yamogenin concentration ratios 1 : 1, 2 : 1, 3 : 1 and 4 : 1 against the absorbance ratio (at 900 to 920 cm^{-1}). The total sapogenin content was the sum of the individual sapogenin values.

DETERMINATION OF INTERFERENCE OCCURRING DURING INFRARED ANALYSIS OF THE CRUDE EXTRACT—

The crude extract from 2·5 g of seed was column chromatographed as above except that the eluate was collected in 5-ml fractions, each fraction being checked for identity by thin-layer chromatography, and then bulked into the fractions (*a*) to (*d*) (see Fig. 1). The residues from these were each dissolved in 5 ml of AnalaR chloroform and the infrared spectra determined (see Fig. 2). The effect on the result of the infrared analysis was determined by mixing equal volumes of the other fractions with fraction (*b*) in the following combinations: $a + b$, $a + b + c$, $a + b + c + d$. Each mixture was evaporated to dryness, dissolved in the same volume of chloroform as the volume of fraction (*b*) taken and assayed for sapogenin content.

RECOVERY OF SAPOGENIN FROM THE COLUMN—

Diosgenin *plus* yamogenin sapogenin mixture was purified by column chromatography and recrystallised from methanol (85 per cent.) or acetone while sapogenin-free fenugreek oil was obtained by extracting powdered Moroccan seed with light petroleum and removing the solvent *in vacuo*. A thin-layer chromatographic check confirmed the presence of fractions (*c*) and (*d*) and the absence of fractions (*a*) and (*b*). Acid-treated oil was then obtained by subjecting oil to the same procedure as was used to obtain the crude sapogenin-containing extract from the whole seed. Weighed amounts of sapogenin-free acid-treated oil and diosgenin *plus* yamogenin sapogenin mixture were dissolved in chloroform so that 4 ml (run in from a bulb pipette) contained 30 mg of sapogenin (equivalent to 1·2 per cent. in the seed) and 250 mg of oil (equivalent to 10 per cent. in the seed). The 4-ml samples were evaporated *in vacuo* and transferred to columns by using 10 ml of a 9 + 1 mixture of hexane - ethyl acetate.

RESULTS AND DISCUSSION

The amount of interference occurring during the infrared analysis when using fenugreek crude extracts was determined by isolating the main components from the extract obtained from 2·5 g of seed and obtaining the infrared spectrum for each fraction over the range of wavenumbers used in the determination. Fig. 2 (*d*) shows that fraction *d* (Fig. 1) possessed a considerable and rapidly changing absorbance over the whole range, the interference being greater at 920 cm^{-1} than at 900 cm^{-1}. Fraction *c* (not shown in Fig. 2) gave a similar but lower absorbance, with a percentage transmission varying between 70 and 80 per cent. over the important 920 to 900 cm^{-1} range. Fraction *a* [Fig. 2 (*a*)] showed the same general shape as fraction *b* [Fig. 2 (*b*)], but at a much lower concentration. The

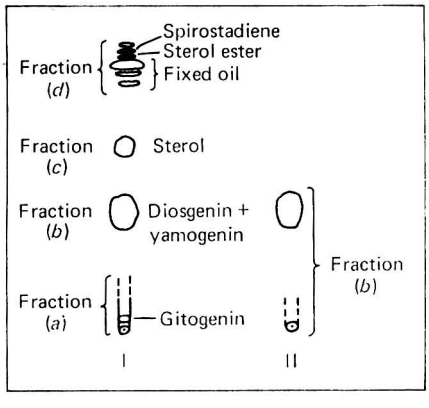

Fig. 1. Thin-layer chromatograms of: I, crude extract; and II, sample for infrared spectrophotometry from the column chromatograph by the routine procedure

fractions *a*, *c* and *d* (Fig. 1) were then added, one at a time, to fraction *b* and assayed for percentage content of sapogenin. The results obtained were as follows: fraction *b*, 1·2 per cent.; fractions $a + b$, 1·3 per cent.; fractions $a + b + c$, 1·6 per cent.; and fractions $a + b + c + d$, 2·1 per cent. Fig. 2 (*e*) also shows the considerable distortion of the sapogenin spectrum when present in the crude extract $a + b + c + d$ compared with pure fraction *b* [Fig. 2 (*b*)]. Thus, all the unwanted components of the crude extract increased the infrared result for diosgenin *plus* yamogenin sapogenin. The analysis of twelve 2·5-g seed crude extracts by infrared spectrophotometry, expressed as a 95 per cent. confidence interval of the mean sapogenin value, gave 1·53 ± 0·06 per cent. for diosgenin *plus* yamogenin, 0·77 ± 0·02 per cent. for diosgenin and 0·75 ± 0·05 per cent. for yamogenin. For duplicate results the corresponding confidence intervals were calculated to be ±0·15 for diosgenin *plus* yamogenin, ±0·04 for diosgenin and ±0·12 for yamogenin.

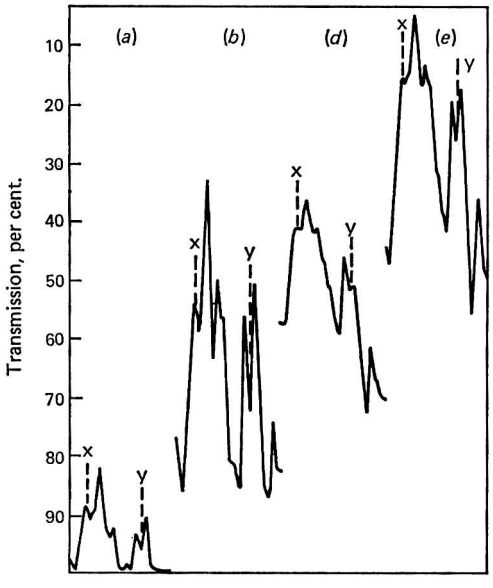

Fig. 2. Infrared spectra of fractions from column chromatograph, all at a concentration equivalent to that occurring in 2·5 g of seed and dissolved in 5 ml of AnalaR chloroform; markers, x = 1000 and y = 900 cm^{-1}. Fractions *a*, *b* (II) and *d* correspond to those in Fig. 1. Fraction *e* corresponds to I, Fig 1 (crude extract $a+b+c+d$)

Alternative methods of eliminating the interference, other than by column chromatography, were also studied. In one method the seed was powdered (to pass a B.S.S. No. 30 sieve) and defatted prior to undergoing infrared spectrophotometric analysis. It was found that Soxhlet extraction for 24 hours with light petroleum completely removed fraction *d* (Fig. 1), but did not completely remove fraction *c* and did not remove any of fractions *a* and *b*. Acidic hydrolysis of the dried, defatted material yielded, after sapogenin extraction, a solution containing more spirostadiene than usual. The second method involved the placing of the extract obtained by defatting the powdered seed in the reference beam during the infrared analysis, but it failed to cancel completely the interference in the whole seed extract.

With experience the sapogenin in the residue from a 5-ml fraction can be detected visually, thus considerably reducing the number of fractions that required the thin-layer chromatographic check. Activity II silica gel gave better resolution and required less solvent than activity I silica gel, as well as being less likely to retain sapogenin. When a

9 + 1 mixture of hexane - ethyl acetate was used throughout the development, 140 ml were needed to collect diosgenin *plus* yamogenin sapogenin instead of the 40 ml required when using a 3 + 1 hexane - ethyl acetate mixture. Thin-layer chromatographic investigation of the subsequent solutions used for infrared determination showed that the change in solvent strength when using the routine procedure resulted in the elution of a faint trace of material, that was left on the base-line of the thin-layer chromatographic plate, but was insufficient to influence the result of the infrared determination. Analysis of a bulk crude extract with six columns by using hexane - ethyl acetate (9 + 1) throughout and with six columns by using the routine method gave identical mean infrared results.

Removal of fractions *c* and *d* during the column elution still leaves a yellow colour on the column which is eluted partly before and partly with fraction *b*. The effect of the yellow colouring matter on the infrared result was studied by isolating all that from 250 mg of acid-treated oil (equivalent to 2·5 g of seed containing 10 per cent. of oil). Infrared spectrophotometry showed that no absorbance occurred between 1050 and 850 cm^{-1}. When all the yellow colouring matter was added to 28·3 mg (found by infrared determination) of diosgenin *plus* yamogenin sapogenins, a result of 28·5 mg was obtained, confirming the absence of significant interference. In practice, it has been found that when the bulked diosgenin *plus* yamogenin sapogenins with yellow pigment are dried *in vacuo* at 50 °C overnight, most of the colour is removed, so that the resulting chloroform solutions are either colourless or pale straw-yellow in colour. Repeated infrared analysis (20 times) of one of these solutions showed that its coefficient of variation was of the same order as that described[6] for the pure epimers.

The recovery of diosgenin *plus* yamogenin sapogenin from the column was tested by using column-purified diosgenin *plus* yamogenin sapogenin and acid-treated, sapogenin-free oil. The recovery obtained by using hexane - ethyl acetate (9 + 1) throughout and six columns was 96 to 103 per cent., giving a mean result of 29·6 mg compared with the theoretical result of 29·5 mg. The recovery obtained with hexane - ethyl acetate, first 9 + 1 and then 3 + 1, and twelve columns was 97 to 104 per cent., giving a mean result of 30·1 mg compared with the theoretical result of 29·8 mg and a coefficient of variation of 2·6 per cent. The reproducibilities of the column and infrared procedural steps were tested by analysing a crude extract by using twelve columns, and expressed as a 95 per cent. confidence interval of the mean sapogenin value. For diosgenin *plus* yamogenin, the mean value was 1·05 $\pm$ 0·017 per cent., for diosgenin, 0·63 $\pm$ 0·005 per cent. and for yamogenin, 0·42 $\pm$ 0·012 per cent. The over-all error of the determination procedure, including sampling, acidic hydrolysis and extraction, was tested by carrying out twelve determinations, each on 2·5 g of seed, and expressing the results as before. The mean value for diosgenin *plus* yamogenin was 0·96 $\pm$ 0·017 per cent., for diosgenin, 0·58 $\pm$ 0·008 per cent. and for yamogenin, 0·38 $\pm$ 0·016 per cent. For duplicate results the corresponding confidence intervals were calculated to be $\pm$0·04 for diosgenin *plus* yamogenin, $\pm$0·02 for diosgenin and $\pm$0·04 for yamogenin. The procedure has been found to be satisfactory for column loadings up to 75 mg of diosgenin *plus* yamogenin sapogenins in the presence of up to 600 mg of fixed oil, which is approximately three times the amount present in a 2·5-g sample of commercial Moroccan seed.

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The Determination of Tin in Steels by Solvent Extraction Followed by Atomic-absorption Spectrophotometry

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A method is described for the determination of 0·001 to 0·25 per cent. of tin in irons and steels. The tin from a 1-g sample of metal is extracted from an aqueous solution, which is 0·5 M in both hydrochloric acid and thiocyanate and 8 per cent. w/v in ascorbic acid, into isobutyl methyl ketone. The organic phase is concentrated to a small volume by evaporation and diluted to 10 ml. The tin content of this solution is determined by atomic-absorption spectrophotometry with a nitrous oxide - acetylene flame. Good results were obtained for the determination of tin in twelve B.C.S. irons and steels. The limit of detection was 0·001 per cent. of tin.

Appreciable concentrations of tin have a detrimental effect on the hot-workability of carbon steels[1] and temper-brittleness can be caused by the presence of trace amounts of tin in alloy steels.[2] For these reasons the concentration of tin in steels is frequently determined. This concentration is usually within the range 0·005 to 0·1 per cent.

With the Unicam SP90, Series 1, atomic-absorption spectrophotometer, the concentration of tin that produced 1 per cent. absorption was 3·0 μg ml^{-1} in a nitrous oxide - acetylene flame when aqueous solutions and the 224·6-nm line were used. If an aqueous solution of a steel (1 per cent. w/v) was analysed by direct atomic-absorption spectrophotometry, the limit of detection for tin in the steel would be 0·03 per cent., assuming realistically that the limit of detection for aqueous solutions might be about the same as the concentration of tin that produces 1 per cent. absorption. Very recently, Thomerson and Price[3] reported that they were able to determine tin in steel down to about 0·01 per cent. by using a 2 per cent. w/v solution of the steel in perchloric acid and the Unicam SP90A, Series 2, atomic-absorption spectrophotometer. Clearly, with atomic-absorption spectrophotometers in the lower price range the direct method is not sensitive enough for the determination of tin in some cast irons and steels.

Headridge and Richardson[4] and Headridge and Smith[5] encountered a similar problem when determining bismuth and antimony, respectively, in steels and cast irons. The problem was overcome by extracting the trace element as an ion-association complex into isobutyl methyl ketone, evaporating the organic phase to small volume, diluting it to a definite volume and spraying the solution into the flame of an atomic-absorption spectrophotometer. It was felt that a similar approach might be adopted for the determination of tin in steels. The solvent-extraction procedure would have to be capable of separating the tin from large amounts of iron and, for alloy steels, of chromium and nickel.

Extraction of tin(IV) as a thiocyanate complex into isobutyl methyl ketone seemed to be a possibility, as tin(IV) is very effectively extracted into diethyl ether from an aqueous solution 0·5 M in hydrochloric acid and containing various concentrations of ammonium thiocyanate.[6] It was expected that iron(III) and molybdenum(V) would also be extracted into the isobutyl methyl ketone in high yield.[6] Most steels do not contain high concentrations of molybdenum, but for all steels the base element is iron. However, despite the fact that the formal electrode potential of the iron(III) - iron(II) couple will be lowered if a complex with thiocyanate is formed, it was felt that the iron(III) might be reduced to non-extractable iron(II) on the addition of excess of ascorbic acid. Preliminary experiments showed that this was so. Only 6 per cent. of the iron is extracted into isobutyl methyl ketone from an aqueous solution that is 0·5 M in hydrochloric acid, 0·5 M in potassium thiocyanate and 8 per cent. w/v in ascorbic acid, and, when the organic phase is shaken with an aqueous solution that contains only hydrochloric acid, potassium thiocyanate and ascorbic acid at these concentrations, almost all of the iron is stripped from the organic phase. In fact, only

0·3 per cent. of the iron originally present remained in the organic phase. A 1-g amount of iron was used in these preliminary extraction studies and the volumes used for all the aqueous and organic phases were 50 ml. Tin(IV) is quantitatively extracted from the aqueous phase in the first shaking with isobutyl methyl ketone and negligible amounts of tin are re-extracted into the aqueous phase in the second shaking.

A method based on the above extraction system is described in this paper for the determination in steels and irons of tin within the concentration range 0·001 to 0·25 per cent.

Experimental

Apparatus—

A Unicam SP90, Series 1, atomic-absorption spectrophotometer was used with a nitrous oxide - acetylene flame.

Reagents—

Analytical-reagent grade reagents were used unless otherwise stated.

Hydrochloric acid, sp. gr. 1·18.

Nitric acid, sp. gr. 1·42.

Ammonium and potassium thiocyanates.

Ascorbic acid solution—Dissolve 40 g of L-ascorbic acid in 250 ml of 0·5 M hydrochloric acid.

Ascorbic acid - potassium thiocyanate solution—Dissolve 40 g of L-ascorbic acid and 24 g of potassium thiocyanate in 500 ml of 0·5 M hydrochloric acid. This solution should be freshly prepared.

Iron metal—Specpure quality (Johnson Matthey Ltd.).

Formic acid, 98 to 100 per cent.—Fisons laboratory-reagent grade.

Isobutyl methyl ketone—General-purpose reagent. Redistil it before use.

Standard tin solution A—Dissolve 0·2500 g of granulated analytical-reagent grade tin metal in 8 ml of concentrated hydrochloric acid *plus* 2 ml of concentrated nitric acid in a 250-ml beaker. Add 1 ml of formic acid and heat the mixture gently until evolution of nitrogen dioxide ceases. Dilute the solution to 250 ml in a calibrated flask with 0·5 M hydrochloric acid.

1 ml of solution ≡ 1·000 mg of tin.

Standard tin solution B—Dilute 10 ml of standard tin solution A to 100 ml in a calibrated flask with 0·5 M hydrochloric acid.

1 ml of solution ≡ 0·1000 mg of tin.

Method

Determination of tin in ferrous alloys—

To 1·0000 g of iron or steel in a 100-ml beaker, add 12 ml of concentrated hydrochloric acid followed by 3 ml of concentrated nitric acid (Note 1). After reaction has ceased, add 5 ml of concentrated hydrochloric acid and heat the mixture gently for 10 minutes. Add 2 ml of formic acid and heat the mixture gently until evolution of nitrogen dioxide ceases. Evaporate the solution until solid first appears. Dissolve the residue in 25 ml of ascorbic acid solution and dilute the solution to approximately 50 ml with 0·5 M hydrochloric acid. Transfer the solution to a separating funnel, rinsing the beaker with a few millilitres of 0·5 M hydrochloric acid, add 2·4 g of potassium thiocyanate crystals (Note 2) and shake the funnel until the crystals dissolve. Add 50 ml of isobutyl methyl ketone and shake the funnel vigorously for 15 s. Run off the lower, aqueous phase and discard it. Add 50 ml of ascorbic acid - potassium thiocyanate solution to the remaining organic phase and shake the funnel vigorously for 2 minutes. Discard the aqueous phase.

Transfer the organic phase to a small distillation flask and distil over the isobutyl methyl ketone at an oil-bath temperature of 140 to 150 °C until a volume of approximately 7 ml remains in the flask (Note 3). Transfer the solution to a 10-ml calibrated flask and make the volume up to the calibration mark with isobutyl methyl ketone. Allow a small precipitate, if present, to settle, draw off about 5 ml of solution with a dry pipette and spray the solution at once into the nitrous oxide - acetylene flame of the atomic-absorption spectrophotometer. Determine the flame absorbance for the solution. The conditions for this spectrophotometric determination are given in Table I.

TABLE I

INSTRUMENTAL CONDITIONS FOR THE DETERMINATION OF TIN

Acetylene flow-rate at 15 p.s.i./l min^{-1}	3·8
Nitrous oxide flow-rate at 30 p.s.i./l min^{-1}	5·0
Wavelength for use with tin lamp/nm	224·6
Slit width/mm	0·06
Lamp current/mA	8
Distance of centre of light path above burner/mm	8

Prepare a calibration graph corresponding to 0 to 0·05 per cent. of tin in an alloy by adding 1-g amounts of Specpure iron to each of six beakers followed by 0, 1, 2, 3, 4 and 5 ml of standard tin solution B. Treat these standards in exactly the same way as described for an iron or steel. Determine the flame absorbances of these solutions under the same conditions as those used for the alloys and draw a calibration graph.

NOTES—

1. If the alloy contains more than 0·05 per cent. of tin (500 $\mu g\ g^{-1}$), take a weight of alloy containing less than 500 μg of tin and make the weight up to 1 g with Specpure iron.
2. If preferred, ammonium thiocyanate can be used throughout instead of potassium thiocyanate.
3. The organic phase should not be left for longer than 2 hours before being concentrated by distillation.

TESTS FOR INTERFERING ELEMENTS—

It was appreciated that certain other elements besides tin would be partly extracted into the organic phase when the above method was used. Various weights of twenty-six elements, most of which might be present in steels, were subjected to the extraction procedure. These inorganic species were originally present in 50 ml of an aqueous solution that was 0·5 M in both hydrochloric acid and potassium thiocyanate and 8 per cent. w/v in ascorbic acid. The aqueous solution was shaken with 50 ml of isobutyl methyl ketone and the extent of extraction was found by determining the amount of each element left in the aqueous phase by well established analytical procedures. These were mainly atomic-absorption spectrophotometric methods, but methods involving absorption spectrophotometry with solutions, gravimetry and titrimetry were also used. The extents of extraction of twenty-

TABLE II

EXTENTS OF EXTRACTION OF VARIOUS INORGANIC SPECIES INTO ISOBUTYL METHYL KETONE FROM 0·5 M HYDROCHLORIC ACID - 0·5 M POTASSIUM THIOCYANATE - 8 PER CENT. w/v ASCORBIC ACID SOLUTION

Inorganic species	Extent of extraction, per cent.	Amount originally present in aqueous phase/mg
Aluminium(III)	0	10
Antimony(V)	5	10
Arsenic(V)	0	10
Bismuth(III)	0	5
Boron(III)	1	10
Calcium(II)	0	10
Chromium(III)	0	200
Cobalt(II)	67	100
Iron(III)	6	1000
Lead(II)	0	10
Magnesium(II)	0	10
Manganese(II)	1	100
Molybdenum(VI)	78	10
Nickel(II)	1	100
Niobium(V)*	2	10
Phosphorus(V)	0	10
Selenium(VI)	0	10
Sulphur(VI)	0	10
Tellurium(VI)	0	10
Tin(IV)	99·9	10
Titanium(IV)*	5	10
Vanadium(V)	27	10
Zinc(II)	99	10

* The aqueous phase contained a trace amount of fluoride.

three of these elements are shown in Table II. The other three inorganic species, *viz.*, copper(II), silver(I) and tungsten(VI), formed precipitates.

It was found that 1 and 10-mg amounts of copper(II) both produced a precipitate of copper(I) thiocyanate, which dissolved completely in the organic phase for 1 mg of copper and partly for 10 mg of copper. With 1 mg of copper, the extent of extraction into the isobutyl methyl ketone layer was greater than 99 per cent. A 10-mg amount of silver(I) produced a precipitate of silver chloride, while 1, 10 and 100-mg amounts of tungsten(VI) produced a cloudiness in the aqueous phase, a slight precipitate and a heavy precipitate, respectively. This precipitate from tungsten(VI) was presumably hydrated tungstic acid; when it was removed from the aqueous phase by filtration and the aqueous phase was shaken with isobutyl methyl ketone, the organic phase was colourless, which indicated that no tungsten had been extracted.

It can be seen from Table II and the preceding paragraph that nine elements are extracted into isobutyl methyl ketone to the extent of 5 per cent. or more, *viz.*, tin, iron, antimony, cobalt, copper, molybdenum, titanium, vanadium and zinc.

The possible interfering effects of the last seven of the above elements were tested for by replacing x g of a 1-g sample of Specpure iron by x g of each element, where $100x$ is the maximum percentage of the element that is likely to be present in a ferrous alloy, by adding 300 μg of tin to the sample and by determining the tin content of the simulated alloy by the described method. The amounts of tin found in each instance are shown in Table III.

TABLE III

RECOVERY OF 300 μg OF TIN ADDED TO SAMPLES OF SIMULATED ALLOYS CONTAINING POSSIBLE INTERFERING ELEMENTS

	Amount of element added/mg	Amount of tin found/μg
Antimony	5	305
Cobalt	100	295
Copper*	10	300
Molybdenum	50	300
Titanium	10	295
Vanadium	20	300
Zinc	5	295

* A suspension of copper(I) thiocyanate in the organic phase was removed by filtration through Whatman 1 PS paper before concentration of this phase.

The results indicate that no interfering effects occur. The slight variations from 300 μg are statistical in nature, as the limit of detection for tin is about 10 μg.

A solution containing 300 μg of tin and 10 mg of tungsten was subjected to the solvent-extraction and concentration procedure, and the tin was determined by atomic-absorption spectrophotometry, the precipitate of hydrated tungstic acid having been removed from the aqueous phase by filtration. Amounts of tungsten up to 10 mg had no effect on the quantitative recovery of tin but with greater amounts of tungsten the recovery of tin was low, presumably because tin was co-precipitated on larger precipitates of hydrated tungstic acid.

RESULTS FOR THE ANALYSIS OF IRONS AND STEELS

The calibration graph for the determination of tin in metal samples passed through the origin and curved down slightly towards the concentration axis. There was no tin in the blank. From this calibration graph, the concentration of tin that produced 1 per cent. absorption was established as 0·8 μg ml^{-1}.

The results for the determination of tin in twelve British Chemical Standard iron and steel samples are shown in Table IV.

Precision data were determined for two alloys, namely B.C.S. 206/1 cast iron and B.C.S. 239/3 carbon steel. The standard deviations from the means for ten determinations in each instance were 0·0004 and 0·0006 per cent., respectively.

DISCUSSION

The results for the twelve alloys examined are considered to be good, all average results being in good agreement with the certificate values. The limit of detection is about 0·0010 per cent. The standard deviation of approximately 0·0005 per cent. was satisfactory for the

TABLE IV

TIN CONTENTS OF METAL SAMPLES DETERMINED BY THE DESCRIBED METHOD

Alloy		Tin content by this method, per cent.	Average tin content by this method, per cent.	Certificate value, per cent.
B.C.S. No.	Type			
149/3	High purity iron	0·0015, 0·0010, 0·0015, 0·0015	0·0015	<0·002
206/1	Cast iron	0·0040, 0·0035, 0·0045, 0·0040	0·0040	~0·005
218/3	Carbon steel	0·0410, 0·0405, 0·0415, 0·0410	0·0410	0·042
239/3	Carbon steel	0·0305, 0·0290, 0·0290, 0·0300	0·0295	0·030
320	Mild steel	0·0875, 0·0860, 0·0880, 0·0860	0·0870	0·085
321	Mild steel	0·0135, 0·0135, 0·0130, 0·0135	0·0135	0·014
322	Mild steel	0·235, 0·245, 0·245, 0·230	0·240	0·24
323	Mild steel	0·0245, 0·0240, 0·0250, 0·0255	0·0250	0·024
324	Mild steel	0·130, 0·145, 0·140, 0·130	0·135	0·13
325	Mild steel	0·0465, 0·0455, 0·0460, 0·0450	0·0455	0·046
219/3	Ni - Cr - Mo alloy steel	0·0160, 0·0155, 0·0165, 0·0170	0·0165	~0·016
224/1	Cr - V alloy steel	0·0100, 0·0105, 0·0120, 0·0100	0·0105	~0·01

determination of tin in these irons and steels, but, if necessary, the standard deviation could be improved by using scale expansion and a recorder to determine absorbances. In this study the absorbances were read directly from the absorbance scale on the instrument without scale expansion.

However, it is essential that the analysis be completed as rapidly as possible after the tin has been extracted into isobutyl methyl ketone as the thiocyanate complex. Thiocyanic acid is not particularly stable in the organic phase. When the solution is evaporated to a small volume in the distillation flask, a yellowish brown precipitate is produced. Most of this precipitate is left in the flask when 7 ml of solution are decanted into the 10-ml calibrated flask. Any precipitate that enters the flask is allowed to settle for 2 minutes before 5 ml of the solution are withdrawn to be sprayed immediately into the flame. These 5-ml volumes of solution will slowly become cloudy if they are allowed to stand before spraying. The precipitate does not appear to absorb any tin.

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The Determination of Zinc in the Feed-water to High Pressure Boilers by Atomic-fluorescence Spectroscopy

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A rapid and sensitive atomic-fluorescence method is described for the determination of zinc in feed-water to high pressure boilers within the range 0·0004 to 0·01 p.p.m. A microwave-excited electrodeless discharge tube is used as the excitation source to stimulate zinc fluorescence at 213·9 nm from a sample solution sprayed into the air - hydrogen flame.

The limit of detection of the final method was 0·0004 p.p.m. of zinc and within-batch coefficients of variation were 1·2 per cent. at the 0·01 p.p.m. level and 3·4 per cent. at the 0·005 p.p.m. level.

Impurities and additives that are likely to be present in the feed-water were examined and it was shown that none interfered.

The method was compared with a solvent-extraction - atomic-absorption method.

INTEREST has been shown recently by the Central Electricity Generating Board (C.E.G.B.) in the zinc content of feed-water to high pressure boilers, because the zinc may be deposited together with iron, copper and nickel on the boiler tubing, superheater tubing and turbine blades of power-station plant with the consequent loss of operational efficiency and plant availability.

The present C.E.G.B. code of practice states[1] that the copper, iron and nickel contents of feed-water to high pressure boilers should together not exceed 10 $\mu g\ l^{-1}$ (0·01 p.p.m.). Zinc occurs in feed-water systems as a result of pick-up from brass components, *e.g.*, feed-heater tubes, and it is likely that its concentration will be similar to those of the other metallic contaminants, *i.e.*, copper, iron and nickel. A method of analysis is therefore needed to determine zinc in the 0 to 0·01 p.p.m. range, with a limit of detection of less than 0·001 p.p.m., and a standard deviation of about 0·0005 p.p.m. within this range.

Until now, a solvent-extraction - atomic-absorption method has been used (Bayley, E.S., unpublished work), based on the work of Malissa and Schoffmann,[2] in which the complex of zinc with ammonium 1-pyrrolidinecarbodithioate is extracted with an organic solvent and the extract is sprayed into the flame of an atomic-absorption spectrophotometer. Although this method gives the required sensitivity, it proved to be rather time consuming and also liable to errors at these low levels because of contamination from glassware and reagents. It was therefore decided to determine zinc by a direct aqueous spray method using the related flame technique, atomic-fluorescence spectroscopy.

Winefordner and Elser[3] have given an excellent review on atomic-fluorescence spectroscopy, and this paper describes the development of an atomic-fluorescence method for zinc in feed-water, outlines the relative sensitivity of a number of high intensity sources, compares the limit of detection with that by atomic-absorption spectroscopy, and examines the effect of a number of impurities or additives that are likely to be present in feed-water.

EXPERIMENTAL

ATOMIC FLUORESCENCE OF ZINC—

Three high intensity line sources were examined in this work, *viz.*, a high intensity hollow-cathode lamp, a vapour-discharge tube and a microwave-excited electrodeless discharge tube.

High intensity hollow-cathode lamp—Previously, the atomic-fluorescence of zinc using a high intensity hollow-cathode lamp as the excitation source had not been investigated. In this study, an Atomic Spectral Lamps Pty. Ltd. high intensity hollow-cathode lamp was supported in a laboratory clamp and positioned as close as possible to the flame and at

right-angles to the optical axis. Atomic-fluorescence measurements were made with the lamp operated at 10 mA and 400 mA boost current, as recommended by the manufacturers. The Southern Analytical A3000 atomic-absorption spectrophotometer used throughout this work was operated in the emission mode, which is permissible because at the wavelength of zinc fluorescence, 213·9 nm, there is no detectable thermal-emission signal at the low concentration levels being investigated. Also, there is very little background emission in this region of the spectrum when the air - hydrogen flame is used.

TABLE I

STANDARD DEVIATIONS, COEFFICIENTS OF VARIATION AND LIMITS OF DETECTION FOR ZINC BY ATOMIC-FLUORESCENCE AND ATOMIC-ABSORPTION SPECTROSCOPY WITH DIFFERENT EXCITATION SOURCES

Technique	Excitation source	Zinc concentration, p.p.m.	Standard deviation, p.p.m.	Coefficient of variation, per cent.	Limit of detection,* p.p.m.
Atomic-fluorescence spectroscopy	High intensity hollow-cathode lamp	0·5	0·014	2·8	0·03
		0·1	0·006	6	
	Vapour-discharge lamp	0·05	0·0008	1·6	0·0024
		0·01	0·0005	5	
	Electrodeless discharge tube	0·01	0·000 12	1·2	0·0004
		0·005	0·000 17	3·4	
Atomic-absorption spectroscopy	High intensity hollow-cathode lamp	0·05	0·0009	1·8	0·002
		0·01	0·0004	4	

* Limit of detection, for 95 per cent. confidence limits, is defined here as $4{\cdot}625S_B$, where S_B is the standard deviation of the blank.[4,5] Because the factors that contribute to noise during nebulisation of the sample include factors additional to those when zinc-free water is nebulised, the value of S_B was calculated from the average recorder deflections of a solution low in zinc that was nebulised on a number of separate occasions. It was assumed that the value of S_B for this solution was the same as that for a blank.

Limits of detection and within-batch standard deviations are given in Table I. The standard deviations were calculated from a series of eleven results obtained by consecutively nebulising solutions at each concentration level. De-ionised, distilled water was nebulised between each standard application. The results were corrected for the blank, *i.e.*, the reference de-ionised, distilled water, by subtracting the mean signal value of the blank from that of the standard, which is the normal procedure in flame methods of analysis. Because there was no difference in the signal with and without the aspiration of water, it was assumed that any zinc present in the water could be considered to be negligible compared with the level of zinc being measured.

Vapour-discharge lamp—This type of source is by far the most commonly used in studies to date. It has been used by Winefordner and co-workers,[6–8] Goodfellow,[9] Dagnall, Thompson and West,[10] Omenetto and Rossi[11] and Vickers and Vaught.[12] In this work a Philips Spectral zinc-vapour discharge tube was used, operated at 0·7 A. This current is slightly below that recommended by the manufacturers, thereby avoiding self-reversal and hence loss of fluorescence intensity.

The effective intensity of the discharge tube can be increased if a cylindrical aluminised mirror is placed behind the source and used to focus the light emitted by the source on to the flame cell. The physical dimensions of the A3000 spectrophotometer and discharge lamp allow a minimum distance of 10 cm between the flame and the lamp and therefore an aluminised mirror with a radius of curvature of 14 cm was placed at an appropriate distance behind the lamp so that radiation from the lamp could be focused on to the flame. By this means a three-fold increase in fluorescent signal was achieved.

Fluorescence measurements were made with the instrument operating under the same experimental conditions as used previously. Limits of detection and within-batch standard deviations are shown in Table I.

Electrodeless discharge tube—The characteristics and properties of microwave-excited electrodeless discharge tubes have been described in detail in the literature.[13,14] In this study an electrodeless discharge tube manufactured by Southern Spectral Sources Ltd. was used and operated in the Electro-Medical Supplies Ltd. 210L cavity.

A slight modification was made to the 210L cavity. The lower tuning stub was removed and the viewing aperture was sealed with a quartz window. This arrangement serves to protect the electrodeless discharge tube from external draughts and also maintains it at a uniform temperature. The 210L cavity can conveniently rest on the optical shelf of the A3000 instrument, close to the burner, so that the maximum amount of excitation radiation is received by the flame.

Fluorescence measurements were made with the electrodeless discharge tube operating at 35 W incident power and about 7 W reflected power. This power rating was found to give a stable and intense output after an initial warm-up time of about 15 minutes.

Limits of detection and within-batch standard deviations are shown in Table I, from which it can be concluded that the electrodeless discharge tube shows by far the best performance as an excitation source for zinc atomic fluorescence, because of its higher line intensity and consequent ability to achieve greater sensitivity. Accordingly, this tube was used in all future work.

EXAMINATION OF POSSIBLE INTERFERENCES—

All the impurities and additives that are likely to be present in the feed-water were investigated to determine whether or not they interfered.

All the analyses were performed in the presence of 5 p.p.m. of impurity or additive. Blank values were also recorded for the impurity or additive alone and these values were subtracted from the sample readings. It was assumed that any positive reading obtained by spraying the impurity or additive alone was due to traces of zinc in the impurity or additive. However, in the presence of 5 p.p.m. of sodium, it is probable that the positive result is due to stray sodium light of wavelengths 589·0 and 589·6 nm entering the entrance slit of the instrument and being reflected off the walls of the monochromator on to the photomultiplier.

The results of this study are given in Table II, and, bearing in mind that the concentrations of the additives examined were vastly in excess of those ever to be expected in practice, it can be concluded that any difference from the true value is unimportant.

TABLE II

EFFECT OF VARIOUS ADDITIVES ON THE FLUORESCENCE OF A 0·05 p.p.m. ZINC SOLUTION

Additive	Concentration, p.p.m.	Equivalent zinc for additive, p.p.m.	Equivalent zinc for 0·05 p.p.m. of Zn + additive, p.p.m.	Difference, p.p.m.	Difference from 0·05 p.p.m. of zinc, per cent.
Copper	5	0·001	0·0525	0·0515	+3
Iron	5	0	0·049	0·049	−2
Nickel	5	0·002	0·053	0·051	+2
Calcium	5	0	0·049	0·049	−2
Magnesium ..	5	0	0·050	0·050	0
Phosphate ..	5	0·001	0·050	0·049	+2
Silicon	5	0	0·049	0·049	−2
Sodium	5	0·004	0·052	0·048	−4
Sodium	0·5	0	0·049	0·049	−2
Sulphate	5	0	0·049	0·049	−2
Chloride	5	0	0·052	0·052	+4
Ammonium ..	5	0	0·052	0·052	+4
Hydrazine ..	5	0	0·052	0·052	+4
Cyclohexylamine ..	5	0	0·050	0·050	0
Morpholine ..	5	0	0·049	0·049	−2

COMPARATIVE ANALYSES—

A series of comparative analyses was carried out with the assistance of other C.E.G.B. laboratories. The purpose of this work was to compare results obtained by atomic-fluorescence spectroscopy at C.E.R.L. with those obtained by a solvent-extraction - atomic-absorption method.

This atomic-absorption method (Bayley, E. S., unpublished work) consists of complexing zinc in a 400-ml sample of acid-stabilised feed-water (0·1 M with respect to hydrochloric acid)

with 2 ml of 2 per cent. ammonium 1-pyrrolidinecarbodithioate solution, adjusting the pH to 4·5 by the addition of ammonia - acetic acid buffer solution and extracting the zinc 1-pyrrolidinecarbodithioate complex with 10 ml of xylene. The organic extract is then sprayed into the flame of the atomic-absorption spectrophotometer. Samples were collected from a number of power stations and analysed to determine zinc, and the results are given in Table III.

TABLE III

COMPARATIVE DETERMINATION OF ZINC BY ATOMIC-FLUORESCENCE SPECTROSCOPY AND SOLVENT EXTRACTION - ATOMIC-ABSORPTION SPECTROSCOPY

		Zinc content	
Sample	Sampling point	by atomic fluorescence, p.p.m.	by atomic absorption, p.p.m.
Battersea No. 4 T/G*	Condenser extraction pump	0·0042, 0·0042	0·0062, 0·0058
Battersea No. 4 T/G	De-aerator	0·0025, 0·0028	0·0031, 0·0035, 0·0038
Bankside No. 2 T/G	Condenser extraction pump	0·0024, 0·0025	0·0025, 0·0020, 0·0025
Bankside No. 2 T/G	De-aerator	0·0007, 0·0005	0·0007, 0·0007, 0·0013
Croydon No. 1 T/G	Condenser extraction pump	0·0013, 0·0011	0·0019, 0·0016, 0·0018
Deptford No. 1 T/G	Condenser extraction pump	0·0085, 0·0087	0·0080, 0·0086, 0·0085
Deptford No. 3 T/G	Condenser extraction pump	0·0035, 0·0034	0·0050, 0·0045
Battersea No. 4 T/G†	De-aerator	0·0020, 0·0018	0·0020, 0·0017, 0·0021
Battersea No. 6 T/G	Condenser extraction pump	0·0020, 0·0022	0·0013, 0·0010, 0·0018
Drakelow No. 11 T/G	Economiser inlet	0·0111, 0·0114	0·0115
Drakelow No. 12 T/G	Economiser inlet	0·0020, 0·0021	0·0013

* T/G = Turbogenerator.
† Sampled on a separate occasion.

It can be seen that in most instances the zinc contents of the feed-waters were within the range 0·001 to 0·01 p.p.m. for the particular power stations selected. The actual value found will, of course, depend on the materials of construction of the condenser and feed systems and also the location at which the sample is taken.

Two conclusions can be drawn from the results of these comparative analyses. Firstly, it appears that the within-batch precision of the atomic-fluorescence technique is superior to the solvent-extraction - atomic-absorption method, because of the greater experimental simplicity of the atomic-fluorescence method compared with the lengthier procedure involved in the solvent-extraction method. Secondly, the results in general are sufficiently close to demonstrate their accuracy within the limits of experimental error.

EXPERIMENTAL OPERATING CONDITIONS—

Collection of samples—Samples were collected in Baird and Tatlock 1-litre, narrow-necked polythene bottles, Type 215/0411/06. A 10-ml volume of Aristar grade hydrochloric acid (B.D.H.) was added to each bottle to prevent the deposition of zinc on the walls of the container. It was noticed that some samples, when they were left standing for a period of time (in 0·1 M hydrochloric acid), tended to show an increase in zinc content. This effect can be attributed either to particulate zinc matter, or to zinc adsorbed on to the surface of particulate iron being slowly released into solution by the action of the acid. It is thought likely that the latter mechanism is dominant, particularly because this effect occurs with samples with high iron contents. Results are shown in Table IV for the analysis of feed-water

TABLE IV

EFFECT OF HEATING POWER-STATION FEED-WATER SAMPLES AT 80 °C ON THE SOLUBILITY OF ZINC

	Zinc concentration, p.p.m.		
		After heating for—	
Sample	In the cold	1 hour	2 hours
A	0·0025, 0·0027, 0·0027	0·0048, 0·0048, 0·0050	0·0052, 0·0052, 0·0051
B	0·0018, 0·0020, 0·0020	0·0018, 0·0018, 0·0020	0·0020, 0·0020, 0·0020
C	0·080, 0·082, 0·081	0·162, 0·162	0·166, 0·168, 0·170

samples in the cold and after heating for periods of 1 and 2 hours at 80 °C, and demonstrate that samples should be heated in a water-bath for about 2 hours at 80 °C before analysis. This treatment ensures that all the zinc is released into solution in a form that is acceptable for atomisation.

Instrumental operating conditions—A Southern Analytical A3000 atomic-absorption spectrophotometer, operating in the emission mode, was used. A Southern Spectral Sources zinc electrodeless discharge tube was supported in an Electro-Medical Supplies 210L cavity by means of a rubber grommet with a quartz window for thermal insulation. The power was supplied to the cavity from an Electro-Medical Supplies Microtron 200 Mk. 2 through a co-axial cable, and initiation of the discharge in the electrodeless discharge tube was achieved by means of an Edwards High Vacuum H.F. Tester T1.

The tube was initially operated at 50 W incident power for a warm-up period of 10 minutes and then at 35 W; stability was achieved after about 15 minutes.

A Southern Analytical emission burner was used instead of a conventional atomic-absorption burner. The following operating conditions were used: burner height, position 1, *i.e.*, the lowest point of travel; flame, air - hydrogen (because of the low radiative background); hydrogen flow-rate, 3·5 l min^{-1} and air flow-rate, 7·5 l min^{-1} (conditions that give the maximum fluorescence signal); slit width, position 6, *i.e.*, 1·0 mm, band pass, 6 nm; wavelength, 213·9 nm; photomultiplier gain, maximum; damping, position 3; and time constant, 2 s. The scale expansion was variable so as to give the required signal, consequent with good stability. No scale expansion was needed in this work because the limit of detection required for our analyses was attained without its use, *i.e.*, the majority of samples occur at levels of approximately 0·005 p.p.m. The recorder was a Kent 1-mV instrument.

PROCEDURE—

Measure the fluorescence signal of a series of standard zinc solutions (in 0·1 M hydrochloric acid) against de-ionised, distilled water. First heat the feed-water samples for about 2 hours at 80 °C in a water-bath and cool them to room temperature. Measure the fluorescence signals of the samples and compare these with the standards, thereby obtaining the zinc level in the samples.

Atomic-absorption spectroscopy of zinc—The atomic-absorption spectroscopy of zinc is well known and has been extensively reported in the literature. A comparison has been made between the limit of detection and coefficient of variation for zinc and the reported results from atomic-fluorescence spectroscopy.

The A3000 instrument was operated in the absorption mode with an Atomic Spectral Lamps Pty. Ltd. hollow-cathode lamp at 10 mA. The emission burner was replaced with the standard 12-cm atomic-absorption burner. All other experimental conditions were identical with those described previously.

Limits of detection and within-batch standard deviations are given in Table I.

CONCLUSIONS

It has been demonstrated that zinc can be determined by atomic-fluorescence spectroscopy very rapidly in feed-water and other high purity waters by a direct aqueous spray method. The method is considerably faster than previous methods involving solvent extraction (Bayley, E. S., unpublished work, and reference 3).

The high intensity hollow-cathode lamp has been found not to be a suitable source for the level of sensitivity required in this work, because it was not capable of achieving a high enough sensitivity as a result of insufficient line intensity. The geometrical design of the lamp also made it difficult to adjust it to a position close to the flame, and thereby light was lost by the inverse square law effect. The vapour-discharge tube gave limits of detection lower than those for the high intensity hollow-cathode lamp, but not quite low enough for the levels required in this work. The microwave-excited electrodeless discharge tube, by virtue of its higher line intensity, gave the greatest fluorescence sensitivity. The limit of detection of the method, 0·0004 p.p.m., with this type of source was low enough to allow the direct determination of zinc between 0 and 0·01 p.p.m., *i.e.*, the range normally required for feed-water analysis.

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Visual Estimation and Spectrophotometric Determination of Zinc in Potable Waters with 4-(2′-Thiazolylazo)resorcinol

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A rapid method is described for the visual estimation and spectrophotometric determination of zinc in potable waters by using 4-(2′-thiazolylazo)-resorcinol. A single addition of a reagent mixture that incorporates buffer, chelating reagent and masking agent is possible. The method is more rapid than existing methods and satisfactory agreement with values from atomic-absorption spectrophotometry is obtained for the range of values normally encountered in potable waters.

Zinc rarely occurs in natural waters and generally enters domestic water supplies as a result of the deterioration of galvanised iron storage tanks or piping. Its toxicity is low, but both the United States Public Health Service[1] and the World Health Organisation[2] quote a limit of 5 mg l^{-1} as the level beyond which the potability of the water may be affected owing to an astringent taste or opalescence in the case of alkaline waters. Zinc is normally estimated in drinking waters by visual comparison of the opalescence resulting from formation of the hexacyanoferrate(II),[3] or determined spectrophotometrically by using diphenylthiocarbazone (dithizone) or 1-(2-hydroxy-5-sulphophenyl)-3-phenyl-5-(2-carboxyphenyl)formazan (zincon).[4] Solvent extraction in the dithizone method and rapid fading of the zinc - zincon colour detracts from rapid spectrophotometric determination on a routine basis. Few colorimetric reagents are available for the determination of zinc; compounds of the thiazolylazophenol type[5] have recently been investigated, one of which, 4-(2′-thiazolylazo)resorcinol (TAR), forms a water-soluble zinc chelate. This reagent has been applied to the determination of zinc oxide fume in air,[6] relevant interference being removed by cation exchange.

A method has been developed in which this reagent enables both the visual estimation and the spectrophotometric determination of zinc in potable waters to be achieved. Levels of other elements that interfere are generally higher than those normally encountered; possible serious interference from copper is masked by thiosemicarbazide.

Method

Reagents—

All reagents should be of analytical-reagent grade. Solutions can be made up with either distilled or de-ionised water.

Methanol.

Hydrochloric acid, sp. gr. 1·18 *and* 2 N.

Thiosemicarbazide.

Sodium acetate - sodium tetraborate solution—Dissolve 136 g of sodium acetate trihydrate and 48 g of sodium tetraborate decahydrate in water and dilute the solution to 2 litres.

4-(2′-*Thiazolylazo*)*resorcinol* (*TAR*), 0·08 *per cent. w/v solution*—Dissolve 400 mg of reagent in 500 ml of methanol.

TAR reagent solution—Dilute 250 ml of the sodium acetate - sodium tetraborate solution to 400 ml with water, add 2 g of thiosemicarbazide and dissolve it by heating. Cool the solution, add 50 ml of 0·08 per cent. w/v TAR solution and, with the aid of a pH meter, adjust the pH to 7·5 with 2 N hydrochloric acid. Dilute the solution to 500 ml with water. The reagent solution is stable for 4 days.

Standard zinc solution—Dissolve 500 mg of granulated zinc in 4 ml of concentrated hydrochloric acid and dilute the solution to 1 litre. A 20-ml volume of this solution made up to 1 litre immediately prior to use will give a standard solution containing 10 mg l^{-1} of zinc.

APPARATUS—

A Unicam SP600 spectrophotometer or an equivalent instrument is suitable for measuring the optical densities.

PROCEDURE—

Prepare a series of standards of known zinc concentrations (0, 0·2, 0·4, 0·6, 0·8, 1·0 and 1·4 mg l^{-1}) by diluting 0, 1, 2, 3, 4, 5 and 7 ml of standard zinc solution to 50 ml in Nessler cylinders. Add 5 ml of TAR reagent solution to each standard, stir the solutions well and allow them to stand for 30 minutes. This series of standards can be used for visual estimations for up to 4 days after their preparation. Measure 50 ml of sample into a Nessler cylinder, or an appropriate aliquot diluted to 50 ml so that the zinc concentration is in the range 0 to 1·0 mg l^{-1}, and add 5 ml of reagent. Stir the mixture, allow it to stand for 30 minutes and compare it with the standards by viewing through the tube, estimating the zinc concentration to the nearest 0·1 mg l^{-1}.

For spectrophotometric determinations the above series of standards, in the range 0 to 1·0 mg l^{-1} of zinc, can be read at 530 nm in 1-cm cells against water in the reference cell and a standard graph obtained by plotting the optical density, corrected for the reagent blank value, against concentration. Samples can be measured in a similar manner, correcting for the reagent blank value, and the concentrations are obtained from this standard graph.

DISCUSSION AND RESULTS

A rapid method for visual estimation with the added facility of spectrophotometric determination for the range 0 to 1 mg l^{-1} of zinc should have a wider effective range of 0 to 2 mg l^{-1} for the visual estimation so that zinc levels of 1 mg l^{-1} can be distinguished from higher levels. Maximum sensitivity and, when applicable, linearity in these ranges would be determined by a number of factors, in particular the strength of the reagent, the pH and, for spectrophotometric determinations, the wavelength of measurement. Kawse[5] reported that the zinc - TAR chelate, formed in aqueous solution within the pH range 7·4 to 8·4, gave a constant absorbance when it was extracted into chloroform and this solution was read at 580 nm; for normal use, he proposed a buffer prepared from sodium acetate and sodium tetraborate adjusted to pH 7·5 with hydrochloric acid. In a subsequent application of this reagent[6] the optimum wavelength for measurement of the zinc chelate in aqueous solution was found to be 530 nm.

This wavelength for maximum sensitivity with change of concentration in aqueous solution has been confirmed and the optimum concentration of the TAR reagent was found to be 7·3 μg ml^{-1} in the final solution, for both visual estimation of the yellow-to-red colour gradation and spectrophotometric determination within the range 0 to 1·0 mg l^{-1} of zinc. A change in the final TAR concentration of $\pm$ 7 per cent. from the optimum value invalidates the colour gradation. A series of potable waters of various compositions and with pH in the range 6·2 to 9·3, adjusted by adding 1 part of the buffer of pH 7·5 to 10 parts of the sample, gave a final pH in the range 7·7 to 7·9. Similarly, de-ionised water adjusted to a pH range of 3·5 to 5·0 and diluted 1 + 10 with the same buffer solution gave a final pH of 7·5 to 7·7. Adjustment of the pH of the buffer with various amounts of hydrochloric acid to give values above and below 7·5 resulted in poorer control of the final pH for the same range of test waters. These conditions of reagent concentration and pH control enable one to distinguish between 1·0 mg l^{-1} and higher concentrations (1·4 and 2·0 mg l^{-1}) of zinc by visual estimation, while a nearly linear relationship exists between the measured optical density and concentration for the range 0 to 1·0 mg l^{-1} of zinc.

INTERFERENCES—

It had previously been noted[6] that iron and copper interfere in the determination of zinc with TAR. Iron in potable waters will invariably be present as iron(III), and at the normal pH of such waters it will be in suspension as colloidal hydrated iron(III) oxide. While iron(II) would interfere at levels as low as 0·2 mg l^{-1}, iron(III) in the colloidal state was found not to interfere greatly for concentrations below 2 mg l^{-1}; at levels of 2 and 5 mg l^{-1} of iron(III) positive errors of 0·1 and 0·2 mg l^{-1}, respectively, of zinc were possible. Attempts to mask iron(III) were unsuccessful owing mainly to the partial complexing of the zinc, or for sulphosalicylic acid and sodium orthophosphate to instability of the combined reagent, which

incorporated buffer, chelating reagent and masking agent. A combined reagent was considered to be necessary for routine practice.

The copper - TAR chelate interfered seriously, giving a high positive response as zinc. Attempted masking with, in particular, thiourea or dimethylglyoxime was unsuccessful, for reasons similar to those given for the attempted masking of iron(III). Thiosemicarbazide was more satisfactory and had the additional advantage of lessening the interference from iron(III) while having no effect on the stability of a composite reagent over 4 days or on standards prepared and read over a period of 4 days. The masking effect of thiosemicarbazide varied with its concentration and with the concentration of copper present, but solubility considerations prevented the use of more than 20 mg per determination. The apparent zinc levels obtained with 50-ml volumes of solutions containing zinc and iron(III) or copper, to which was added 5 ml of a composite buffer - reagent solution incorporating 20 mg of thiosemicarbazide, are shown in Table I for both visual estimation and spectrophotometric determination.

TABLE I

MASKING OF INTERFERENCE FROM VARIOUS CONCENTRATIONS OF IRON(III) AND COPPER WITH THIOSEMICARBAZIDE IN THE DETERMINATION OF ZINC

Zinc added/mg l^{-1} ..	0·00	0·20	0·60	1·00	0·00	0·20	0·60	1·00
	Zinc measured							
	by visual estimation/mg l^{-1}				by spectrophotometric determination/mg l^{-1}			
Iron(III)/mg l^{-1}—								
0·2	Nil	0·2	0·6	1·0	Nil	0·19	0·59	1·00
0·6	Nil	0·2	0·6	1·2	Nil	0·20	0·57	1·01
2·0	Nil	0·2	0·6	1·2	Nil	0·22	0·60	0·99
5·0	Nil	0·3	0·7	1·0	0·04	0·28	0·60	0·92
Copper/mg l^{-1}—								
0·2	Nil	0·2	0·6	1·0	0·05	0·23	0·62	1·06
0·6	0·1	0·3	0·6	1·0	0·11	0·28	0·64	1·04
2·0	0·1	0·3	0·6	1·0	0·13	0·32	0·66	1·04
5·0	0·1	0·3	0·5	0·8	0·16	0·32	0·69	1·08

By using this composite reagent, a graph of optical density against concentration of zinc gives an essentially linear relationship over the range 0 to 0·8 mg l^{-1} of zinc, with slight curvature from 0·8 to 1·0 mg l^{-1} of zinc; the response is an optical density of approximately 0·050 per 0·1 mg l^{-1} of zinc.

Other ions present in potable waters, such as Ca^{2+}, Mg^{2+}, Al^{3+}, Cr^{6+}, F^-, PO_4^{3-} and SO_4^{2-}, or silica and chlorine do not interfere in the determination even when they are present

TABLE II

RECOVERY OF ZINC ADDED TO POTABLE WATERS

Zinc added/mg l^{-1}					0·00	0·20	0·60	1·00	5·0*	0·00	0·20	0·60	1·00	5·0*
					Zinc measured									
Sample	Hardness/ mg l^{-1} $CaCO_3$	pH	Cu^{2+}/ mg l^{-1}	Fe^{3+}/ mg l^{-1}	by visual estimation/mg l^{-1}					by spectrophotometric determination/mg l^{-1}				
A	600	7·2	—	0·8	0·3	0·5	0·9	1·4	6·0	0·23	0·46	0·82	1·18	5·9
B	410	7·3	0·02	—	0·1	0·3	0·7	1·2	6·0	0·11	0·35	0·70	1·10	6·1
C	320	7·9	—	—	0·2	0·4	0·7	1·2	6·0	0·16	0·35	0·73	1·26	5·8
D	180	8·6	—	0·2	0·0	0·2	0·6	1·0	5·0	0·05	0·24	0·61	0·96	4·8
E	180	8·7	—	—	0·0	0·2	0·6	1·2	5·0	0·06	0·23	0·60	0·97	4·8
F	130	7·3	0·06	0·04	0·0	0·3	0·6	1·0	5·0	0·05	0·26	0·63	0·98	4·7
G	80	8·1	0·2	0·3	0·2	0·4	0·8	1·2	5·0	0·17	0·39	0·77	1·23	5·2
H	65	7·4	—	0·1	0·2	0·3	0·7	1·4	5·0	0·17	0·32	0·70	1·15	5·0
J	40	6·2	0·1	—	0·1	0·3	0·7	1·2	5·0	0·13	0·33	0·70	1·05	5·3

* An aliquot diluted 10-fold was used for determination of this level.

at levels many times those normally found. At the 1 mg l^{-1} level, both lead (Pb^{2+}) and manganese (Mn^{2+}) interfere, giving apparent zinc levels of 0·3 and 0·05 mg l^{-1}, respectively. In solutions that contain 0·1 mg l^{-1} of lead or manganese, a more likely practical level, there is no interference from either element.

RESULTS OF RECOVERY EXPERIMENTS—

A series of recovery experiments at four levels of zinc for each of nine potable waters was undertaken, in which the recommended procedure was used. These potable waters were divided into three groups of three, with hardnesses of 300 to 600, 130 to 180 and 40 to 80 mg l^{-1} of calcium carbonate, respectively. In each group, at least one water contained a measurable amount of iron(III) (in the range 0·2 to 0·8 mg l^{-1}) and another contained a measurable amount of copper (not exceeding 0·1 mg l^{-1}). The pH was in the range 6·2 to 8·7 and the original zinc level did not exceed 0·3 mg l^{-1}. Details of recoveries are shown in Table II. The average recovery, calculated from the more accurate spectrophotometric results, was 99 per cent. with a coefficient of variation of 9 per cent. No great significance could be attached to different levels of recovery obtained when averaging for a particular variation, but it was noted that the recovery from potable waters of hardness 300 to 600 mg l^{-1} was 106 per cent.

Comparison of the results obtained by both the visual and spectrophotometric methods with those obtained by atomic-absorption spectrophotometry were satisfactory, and are listed for a range of levels in Table III. This method has been used successfully for the monitoring of zinc in potable waters in this laboratory for nearly 12 months.

TABLE III

COMPARISON OF ZINC LEVELS DETERMINED IN POTABLE WATERS BY DIFFERENT METHODS

By visual estimation/mg l^{-1}	By spectrophotometric measurement/mg l^{-1}	By atomic-absorption spectrophotometry/mg l^{-1}
0·0	0·06	0·04
0·1	0·12	0·07
0·3	0·28	0·25
0·4	0·42	0·44
0·5	0·50	0·51
0·7	0·64	0·68
1·0	0·93	0·81
1·0	1·19	1·10
2·0	1·85	1·93
2·5	2·30	2·31
2·5	2·55	2·75
6·0	6·2	6·8

We thank Mr E. J. David for carrying out the atomic-absorption measurements. This paper is published by permission of the Government Chemist.

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The Determination of Calcium in Rain Waters by Using High-temperature Flame-emission Spectroscopy

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A high-temperature flame-emission method involving the use of the nitrous oxide - acetylene flame is described for determining calcium in rain waters. The method described is compared with the di(2-hydroxyphenylimino)ethane [glyoxalbis(2-hydroxyanil)] colorimetric method over the 0 to 2 μg ml^{-1} calcium concentration range and is shown to be more accurate and less susceptible to contamination.

DETERMINATION of the alkali and alkaline earth elements in natural waters such as rain water can be carried out accurately and quickly by using atomic-absorption and flame-emission techniques. However, techniques in which flames of relatively low temperature (*e.g.*, air - acetylene) are used have inadequate detection limits for accurate determinations of calcium in the 0 to 2 μg ml^{-1} range[1,2] and generally colorimetric methods are used because they are more sensitive. In this laboratory the di(2-hydroxyphenylimino)ethane [glyoxalbis(2-hydroxyanil)] colorimetric method described by Kerr[3] has been used for the determination of calcium in rain water, the method being based upon the formation of a red complex with calcium. This complex is stable in an aqueous alcoholic medium, no significant interferences being encountered from magnesium[4] in concentrations up to 40 μg ml^{-1} or from chloride, nitrate and sulphate in concentrations up to 500 μg ml^{-1}. Barium and strontium interfere at concentrations greater than 4 μg ml^{-1}.[3] This colorimetric reaction has been studied in detail by Florence and Morgan[5] and has since found applications in the determination of calcium in magnesium carbonate,[6] milk[4] and plant tissue.[7]

Willis[8] introduced the pre-mixed nitrous oxide - acetylene flame for atomic-absorption analysis and since then the potential of this flame as an emission source has been extensively examined.[9–11] It is now well established that high-temperature flame-emission techniques give lower detection limits for many elements than colorimetric and atomic-absorption methods, especially when high-resolution monochromators and laminar-flow burner systems are used.[12] High-temperature flame-emission has already found application in the analytical chemistry of the alkali metals[13] and this paper examines the possibility of using it for the determination of calcium in rain waters.

EXPERIMENTAL

Flame and colorimetric determinations were made on the same solutions to permit a direct comparison of the methods. Solutions of samples and standards were poured up to the mark into a 25-ml calibrated test-tube and a standard addition of ionisation suppressant (0·1 ml of a 125 mg ml^{-1} solution of potassium as the chloride) was made with a syringe. Because of the very small volume of potassium chloride solution added to the sample the error introduced by slightly different sample volumes is negligible.

For the colorimetric method a 10-ml aliquot was removed by pipette from the tube after the flame analysis. The determination was carried out as described by Kerr[3] and the absorbance was read at 520 nm on a Beckmann D.U. monochromator modified with Gilford attachments to meet Gilford, Model 200, spectrophotometer specifications.

Emission analysis was carried out with a Techtron AA4 atomic-absorption spectrophotometer with an emission-chopper attachment, and a laminar-flow burner system with a grooved titanium burner head. With the atomisation system used the nitrous oxide and acetylene flow-rates were 7 and 3 l min^{-1}, respectively. These conditions produced a flame with a red "feather" zone 8 mm high. A 6-mm vertical section of the flame starting 2 mm above the primary reaction zone was viewed lengthwise by the monochromator. With the calcium 422·7-nm atomic resonance line the detection limit was 0·0002 μg ml^{-1}, which is comparable with values given in the literature.[12] In the range 0 to 2 μg ml^{-1} of calcium,

a monochromator exit slit of 50 μm and a voltage supply of 500 V to the photomultiplier (HTV, Type R213) produced the best signal-to-noise ratio. The flame was allowed to burn for 5 to 10 minutes prior to analysis to stabilise burner temperature and so reduce background-noise fluctuations.

In rain water no spectral or physical interferences were experienced; however, chemical nterferences could occur if aluminium was present. High-temperature flame-emission determinations with the aluminium 396·2-nm atomic line showed no detectable amounts of aluminium. Ionisation in the flame was suppressed by the addition of potassium as chloride to give a concentration of 500 μg ml^{-1} in the sample solution, which proved as efficient as a concentration of 1000 μg ml^{-1}, a commonly recommended value. The lower level of added potassium was used in this work because of the chloride tolerance limit of the di(2-hydroxyphenylimino)ethane method.

Method

Apparatus—

Emission instrument—A Techtron, Model AA4, atomic-absorption spectrophotometer fitted with a Techtron, Type FE-4, beam chopper and a grooved titanium burner head, Techtron, Type AB50, was used. Read-out was on a Beckmann 10-inch flat-bed recorder, Catalogue No. 100505.

Glassware—Exelo 25-ml graduated test-tubes with stopper, Catalogue No. T2/265.

Syringe—Hamilton, 250 μl, Catalogue No. 700 Series, or equivalent.

Reagents—

Calcium stock solution, 0·1 N—Weigh 5·004 g of analytical-reagent quality calcium carbonate that has been freshly dried for 4 hours at 110 °C. Dissolve it in about 20 ml of 1 + 1 hydrochloric acid (analytical-reagent quality) and make the volume up to 1 litre with de-ionised water.

Calcium working stock solution, 0·001 N—Dilute an aliquot of the 0·1 N stock solution 100-fold with de-ionised water.

Working calcium standards—By pipette, transfer 0, 5, 10, 20, 30, 40 and 50-ml aliquots of the 0·001 N stock solution into 500-ml flasks and make up to volume with de-ionised water. These solutions then contain 0·00, 0·20, 0·40, 0·80, 1·20, 1·60 and 2·00 μg ml^{-1} of calcium, respectively.

Ionisation suppressant solution (125 *mg ml*$^{-1}$ *of potassium*)—Dissolve 24·86 g of dry analytical-reagent quality potassium chloride in de-ionised water and make the volume up to 100 ml.

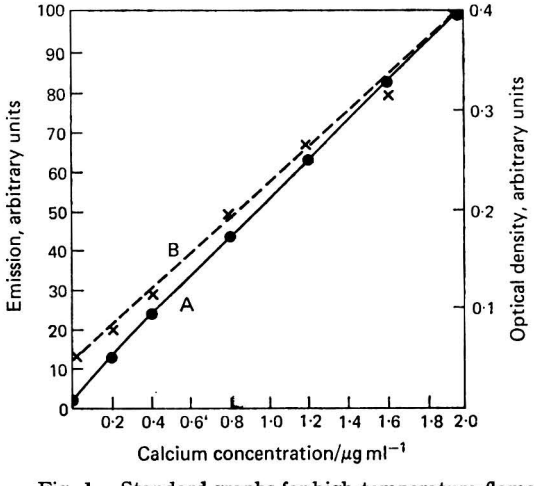

Fig. 1. Standard graphs for high-temperature flame-emission (A) and colorimetric di(2-hydroxyphenylimino)-ethane (B) methods

PROCEDURE—

Transfer samples and standards to 25-ml calibrated test-tubes, making the volumes up to the mark. With a syringe, add 0·1 ml of the potassium chloride solution to all tubes; stopper and shake them well. Measure the emission intensities of the samples against those of the standards in the nitrous oxide - acetylene flame by using the 422·7-nm calcium resonance line. Prepare a standard graph from the readings for the standards and read off the sample concentration directly as micrograms per millilitre.

RESULTS AND DISCUSSION

The standard graphs for both methods were nearly linear over the 0 to 2 μg ml^{-1} range (see Fig. 1); the graph for the colorimetric method intercepted the y-axis above the origin because of contamination from the added buffer solution. The graph for emission passed more closely to the origin, showing less contamination from reagents.

TABLE I

COMPARISON OF CALCIUM VALUES OBTAINED FOR RAIN WATERS BY COLORIMETRIC AND FLAME-EMISSION METHODS

	Calcium/μg ml^{-1}			
	High-temperature flame emission*		Di(2-hydroxyphenylimino)-ethane*	
Sample	Mean	Standard deviation	Mean	Standard deviation
R77–1	1·97	0·00	2·09	0·20
R78–1	1·09	0·01	1·12	0·05
R81–1	1·69	0·03	1·78	0·02
R83–1	1·16	0·03	1·20	0·09
R84–1	0·31	0·02	0·30	0·04
R87–1	0·39	0·01	0·43	0·05
R88–1	0·66	0·03	0·64	0·08
R89–1	1·66	0·04	1·76	0·09
R91–1	0·52	0·01	0·59	0·07
R93–1	1·63	0·02	1·79	0·11
Over-all mean	1·11	0·02	1·17	0·08

* Mean and standard deviation values calculated from four determinations.

In Table I results for calcium in rain water by the di(2-hydroxyphenylimino)ethane and high-temperature flame-emission methods are compared for a range of calcium concentrations; these show generally good agreement. The colorimetric method generally gave higher mean values and a higher standard deviation.

TABLE II

RECOVERY OF STANDARD ADDITIONS OF CALCIUM TO RAIN WATERS

		Calcium/μg ml^{-1}			
		High-temperature flame emission		Di(2-hydroxyphenyl-imino)ethane	
Sample	Added	Total present	Recovered	Total present	Recovered
R84–2	0·00	0·13	—	0·19	—
	0·20	0·34	0·21	0·39	0·20
	0·40	0·53	0·40	0·58	0·39
	0·60	0·73	0·60	0·82	0·63
	1·00	1·13	1·00	1·26	1·07
R87–2	0·00	0·39	—	0·46	—
	0·20	0·59	0·20	0·68	0·22
	0·40	0·78	0·39	0·88	0·42
	0·60	0·99	0·60	1·16	0·70
	1·00	1·38	0·99	1·58	1·12
R89–2	0·00	0·75	—	0·84	—
	0·20	0·94	0·19	1·06	0·22
	0·40	1·17	0·42	1·28	0·44
	0·60	1·35	0·60	1·44	0·60
	1·00	1·75	1·00	1·96	1·12

Recovery of standard additions of calcium by the emission method was satisfactory at all levels of addition (Table II), whereas the colorimetric method again tended to give high values. Measured levels of barium and strontium in the rain waters by high-temperature flame-emission were between 0·1 and 0·4 μg ml^{-1} and 0·2 and 0·6 μg ml^{-1}, respectively, which were well below the stated[3,5] interference level of 4 μg ml^{-1}.

Determination of calcium in rain water by high-temperature flame-emission presents few problems other than that of contamination prior to the determination step. Provided an instrument with adequate sensitivity and stability is available the method described is accurate, free from interferences and offers advantages over colorimetric methods and methods involving evaporation because of its speed and simplicity.

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The Analysis of Titanium Dioxide Pigments by Automatic Simultaneous X-ray Fluorescence Spectrometry*

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The use of an automatic fourteen-channel simultaneous X-ray spectrometer for the analysis of titanium dioxide pigments is described. The selection of channel parameters for the determination of calcium, aluminium, silicon, phosphorus, tin, iron, sulphur, chlorine, potassium, zinc, zirconium, niobium, antimony and lead is discussed, and statistical data are given on the accuracy of the determination of these elements.

In the manufacture of titanium dioxide pigments, additives are introduced at various stages of manufacture in order to produce different grades of pigment, each designed for a special application. Careful control must be maintained on these additives and it must also be ensured that deleterious impurities are kept below critical levels.

X-ray fluorescence spectrometry has been used for the analysis of titanium dioxide pigments since the first commercial equipment became available. This technique is ideally suited for pigment analysis[1,2] as the following problems, which are normally experienced with this method of analysis, do not apply.[3]

Inter-element effects—As the concentrations of all the elements to be determined are low, this effect is negligible and in most instances can be ignored.

Particle size—Titanium dioxide pigments have a crystal size distribution within the range 0·1 to 0·3 μm and are reasonably uniform in composition. Errors due to particle-size effects are therefore negligible.

Sample preparation—Titanium dioxide pigment is easily compressed into discs of adequate strength, without the need for a binding agent.

The introduction of this technique into the industry has resulted in a considerable saving in laboratory personnel and has also enabled certain elements to be determined with much greater precision than was possible by existing methods.

The need for closer analytical control, and also the increasing number of elements to be determined by this technique, overloaded the system to such an extent that some form of automation became desirable. As speed of analysis was considered to be an important factor, a simultaneous spectrometer was preferred although it was appreciated that the number of elements that could be determined would be limited to the number of channels in the instrument. The advantages of this instrument over the manual type can be summarised as follows.

(*i*) The spectrometer parameters (*i.e.*, crystal, collimator and detector) are pre-set for each element. Therefore, once the instrument has been aligned it can be used by semi-skilled operators.

(*ii*) With the exception of the anode of the X-ray tube, optimum conditions can be selected for each element; however, the tube can be changed as easily as in a manual instrument.

(*iii*) Automatic sample handling with facilities for data processing and print-out of results enables the instrument to be operated without supervision for an extended working day.

(*iv*) The instrument can cope with a very high work load for basically similar samples and it is therefore very suitable for use in routine control work.

The major disadvantage of this type of instrument is the lack of flexibility in the number of elements that can be determined. The elements selected can be changed but this would require a range of crystals, collimators and detectors to be available and also this change

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would take about one working day to carry out, so that frequent changes are not desirable. A further disadvantage is the complexity of the instrument, which makes electronic fault-finding and the tracing of vacuum leaks more difficult.

The following three measuring techniques can be used on the instrument.

(*i*) *Absolute*—In this method, all the elements are counted for a fixed time and the total count is printed out.

(*ii*) *Monitor*—This is a fixed time measurement for up to thirteen elements, the length of this time being determined by the fourteenth, or monitor, element. The period of measurement is the time required to collect a pre-set number of counts for the monitor element, which is usually added prior to the determination. By this technique, elements adjacent to the monitor element can be determined with increased precision.

(*iii*) *Ratio*—In this method, a standard sample of known composition is placed in the beam and the time required for each element channel to accumulate a fixed number of counts is recorded. The number of counts is not necessarily the same for each element and the time recorded, *t*, will therefore be different for each channel. The standard is then replaced by the sample and the number of counts within the time *t* previously determined for each channel is recorded. By this method any errors due to long-term drift are eliminated.

The advantages of a simultaneous spectrometer clearly outweigh the disadvantages, and the use of such an instrument for the analysis of titanium dioxide pigments was investigated.

Experimental

Apparatus—

The equipment used for this work was a Philips PW1270 automatic simultaneous X-ray spectrometer fitted with data-processing equipment and an automatic sample changer. A chromium-anode X-ray tube rated at 2700 W was selected for use. The spectrometer chamber can be evacuated so that elements that produce X-radiation of long wavelength, which is strongly absorbed by air, can be determined, and its temperature is maintained at 35 $\pm$ 1 °C so as to minimise drift in the case of the pentaerythritol crystal. Polypropylene film of 1-μm thickness is used for the windows in the flow-proportional counters of channels 4, 5, 7, 8 and 14 (see Table I) so as to reduce absorption of the corresponding fluorescent beam.

The instrument incorporates three separate banks of data-processing equipment[4] so that it is possible to analyse three different types of material at one loading, micro-switches being used to initiate the selection of the appropriate standard and data processor. The ratio method was used in this work.

Fourteen channels are available and each was used for the determination of one element, *i.e.*, it was not found necessary to make any background measurements. The list of elements together with the spectrometer parameters are shown in Table I.

Table I

Spectrometer parameters for the analysis of titanium dioxide pigments using the PW1270 spectrometer fitted with a chromium-anode X-ray tube operating at 60 kV and 40 mA

Sequence number	Determination	Radiation	Take-off angle/degrees	Primary collimator/μm	Crystal	Detector
1	Calcium	Ca Kα	37·00	240	Lithium fluoride	Flow proportional
2	Antimony	Sb Kα	45·35	240	Lithium fluoride	Scintillation
3	Iron	Fe Kα	50·35	900	Lithium fluoride	Flow proportional
4	Aluminium	Al Kα	54·00	450	Pentaerythritol	Flow proportional
5	Silicon	Si Kα	50·35	450	Pentaerythritol	Flow proportional
6	Tin	Sn Kα	45·35	240	Lithium fluoride	Scintillation
7	Phosphorus	P Kα	37·00	450	Pentaerythritol	Flow proportional
8	Sulphur	S Kα	22·30	450	Germanium	Flow proportional
9	Zirconium	Zr Kα	29·40	240	Lithium fluoride	Scintillation
10	Lead	Pb Lβ	34·30	450	Lithium fluoride	Scintillation
11	Potassium	K Kα	36·00	450	Lithium fluoride	Flow proportional
12	Niobium	Nb Kα	34·30	240	Lithium fluoride	Scintillation
13	Zinc	Zn Kα	29·40	240	Lithium fluoride	Scintillation
14	Chlorine	Cl Kα	22·30	450	Pentaerythritol	Flow proportional

As the concentration level of the elements to be determined was relatively low, the most intense X-ray line of each element was examined, whenever possible, in order to keep the counting time to a reasonable level. With lead, the Lβ line had to be used because of interference from the arsenic Kα line. The fourteen channels are arranged round the sample in the form of a clam-shell, and the intensity can be shown to be directly proportional to the take-off angle between the fluorescent beam and the primary collimator.

From Table I it can be seen that channel 4 is the most sensitive position and should be used for elements that have a low fluorescent yield, such as aluminium, or that are present at a low concentration, such as iron, for which high accuracy is required.

Overlap of lines normally occurs between a second-order titanium line, titanium 2Kα, and the sulphur Kα line. This overlap was eliminated by using a germanium crystal, which does not give second-order reflections.

Selective pulse-height analysis was used in all instances in order to remove as much unwanted radiation as possible.

Automatic sample changer—An automatic sample changer capable of holding up to 160 samples in a sixteen-disc store was included in the apparatus. The samples were automatically inserted into the spectrometer, analysed and then replaced in their original positions in the store. An electromechanical counter increased by one step every time a sample was received and one symbol was printed by the typewriter to act as a means of sample identification.

Sample preparation—

Titanium dioxide pigments can readily be compressed into solid discs that are suitable for X-ray fluorescence examination without the addition of a binding agent. About 10 g of sample were placed in an aluminium cup and compressed by using a die and plunger with a pressure of approximately 5 ton inch^{-2}. The compression pressure was varied but was found to have no effect on the radiation intensities.

The preparation of the large number of samples that can be processed by the instrument is tedious when a hand-operated press is used. The process was speeded up considerably by fitting an electrically operated hydraulic pump to the press so that pressure could be applied or released from the sample by operating a simple lever. Samples prepared in this way were sufficiently stable to allow measurements to be made but they tended to break down after a few days. Although approximately 10 g of sample were required to give a sample that was infinitely thick to X-radiation when using the standard sample holder, it was possible to obtain results by using smaller amounts (about 0·5 g) of sample. In this instance, a titanium disc with a well of suitable diameter and depth to give an infinitely thick layer was used, the actual size of the well being determined by the amount of sample available. The specimens were prepared by compressing the sample in the well by using a spatula and then smoothing the surface with a microscope slide. The absolute method was used, the counts for each element being accumulated for 100 s and then compared with calibration graphs that had been prepared for standards measured in the same holder.

Method of measurement—

The ratio method, in which the sample is compared with a standard of known composition, was selected as being the most suitable for pigment analysis. By this means, any errors due to long-term drift in the instrument were eliminated. The standard, which is most important in this method of measurement, was prepared by adding accurately known amounts of each element to a measured amount of titanium tetrachloride and then converting the mixture into titanium dioxide. The concentrations of each element were then carefully checked by chemical methods so as to ensure that the correct amounts of each element were present. The stability of the standard disc is important and it was replaced frequently as it would have deteriorated under the intensive X-ray exposure that it received. In practice, the disc was replaced at 3-day intervals when the instrument was being used to its full extent. A more permanent standard is desirable and the possibility of using a plastics or enamel matrix is being investigated.

Data processing and results

The data processing was simplified as the calibration graphs were linear over the range of concentrations of elements that were of interest. A series of accurately analysed standards

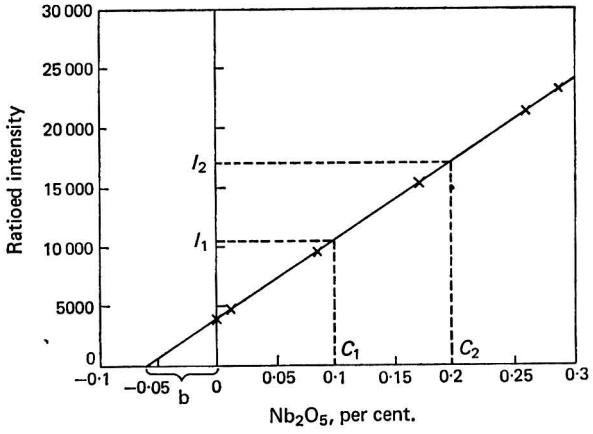

Fig. 1. Calibration graph for the determination of niobium in titanium dioxide pigment

$$\frac{C - C_1}{C_2 - C_1} = \frac{I - I_1}{I_2 - I_1}$$

$$C = mI - b$$

for each element was measured against the ratio standard and the resulting intensities were plotted against concentration to give a straight line.

The slope and intercept of this graph were calculated and the values inserted in the data processor, this procedure being repeated for each element. The process is illustrated in Fig. 1, which shows the calibration graph for niobium. In this instance, the number of counts accumulated was set at 40×10^4 and these counts were accumulated in 68·0 s for the ratio standard. All the standards were therefore counted for the same period. It should be understood that the values of slope and intercept of each element applied only for the corresponding fixed count setting and for this particular ratio standard.

TABLE II

X-RAY INTENSITY AND TIME TO ACHIEVE FIXED COUNTS FOR VARIOUS ELEMENTS IN A TITANIUM DIOXIDE MATRIX

Determination	Sensitivity, counts s^{-1} for 1 per cent. of element	Background count-rate/ counts s^{-1}	Fixed counts	Time to accumulate fixed counts/s
Calcium	10 700	70	10×10^4	73·0
Antimony	12 600	1390	10×10^4	33·8
Iron	7280	220	10×10^4	102·7
Aluminium	580	91	40×10^4	158·5
Silicon	810	59	10×10^4	81·4
Tin	16 600	1660	40×10^4	140·2
Phosphorus	870	15	10×10^4	49·1
Sulphur	1360	30	1×10^4	25·6
Zirconium	9300	604	10×10^4	56·0
Lead	13 600	1190	40×10^4	127·5
Potassium	15 100	47	40×10^4	54·6
Niobium	9850	888	40×10^4	68·0
Zinc	6500	710	40×10^4	78·2
Chlorine	2500	55	1×10^4	37·7

The sensitivities, background count-rates, fixed count settings and times to accumulate the fixed counts are shown in Table II for each element. The time taken for a complete analysis is dependent on the element that takes the longest time to accumulate its fixed number of counts, which, in the example shown, is aluminium (158·5 s). The total time required for the analysis of a sample is approximately 5 minutes.

As all fourteen channels were used for elemental determinations, an arbitrary background level was used in the data processor. This level will cause small errors in the determination of trace amounts of antimony and tin, whose characteristic X-radiation occurs at high background regions. This effect can be avoided by using the Lα lines for these elements, but in this instance coarse collimators are required because the radiation is less intense. Alternatively, correction factors can be applied either by using the data-processing equipment or by hand.

The performance of the instrument is shown in Table III, which shows the results of one hundred determinations on the same sample, separate discs being prepared in each instance. The table shows, for each element: (*a*) the limit of detection,[5] which is taken to be three times the standard deviation of the background variation; (*b*) the mean value of the results compared with the value obtained by alternative methods; and (*c*) the relative 2σ deviation of the results. The precision and accuracy of the method are clearly demonstrated by these results.

TABLE III

STATISTICAL ANALYSIS OF THE RESULTS FOR 100 DETERMINATIONS ON THE SAME SAMPLE OF TITANIUM DIOXIDE PIGMENT

Determination	Limit of detection, per cent.	Analysed value by alternative methods, per cent.	Mean X-ray value, per cent.	Relative standard deviation, per cent.
Calcium as CaO	0·0003	0·076	$0·075_2$	1·3
Antimony as Sb_2O_3	0·0015	0·065	$0·065_3$	2·4
Iron as Fe	0·0006	0·0099	$0·010_4$	5·8
Aluminium as Al_2O_3	0·0039	2·10	$2·07_0$	0·7
Silicon as SiO_2	0·0032	1·30	$1·27_7$	1·5
Tin as Sn	0·0006	0·011	$0·010_2$	5·9
Phosphorus as P_2O_5	0·0019	0·17	$0·167_0$	2·4
Sulphur as SO_3	0·0023	0·12	$0·119_0$	12·6
Zirconium as ZrO_2	0·0011	0·014	$0·014_8$	7·4
Lead as Pb	0·0007	0·0099	$0·010_1$	6·9
Potassium as K_2O	0·0002	0·006	$0·006_1$	3·3
Niobium as Nb_2O_5	0·0011	0·21	$0·212_4$	1·3
Zinc as ZnO	0·0013	0·96	$0·964_9$	1·7
Chlorine as Cl	0·0014	0·01	$0·011_2$	17·0

CONCLUSIONS

X-ray fluorescence spectrometry has been shown over the last fifteen years to be an essential tool for the process-control analyst. When several determinations are required to be carried out on the same sample, the manual technique tends to be tedious and slow by present standards and requires a full-time operator. The possibility of errors arising when transposing and calculating the results must always be considered.

The results reported in this paper clearly demonstrate the potential of simultaneous X-ray spectrometry, particularly when it is used in combination with automatic sample-feed equipment, which enables samples to be analysed without the attention of an operator.

The Directors of Tioxide International Limited are thanked for permission to publish this paper.

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The Determination of the Iodine Content of Organic Compounds as Soluble Iodides with *N*-Bromosuccinimide

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A method is proposed for the determination of iodine in organic compounds as soluble iodides with *N*-bromosuccinimide. Details of the procedure are given for the determination of the iodine content of thyroxine sodium, diiodohydroxyquinoline, iodochlorohydroxyquinoline and iodoform. The experimental error does not exceed ±2 per cent. The application of the proposed method to the determination of the iodine content of pharmaceutical preparations such as tablets is also described.

Previously all known methods for the determination of iodine in organic substances involved destruction of the organic matter, separation of iodine from the residue and its concentration into relatively small bulk. The iodine was then either determined colorimetrically after dissolving it in chloroform or carbon disulphide,[1] or it was oxidised to iodic acid, which was allowed to react with added potassium iodide, the liberated iodine being titrated with standard sodium thiosulphate.[2,3]

Although the colorimetric method is highly sensitive, it has certain limitations.[4] The titrimetric method has the advantage that for each atom of iodine in the material under investigation six atoms are actually titrated as follows.

$$HIO_3 + 5HI \rightarrow 6I + 3H_2O$$

The use of *N*-bromosuccinimide for the determination of iodine (and iodide) in pharmaceutical products has also been described.[5]

The present work involves the determination of small amounts of iodine in certain organic compounds of medical interest by the use of standard *N*-bromosuccinimide solution. The reaction between *N*-bromosuccinimide and potassium iodide at the low acid concentration used can be represented as follows.

Experimental

The method recently reported for the microdetermination of soluble iodides in biological fluids[6] with standard *N*-bromosuccinimide is extended to the determination of iodine in organic compounds.

Reagents—

Standard N-*bromosuccinimide solution, 0·02 and* 0·002 N—Pure *N*-bromosuccinimide (Hopkin & Williams, Chadwell Heath, Essex) was used and the standard solution was freshly prepared. The 0·002 N solution was used for determinations of amounts in the microgram range.

The organoiodine compounds examined included thyroxine sodium (B.D.H. Chemicals Ltd., Poole, Dorset), diiodohydroxyquinoline (B.P. grade, B.P. 1963) (I.U.P.A.C. systematic name 8-hydroxy-5,7-diiodoquinoline), iodochlorohydroxyquinoline (U.S.P. grade, U.S.P. XVI, 1960) (I.U.P.A.C. systematic name 5-chloro-8-hydroxy-7-iodoquinoline) and iodoform (B.P. grade, B.P. 1932).

Dilute hydrochloric acid, 10 per cent. v/v.

Soluble starch solution, 1 per cent., aqueous.

Procedure—

An accurately weighed amount of the organic material (100 or 250 mg) was placed in a porcelain crucible of 50-ml capacity and covered with a layer (10 g) of anhydrous potassium carbonate, the double crucible technique being used. The mixture was heated in a Wild -

Barfield muffle furnace at a temperature[7] of 400 °C (the temperature should not exceed 425 °C) for a period of 4 hours. After allowing the crucible to cool, the ash was dissolved in distilled water and the volume was made up to 100 ml with distilled water in a calibrated flask. Various known volumes of this solution were accurately measured and the iodine content of the organic material, which is now present as potassium iodide, was determined by the *N*-bromosuccinimide method.[6]

To an accurately measured volume, *e.g.*, 10 ml, dilute hydrochloric acid was gradually added until no effervescence was observed. A few drops of starch solution were then added as indicator. A 0·02 N *N*-bromosuccinimide solution was added dropwise, with continuous shaking, until the blue colour just disappeared and the titre of the standard *N*-bromosuccinimide solution was recorded. The iodine content of the organic material was readily calculated as follows.

$$\text{Iodine content (mg or } \mu g) = V \times C \times \frac{126{\cdot}9}{178} \times \frac{100}{A}$$

where V ml is the titre of *N*-bromosuccinimide solution, C mg ml^{-1} or μg ml^{-1} is the concentration of the *N*-bromosuccinimide solution and A mg is the amount of organic material in the volume taken.

Applications and Results

Determination of the iodine content of thyroxine sodium—

A 100-mg amount of thyroxine sodium was ashed as previously described, the residue was dissolved in distilled water and the volume adjusted with distilled water to 100 ml in a calibrated flask. The iodine content, corresponding to known amounts of thyroxine sodium ranging from 10 to 5 mg, was then determined as above with 0·02N *N*-bromosuccinimide solution. The percentage iodine content of thyroxine sodium was then readily calculated and the results are shown in Table I.

The iodine contents of 250 mg of diiodohydroxyquinoline (the iodine content corresponding to known amounts of diiodohydroxyquinoline varying from 25 to 12·5 mg), 250 mg of iodochlorohydroxyquinoline and 100 mg of iodoform were determined by using exactly the same procedure as that used for thyroxine sodium. The results obtained are given in Tables II, III and IV, respectively.

Table I

Determination of iodine content of thyroxine sodium by the proposed method

Amount of thyroxine sodium taken/mg	Titre of 0·02 N* *N*-bromosuccinimide/ml	Iodine found in amount taken/mg	Iodine found in thyroxine sodium (theoretical content 63·55 per cent.), per cent.	Error, per cent.
10	5·00	6·345	63·45	−0·10
9	4·51	5·723	63·59	0·04
8	4·01	5·089	63·61	0·06
7	3·50	4·442	63·46	−0·09
6	3·00	3·807	63·45	−0·10
5	2·50	3·173	63·46	−0·09

* 1 ml of 0·02 N *N*-bromosuccinimide solution ≡ 1·269 mg of iodine.

Diiodohydroxyquinoline tablets—

Each tablet contains 250 mg of diiodohydroxyquinoline. Twenty tablets were accurately weighed, then pulverised and the powder was mixed well. The average weight of one tablet (620 mg) was analysed to determine the iodine content as previously described. The results are shown in Table V.

Iodochlorohydroxyquinoline tablets—

Each tablet contains 250 mg of iodochlorohydroxyquinoline. The average weight of one tablet (380 mg) was similarly analysed and the iodine content determined according to the proposed method. The results are recorded in Table VI.

TABLE II

DETERMINATION OF IODINE CONTENT OF DIIODOHYDROXYQUINOLINE BY THE PROPOSED METHOD

Amount of compound taken/mg	Titre of 0·02 N *N*-bromosuccinimide/ml	Iodine found in amount taken/mg	Iodine found in diiodohydroxyquinoline (theoretical content 63·96 per cent.), per cent.	Error, per cent.
25·0	12·61	16·002	64·01	0·05
22·5	11·35	14·403	64·01	0·05
20·0	10·09	12·804	64·02	0·06
17·5	8·83	11·205	64·03	0·07
15·0	7·57	9·606	64·04	0·08
12·5	6·33	8·033	64·26	0·30

INTERFERING SUBSTANCES—

No interference is caused by the presence of fluoride, chloride, bromide, sulphate, nitrate, nitrite and acetate. However, sulphite, thiosulphate and hydrosulphite interfere.

EXPERIMENTAL ERROR—

The experimental error of the proposed method does not exceed ± 2 per cent. when determining the iodine content of amounts ranging from 25 to 5 mg of the organic material (Tables I to IV).

TABLE III

DETERMINATION OF IODINE CONTENT OF IODOCHLOROHYDROXYQUINOLINE BY THE PROPOSED METHOD

Amount of compound taken/mg	Titre of 0·02 N *N*-bromosuccinimide/ml	Iodine found in amount taken/mg	Iodine found in iodochlorohydroxyquinoline (theoretical content 41·57 per cent.), per cent.	Error, per cent.
25·0	8·18	10·380	41·52	−0·05
22·5	7·37	9·353	41·57	0·00
20·0	6·53	8·287	41·44	−0·13
17·5	5·72	7·259	41·48	−0·09
15·0	4·91	6·231	41·54	−0·03
12·5	4·09	5·190	41·52	−0·05

TABLE IV

DETERMINATION OF IODINE CONTENT OF IODOFORM BY THE PROPOSED METHOD

Amount of iodoform taken/mg	Titre of 0·02 N *N*-bromosuccinimide/ml	Iodine found in amount taken/mg	Iodine found in iodoform (theoretical content 96·67 per cent.), per cent.	Error, per cent.
10	7·65	9·708	97·08	0·41
9	6·86	8·705	96·72	0·05
8	6·11	7·754	96·93	0·26
7	5·31	6·738	96·26	−0·41
6	4·62	5·863	97·72	1·05
5	3·81	4·835	96·70	0·03

DISCUSSION

The destruction of organic matter in a porcelain crucible at a temperature of 400 °C in the presence of an excess of anhydrous potassium carbonate when using the double crucible technique has been shown to minimise the loss of iodine. This finding is supported in previous reports on the determination of trace amounts of iodine in organic substances.[8,9] Further, Scheffer[9] showed that the volatility of iodine in the presence of organic material was increased in the absence of an alkali.

During ashing, the iodine content of an organic material is converted into potassium iodide. No iodate is formed by prolonged heating in the presence of a large excess of oxidising agent, or it is formed only in undetectable amounts.[8] The mixture of potassium iodide and potassium carbonate obtained is soluble in water and, after neutralisation of the solution with

TABLE V

DETERMINATION OF IODINE CONTENT OF DIIODOHYDROXYQUINOLINE TABLETS BY THE PROPOSED METHOD

One 620-mg tablet contains 250 mg of diiodohydroxyquinoline, equivalent to 159·90 mg of iodine

Volume taken/ml	Titre of 0·02 N *N*-bromosuccinimide/ml	Iodine found in volume taken/mg	Iodine found per 100 ml or one tablet/mg
10	12·84	16·278	162·78
9	11·30	14·340	159·33
8	10·15	12·880	161·00
7	8·89	11·281	161·16
6	7·63	9·682	161·37
5	6·33	8·033	160·66

dilute hydrochloric acid, the potassium iodide can be titrated directly with standard *N*-bromosuccinimide in the presence of starch solution indicator.[6]

However, the extraction of iodide from such ash mixtures by shaking them with 93 per cent. ethanol appears to be practical,[7,9] the residual ash of potassium carbonate being left in a pasty condition. The methods known so far consist in heating this ash for 2 minutes with a very small amount of potassium nitrate to ensure the complete absence of organic matter.[8] Trace amounts of organic matter remaining in the residue after the ethanol has been evaporated off will cause low results, probably because of the premature reduction of iodate at a later stage with consequent loss of iodine. Any nitrite formed must be removed, as it would interfere with the quantitative oxidation of hydriodic acid to iodic acid by bromine and thus lead to low results.

TABLE VI

DETERMINATION OF IODINE CONTENT OF IODOCHLOROHYDROXYQUINOLINE TABLETS BY THE PROPOSED METHOD

One 380-mg tablet contains 250 mg of iodochlorohydroxyquinoline, equivalent to 103·91 mg of iodine

Volume taken/ml	Titre of 0·02 N *N*-bromosuccinimide/ml	Iodine found in volume taken/mg	Iodine found per 100 ml or one tablet/mg
10	8·09	10·266	102·66
9	7·29	9·251	102·79
8	6·57	8·337	104·21
7	5·75	7·297	104·24
6	4·89	6·205	103·42
5	4·06	5·152	103·04

The results given by the proposed method are not influenced by the presence of either organic matter or nitrite. In addition, the step in which iodide is oxidised to iodate is avoided. Also, the method has the advantage that no potassium iodide is added, and in consequence there is no risk of obtaining false results from the accidental presence of oxidising agents in the final stage.

The proposed method is sensitive and enables minute amounts of iodine ranging from 20 to 5 mg to be accurately determined with an experimental error that does not exceed ±2 per cent. (Tables I to IV). It has also been applied to the assay of two types of tablets available on the market, and gave reproducible results.

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The Determination of Some Aromatic Phenols with *N*-Bromosuccinimide

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A method for the determination of phenol, vanillin and thymol by titration with standard *N*-bromosuccinimide solution is described. The method is sufficiently sensitive to enable amounts ranging from 0·1 to 10 mg of the phenols to be determined. The experimental error does not exceed ±2 per cent. Results for determinations by the proposed method and by previously recognised methods are compared.

Phenols that are extensively used in medicine as well as in industry include phenol, vanillin and thymol.[1] The recognised method in which bromine is used is carried out on at least 50 mg of phenol.[2] No reports have been found in the literature on the titrimetric determination of vanillin, but previously known methods include the use of colorimetric,[3] gravimetric[4] and polarographic[5] techniques. In the determination of phenols by titration with iodine an accuracy of 3 per cent. is claimed for thymol.[6]

The present work describes a method for the determination of these phenols in amounts as little as 100 μg that involves the use of standard *N*-bromosuccinimide solution.

Experimental

Reagents—

The three aromatic phenols used in the present study conform to the requirements of the British Pharmacopoeia, 1963. Pure *N*-bromosuccinimide (Hopkin & Williams Ltd., Chadwell Heath, Essex) was used and the standard aqueous solution was freshly prepared.

Procedure—

To an accurately measured volume, *e.g.*, 5 ml, of phenol or vanillin solution contained in a 100-ml conical flask, and equal volume of 0·1 M disodium hydrogen orthophosphate solution and 5 drops of bromothymol blue indicator solution were added. For thymol solution, equal volumes of 10 per cent. v/v dilute sulphuric acid and 10 per cent. w/v potassium bromide solution were added followed by 3 drops of methyl red indicator solution.

The mixture was titrated with 0·5, 0·1 or 0·05 per cent. w/v *N*-bromosuccinimide solution, depending on the phenol and its concentration. The titrant was added dropwise from a microburette, the mixture being continuously shaken. The end-point is easily detected when the blue colour of bromothymol blue just changes to yellow with phenol and vanillin and the red colour of the methyl red is just discharged with thymol. The volume of the titrant is noted. A blank experiment is carried out simultaneously and the reading is subtracted from the titre before calculation, the content of the sample solution being calculated as follows.

$$\text{Phenol content (mg or } \mu\text{g)} = V \times C \times \frac{94{\cdot}11}{534}$$

$$\text{Vanillin content (mg or } \mu\text{g)} = V \times C \times \frac{152{\cdot}15}{178}$$

$$\text{Thymol content (mg or } \mu\text{g)} = V \times C \times \frac{150{\cdot}22}{356}$$

where V ml is the titre of *N*-bromosuccinimide solution and C mg ml^{-1} or μg ml^{-1} is the concentration of the *N*-bromosuccinimide solution.

Stoicheiometry of the reactions—

When the recommended procedure was used, phenol (10 to 50 μmol), vanillin (10 to 100 μmol) and thymol (10 to 100 μmol) reacted with exactly three, one and two equivalents of *N*-bromosuccinimide, respectively.

TABLE I

DETERMINATION OF PHENOL BY THE PROPOSED METHOD

Volume of solution taken/ml 0·1 per cent. solution	Phenol content/mg	Titre of *N*-bromosuccinimide solution/ml 0·5 per cent. w/v solution*	Phenol found/mg	Error, per cent.
5	5	5·72	5·04	0·80
4	4	4·52	3·98	0·50
3	3	3·42	3·01	0·33
2	2	2·27	2·00	0·00
1	1	1·15	1·01	1·00
0·01 per cent. solution	Phenol content/μg	0·1 per cent. w/v solution†	Phenol found/μg	Error, per cent.
10	1000	5·69	1003	0·30
9	900	5·12	902	0·22
8	800	4·55	802	0·25
7	700	3·97	700	0·00
6	600	3·41	601	0·17
5	500	2·84	500	0·00
4	400	2·27	400	0·00
3	300	1·70	300	0·00
2	200	1·13	199	0·50
1	100	0·56	99	1·00

* 1 ml of 0·5 per cent. *N*-bromosuccinimide solution ≡ 0·8812 mg of phenol.
† 1 ml of 0·1 per cent. *N*-bromosuccinimide solution ≡ 176·2 μg of phenol.

APPLICATIONS AND RESULTS

DETERMINATION OF THE PHENOLS—

Phenol, vanillin and thymol were determined independently in solutions at concentrations varying from 0·1 to 0·01 per cent. by the proposed methods with 0·5 or 0·1, 0·1 or 0·05 and 0·1 or 0·05 per cent. w/v *N*-bromosuccinimide solution, respectively. The results are shown in Tables I, II and III, respectively.

TABLE II

DETERMINATION OF VANILLIN BY THE PROPOSED METHOD

Volume of solution taken/ml 0·1 per cent. solution	Vanillin content/mg	Titre of *N*-bromosuccinimide solution/ml 0·1 per cent. w/v solution*	Vanillin found/mg	Error, per cent.
10	10	11·75	10·04	0·40
9	9	10·55	9·02	0·22
8	8	9·35	7·99	0·13
7	7	8·20	7·01	0·14
6	6	7·00	5·98	0·33
5	5	5·85	5·00	0·00
4	4	4·65	3·97	0·75
3	3	3·55	3·03	1·00
2	2	2·30	1·97	1·50
1	1	1·15	0·98	2·00
0·01 per cent. solution	Vanillin content/μg	0·05 per cent. w/v solution†	Vanillin found/μg	Error, per cent.
10	1000	2·33	996	0·40
9	900	2·13	910	1·11
8	800	1·88	804	0·50
7	700	1·65	705	0·71
6	600	1·41	603	0·50
5	500	1·18	504	0·80
4	400	0·93	397	0·75
3	300	0·70	299	0·33
2	200	0·47	201	0·50
1	100	0·23	98	2·00

* 1 ml of 0·1 per cent. *N*-bromosuccinimide solution ≡ 0·8548 mg of vanillin.
† 1 ml of 0·05 per cent. *N*-bromosuccinimide solution ≡ 427·4 μg of vanillin.

TABLE III

DETERMINATION OF THYMOL BY THE PROPOSED METHOD

Volume of solution taken/ml 0·1 per cent. solution	Thymol content/mg	Titre of *N*-bromosuccinimide solution/ml 0·1 per cent. w/v solution*	Thymol found/mg	Error, per cent.
10	10	23·80	10·04	0·40
9	9	21·40	9·03	0·33
8	8	19·00	8·02	0·25
7	7	16·60	7·01	0·14
6	6	14·25	6·01	0·17
5	5	11·85	5·00	0·00
4	4	9·50	4·01	0·25
3	3	7·15	3·02	1·67
2	2	4·75	2·00	0·00
1	1	2·40	1·01	1·00
0·01 per cent. solution	Thymol content/μg	0·05 per cent. w/v solution†	Thymol found/μg	Error, per cent.
10	1000	4·75	1002	0·20
9	900	4·27	901	0·11
8	800	3·80	802	0·25
7	700	3·33	703	0·43
6	600	2·87	606	1·00
5	500	2·40	506	1·20
4	400	1·92	405	1·25
3	300	1·42	300	0·00
2	200	0·95	200	0·00
1	100	0·48	101	1·00

* 1 ml of 0·1 per cent. *N*-bromosuccinimide solution ≡ 0·422 mg of thymol.
† 1 ml of 0·05 per cent. *N*-bromosuccinimide solution ≡ 211 μg of thymol.

COMPARISON OF DETERMINATION OF PHENOL BY THE PROPOSED METHOD AND BY THE OFFICIAL B.P. METHOD—

Results obtained by the proposed method and by the official bromine method[2] for the determination of known amounts of phenol ranging from 50 to 10 mg with 1 per cent. *N*-bromosuccinimide solution and 0·1 N bromine solution, respectively, are compared in Table IV.

TABLE IV

COMPARISON OF RESULTS FOR DETERMINATION OF PHENOL BY THE PROPOSED METHOD AND BY THE OFFICIAL BROMINE METHOD

Content/mg	Found by the proposed method*/mg	Error, per cent.	Found by the bromine method†/mg	Error, per cent.
50	49·31	1·38	50·33	0·66
40	40·09	0·23	40·30	0·75
30	30·29	0·97	30·26	0·87
20	20·08	0·40	20·23	1·15
10	10·04	0·40	9·72	2·80

* 1 ml of 1 per cent. *N*-bromosuccinimide solution ≡ 1·762 mg of phenol.
† 1 ml of 0·1 N bromine solution ≡ 1·568 mg of phenol.

COMPARISON OF DETERMINATION OF THYMOL BY THE PROPOSED METHOD AND BY THE IODINE METHOD—

The determination of thymol in amounts varying from 10 to 1 mg was carried out simultaneously by the proposed method and by the previously known method.[6] The results obtained are compared in Table V.

EXPERIMENTAL ERROR—

The experimental error of the proposed method does not exceed ±2 per cent. when determining amounts of phenol, vanillin or thymol ranging from 10 mg to 100 μg (Tables I, II and III).

TABLE V

COMPARISON OF RESULTS FOR DETERMINATION OF THYMOL BY THE PROPOSED METHOD AND BY THE IODINE METHOD

Content/mg	Found by the proposed method/mg	Error, per cent.	Found by the iodine method*/mg	Error, per cent.
10	10·02	0·20	10·13	1·30
9	9·01	0·11	9·15	1·67
8	8·02	0·25	8·09	1·13
7	7·01	0·14	7·08	1·14
6	6·01	0·17	6·14	2·33
5	5·00	0·00	5·15	3·00
4	4·01	0·25	4·07	1·75
3	3·00	0·00	3·07	2·33
2	2·00	0·00	2·07	3·50
1	1·00	0·00	1·03	3·00

* 1 ml of 0·01 N iodine solution ≡ 0·7511 mg of thymol.

DISCUSSION

It is obvious that the experimental error of the previously known methods increases with a decrease in the amount of the aromatic phenol (Tables IV and V).

The proposed method is based on the fact that *N*-bromosuccinimide acts on phenols as a brominating agent, as previously reported.[7] *N*-Bromosuccinimide reacts with phenol, vanillin and thymol to form 2,4,6-tribromophenol, 5-bromovanillin[8,9] and 2,4-dibromothymol,[10] respectively. In each instance, succinimide is also formed as a reaction product.

The proposed method is simple, rapid and sufficiently sensitive to permit amounts as little as 100 μg of the aromatic phenol to be determined. The experimental error does not exceed ± 2 per cent. (Tables I, II and V).

Comparative determinations of phenol and thymol by the proposed method and by previously known methods have shown that the experimental error of the iodine method increases to about 3·5 per cent. with small amounts of thymol (Table V).

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Automated Enzymic Determination of Oxalic Acid in Human Serum*

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An automated enzymic method is described for the determination of microgram amounts of oxalic acid. The acid is decarboxylated by a specific enzyme, oxalate decarboxylase (E.C.4.1.1.2), and the carbon dioxide evolved is measured colorimetrically in a modified Technicon AutoAnalyzer. The minimum detectable concentration of oxalic acid was about 5 μg per 100 ml. For serum it was necessary to remove protein first by ultrafiltration as it interfered with the recorder base-line readings. Phosphate and sulphate ions inhibited enzymic activity, which was allowed for by adding appropriate concentrations of these ions to the standard oxalate solutions used for calibration. The concentration of oxalic acid in normal human serum ultrafiltrates ranged from 80 to 140 μg per 100 ml (mean 118 μg per 100 ml). Similar values were observed for horse serum. The recovery of microgram amounts of oxalic acid added to serum averaged 110 per cent. The coefficient of variation of replicate determinations of oxalic acid was ±2 per cent. for aqueous solutions and ±5 per cent. for serum ultrafiltrates.

Early attempts to measure the oxalate content of human blood relied upon the precipitation of oxalic acid as its calcium salt, followed by titration with permanganate.[1–3] These methods yielded values ranging from 3 to 10 mg per 100 ml but they were soon shown to be too high[4] and subsequent improvements in analytical techniques led to a progressive lowering of the estimated levels in normal blood.[5–8] The most recent values observed in this laboratory, by using a chemical method, ranged from 100 to 235 μg per 100 ml (mean 146 μg).[9,10] Still lower values, ranging from 3 to 100 μg per 100 ml, have been derived from measurements with ^{14}C-labelled oxalic acid.[11–13]

Crawhall and Watts[14] used a specific enzyme, oxalate decarboxylase, to determine oxalic acid in human plasma. They measured the carbon dioxide evolved in a Warburg constant-volume respirometer and concluded that the normal level is less than 0·8 mg per 100 ml, this being the limit of detection of their method. We have used the principle of enzymic decarboxylation in the present study, but the sensitivity of the method was greatly increased by using a modified Technicon AutoAnalyzer procedure for the measurement of carbon dioxide.

Method

Principle—

Serum was de-proteinised by ultrafiltration and, after removal of carbon dioxide, it was sampled in a Technicon AutoAnalyzer. The sample stream was mixed with buffer and enzyme and the liberated carbon dioxide was passed into a weakly alkaline solution of phenolphthalein. The colour change was recorded automatically.

Reagents—

All reagents were prepared with de-mineralised distilled water.

Horse blood—Fresh defibrinated blood from individual horses was supplied by Tissue Culture Services Ltd., Slough, Bucks.

Oxalate decarboxylase—Sigma Chemical Co. Ltd. The enzyme was dissolved in 0·001 N hydrochloric acid that had been freshly boiled and cooled. This solution contained approximately 2 units ml^{-1} of activity. (One unit will convert 1 μmol min^{-1} of oxalate into formate and carbon dioxide at pH 3·0 and 37 °C.[15]) After determining the activity in the Auto-Analyzer, by using a 600 μg per 100 ml oxalic acid standard (see below), the solution was diluted with 0·001 N hydrochloric acid to give 1 unit ml^{-1} of activity and a recorder peak of approximately 80 per cent. transmission above the base-line.

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

Citrate buffer solution, pH 3·0—A 1 M stock buffer solution was prepared by dissolving 210 g of citric acid dihydrate and 18·5 g of sodium hydroxide in 1 litre of water. A working buffer was prepared by mixing 50 ml of the stock buffer solution with 6 ml of 0·9 per cent. EDTA (dipotassium salt) in a stoppered flask and diluting the mixture to 5 litres with 0·001 N hydrochloric acid. After adding a few anti-bumping granules, the solution was boiled gently for 5 minutes, and the flask was stoppered and connected to a soda lime trap containing 20 g of coarse-grade Carbosorb (B.D.H. Ltd.) and allowed to cool overnight. Immediately before use a rapid stream of carbon dioxide free air was bubbled through the solution to remove the last traces of carbon dioxide.

Phenolphthalein reagent—This was prepared electrolytically in a two-compartment cell constructed from Perspex (Fig. 1). The compartments were separated by a Permaplex C20 cation-exchange membrane and the assembly was held together with clamps (not shown in the figure).

The electrolytes were prepared the day before use as follows.

Anode electrolyte—A few anti-bumping granules were added to 2 litres of freshly distilled de-mineralised water contained in a flask. A saturated aqueous solution of sodium chloride (50 ml) was added and the mixture was boiled for 2 minutes; 2 ml of 1 N hydrochloric acid were then added and boiling was continued for a further 2 minutes, after which time the flask was closed with a Carbosorb trap and allowed to cool.

Cathode electrolyte—A few anti-bumping granules were added to another 2-litre volume of freshly distilled de-mineralised water. The water was boiled for 2 minutes, then 36 mg of phenolphthalein were added with a microscope cover-slip. After the phenolphthalein had dissolved completely, 2 ml of 1 N hydrochloric acid were added and boiling was continued

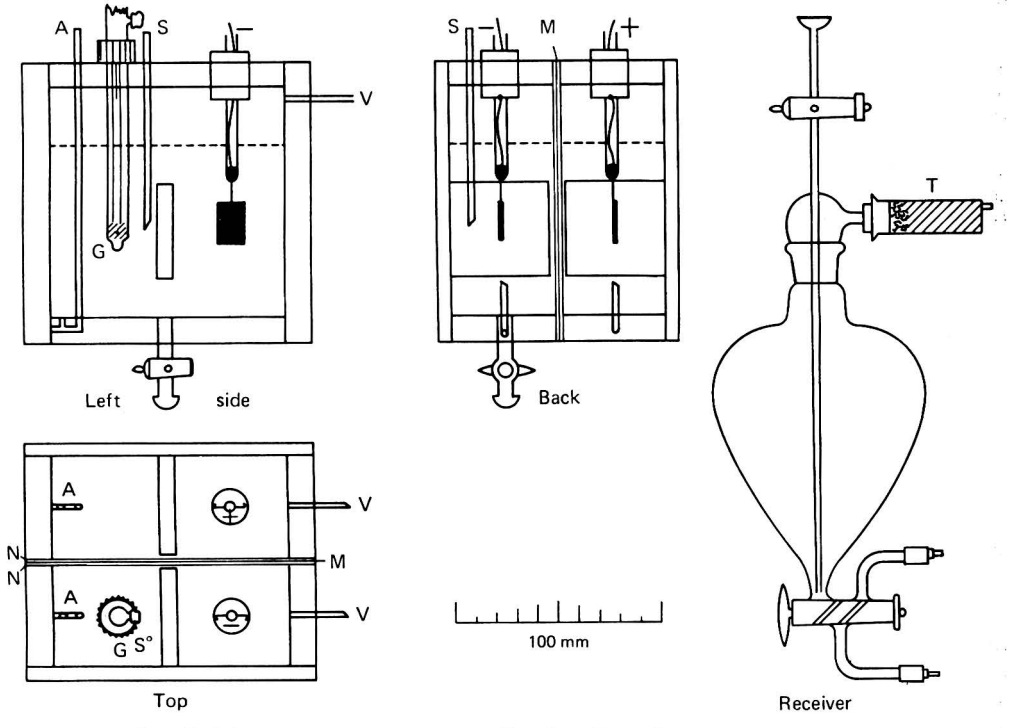

Fig. 1. Electrolytic cell for preparation of phenolphthalein reagent

for a further 2 minutes, after which time the flask was closed with a Carbosorb trap and allowed to cool.

The next day, the cell, with the receiver connected below, was flushed well with carbon dioxide free air and the electrode was standardised to pH 3·0 with 0·001 N hydrochloric acid. Both compartments were filled to the 500-ml level with their respective electrolytes and the filling holes plugged with bungs carrying the platinum electrodes. Aeration was then continued for 5 hours. The platinum electrodes were connected to a 70-V dry battery and, after temporarily disconnecting the pH meter to avoid damage, a current was passed until a faint pink colour persisted. The pH was now 7·0 to 7·5 and further current was passed for periods of 10 s at a time until the pH reached 9·5 and remained stable at this value for at least 5 minutes.

About 5 ml of reagent were passed into the receiver and discarded. This procedure was repeated several times until the reagent showed no loss of colour on standing for 20 s. The remaining reagent was then collected and, after closing the taps, the receiver was removed and connected to the AutoAnalyzer. The top opening of the two-way tap was connected to a short Tygon tube that dipped into de-mineralised distilled water and, by attaching a syringe to the pump connection, the section to the pump was primed with bubble-free water. On starting, the sytem pumped water for a few minutes while final checks were made; then the two-way tap was turned through 180° to allow the reagent to flow.

Antifoam emulsion—Antifoam emulsion M30 (Midland Silicones Ltd.) was diluted with 0·001 N hydrochloric acid to give an approximately 5 per cent. v/v solution. The mixture was shaken in a flask, which was then placed in an ultrasonic cleaning bath for several minutes to form a stable emulsion. Any unemulsified material was separated by decanting the homogeneous liquid.

Oxalic acid standards—Oxalic acid dihydrate (0·14 g) was dissolved in 100 ml of 0·001 N hydrochloric acid. This solution was prepared freshly before use and contained 1 mg ml^{-1} of anhydrous oxalic acid. Working standards were prepared by mixing 0·2, 0·4 and 0·6 ml of the concentrated oxalic acid solution with 1 ml of phosphate - sulphate solution and diluting the mixture to 100 ml with 0·001 N hydrochloric acid. These solutions contained 200, 400 and 600 μg of anhydrous oxalic acid per 100 ml, respectively. The phosphate - sulphate solution contained 15·35 g of potassium dihydrogen orthophosphate and 67·1 g of potassium sulphate per litre of 0·001 N hydrochloric acid. After this 100-fold dilution, the concentrations of phosphate and sulphate in the solution corresponded to those in normal serum, namely, 3·5 mg of phosphate (as phosphorus) and 1·2 mg of sulphate (as sulphur) per 100 ml.

APPARATUS—

This was a modification of the Technicon apparatus used for the determination of carbon dioxide in serum or plasma (Method N-8a). The sample was passed into a segmented stream of buffer at pH 3·0 and oxalate decarboxylase was then added (Fig. 2). After passing through a small mixing coil the stream entered a standard 40-foot coil at 37 °C and the carbon dioxide evolved from oxalic acid entered the gas phase.

$$(COOH)_2 \rightarrow CO_2 + H.COOH$$

Approximately 83 per cent. of the gas phase was recovered in a modified gas separator (see below) and was used to segment a stream of phenolphthalein reagent at pH 9·5. The stream was then passed through a small mixing coil to the de-bubbler and flow-through cell of the colorimeter, and the loss in optical density at 550 nm was recorded.

Sensitivity was approximately 600 times greater than that of the original Technicon method (N-8a). This was achieved by using 2·0 ml of sample instead of 0·2 ml, a very sensitive phenolphthalein reagent and electrical scale expansion by means of a selection of resistors, ranging from 300 to 1500 Ω, in series with the recorder slide-wire. In order to obtain accurate and reproducible results at this high level of sensitivity it was necessary to provide the sampler with a carbon dioxide free environment. This was achieved by fitting a Perspex glove-box, with a capacity of 120 litres, over the sampler and providing this with carbon dioxide free air at the rate of 12 l min^{-1}.

Polythene and PVC tubing were found to be slightly permeable to carbon dioxide and glass tubing was therefore used, where possible. However, polythene was found to be satisfactory for the tubes carrying buffer and enzyme solutions at pH 3·0 because atmospheric

carbon dioxide was not absorbed. Polythene tubing was also suitable for short connections if wrapped with adhesive aluminium tape. A piece of Portex PP260 polythene tubing was used to carry the phenolphthalein reagent from the receiver to the peristaltic pump where flexibility was required. This tube was covered loosely with a length of PVC sleeving, which was supplied with a stream of carbon dioxide free air.

The air used for segmentation was first washed with water to remove any vapours from the laboratory to which the reagent was sensitive, particularly those of diethylamine, cyclohexanone and acetone. After passing through a large Carbosorb trap the air passed through Tygon tubing to the pump and then through a miniature Carbosorb trap and cotton-wool filter immediately before injection into the glass section of the manifold.

Gas separator and filter—The Technicon gas separator was re-designed in order to minimise the formation of aerosol (containing pH 3·0 buffer), and the last traces of aerosol were removed by passing the gas through a suitable filter (Fig. 3). The gas separator was constructed from Perspex. The tubing from the heating bath to the interior of the gas separator had a constant bore so as to ensure a steady minimum pressure on the segmented stream that entered the unit. In addition, an ultrasonically treated thin emulsion of silicone antifoam was injected into the stream just before it entered the unit, and buffer solution (pH 3·0) was pumped into the upper part of the unit at the rate of 3·9 ml min^{-1}. These measures effectively prevented the rising of surface films and minimised the formation of aerosols. The vent was approximately $\frac{1}{4}$ inch long and was made to empty steadily, and without surging, by the passage of a slow stream of carbon dioxide free air.

The filter consisted of 14-mm Whatman glass paper GF/C, which was held in a Perspex block heated to 36 °C (Fig. 3). Heating was accomplished by including a second 40-foot coil in the heating bath module and circulating water by means of a small centrifugal pump. The gas passed down a central narrow-bore tube where it was warmed before passing through the glass paper.

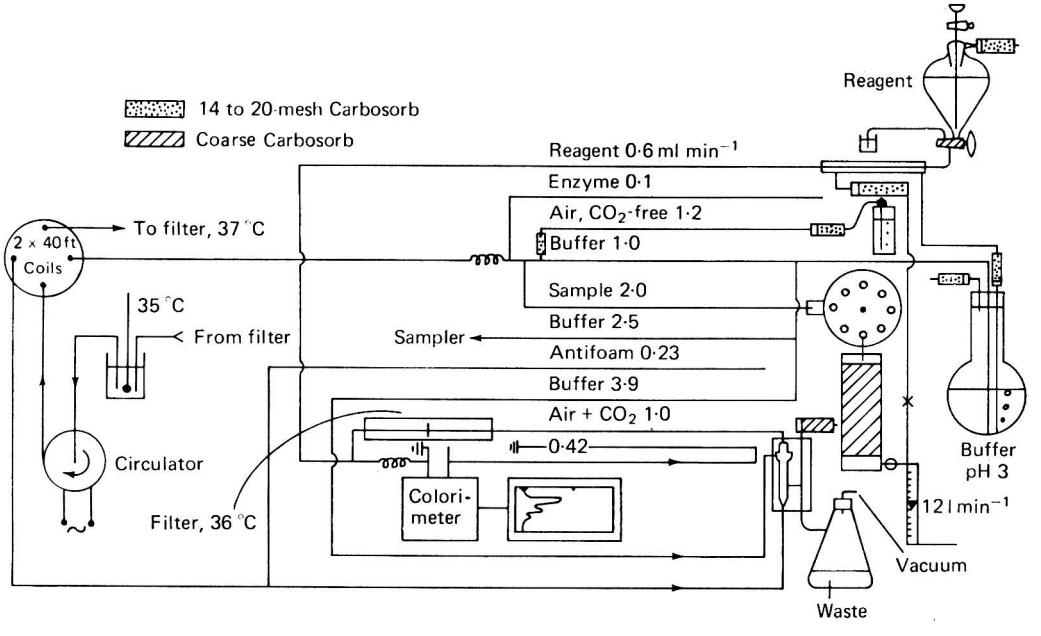

Fig. 2. Flow diagram for the automatic determination of serum oxalic acid

PROCEDURE—

Serum (10 ml) was de-proteinised by ultrafiltration through a Centriflo CF50 Membrane Ultrafilter (Amicon Corporation, Lexington, U.S.A.) and the ultrafiltrate was collected in a 25-ml test-tube fitted with a glass stopper. After testing it with salicylsulphonic acid for

the absence of protein, the solution was adjusted to pH 3·0 with concentrated hydrochloric acid and 50 per cent. w/v sodium hydroxide solution by using a glass electrode. A single anti-bumping granule was added and the solution was boiled gently for 30 s to denature any residual protein that might be present and to assist in the removal of carbon dioxide present as hydrogen carbonate. After cooling, the tube was agitated in a Vortex mixer for 3 minutes, during which time a strong jet of carbon dioxide free air from a pipette was introduced at the base of the vortex. This operation was found to remove all but occasional traces of carbon dioxide. The tube was then stoppered and stored at 4 °C in a sealed jar containing Carbosorb soda-lime until the determination could be completed. Standard solutions adjusted to pH 3·0 were treated in a similar manner.

For analysis, the tube was re-opened inside the carbon dioxide free glove-box and any additions of oxalate solutions for recovery checks were made at this stage. The apparatus was run first without enzyme at thirty samples per minute in order to obtain blank readings for all the samples, standards and additives. The sample plate was then re-loaded and the run was repeated with enzyme present.

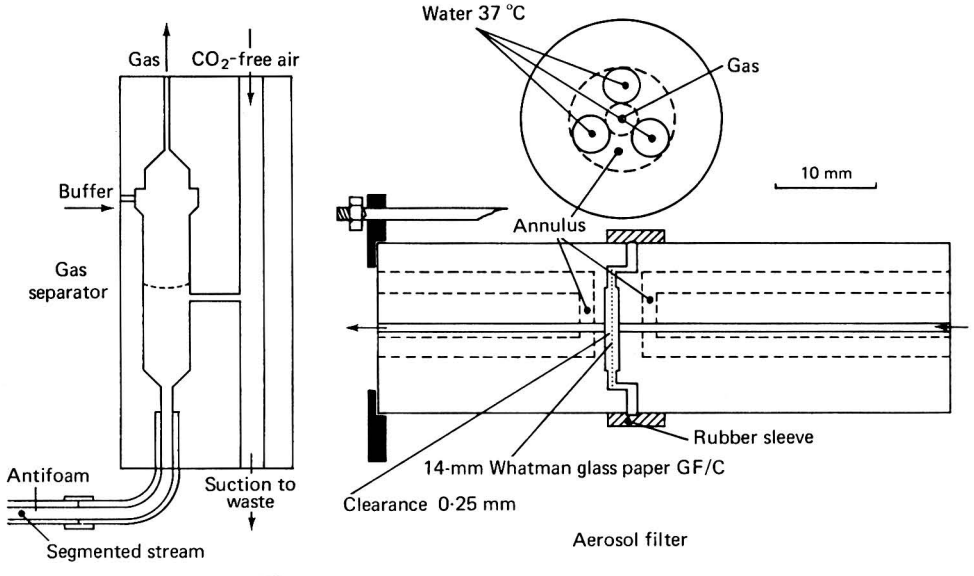

Fig. 3. Details of gas separator and aerosol filter

Results

Sensitivity and reproducibility of the method—

Fig. 4 shows a typical calibration graph and also the blank readings given by the samples without enzyme present. A series of repeated determinations on the same sample of serum ultrafiltrate is also shown to indicate the reproducibility of the method.

The minimum detectable concentration of oxalic acid was about 5 μg per 100 ml and full-scale deflection occurred with about 600 μg per 100 ml. The coefficient of variation of replicate determinations of oxalic acid was ± 2 per cent. for aqueous solutions and ± 5 per cent. for serum ultrafiltrates.

Inhibition by phosphate and sulphate ions—

The effect of different concentrations of phosphate and sulphate ions on the recovery of oxalic acid from aqueous solution is shown in Fig. 5. The two ions had an equal effect on oxalate recovery, when expressed in molar concentrations. The concentrations of phosphate and sulphate in urine (shown in the figure) are sufficient to cause losses of 50 per cent. or more in the recovery of oxalic acid. Serum, on the other hand, contains much lower concentrations of these ions, with correspondingly smaller losses.

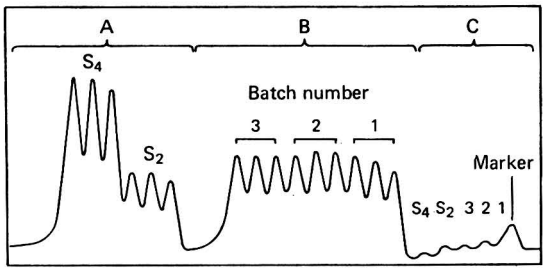

Fig. 4. Recorder tracing of standard oxalic acid solutions and replicate determinations on the same sample of fresh horse serum: A, standards $S_4 = 400$ and $S_2 = 200$ μg per 100 ml; B, horse serum ultrafiltrate; and C, blanks. The small peaks on the right are reagent blanks. Batch numbers refer to different samples of horse serum

Preliminary studies of enzyme kinetics indicated that both phosphate and sulphate ions are competitive inhibitors of oxalate decarboxylase activity. Thus, a graph[16] of $1/v$ against $1/s$ became steeper in the presence of 10 and 20 mM phosphate or sulphate ions but the intercept on the vertical axis remained almost the same.

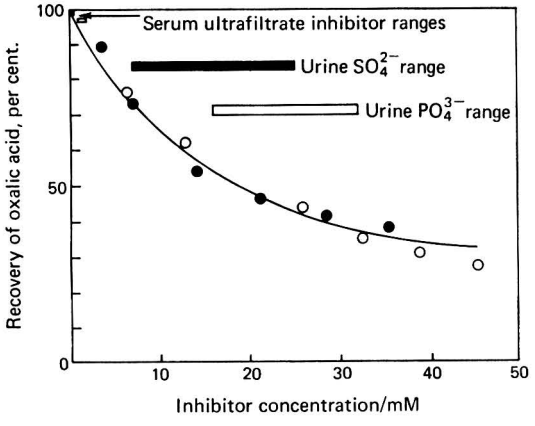

Fig. 5. Effect of different concentrations of phosphate and sulphate ions on the recovery of oxalic acid from aqueous solution: o, sulphate; and ●, phosphate

RECOVERY OF OXALIC ACID FROM SERUM AND SERUM ULTRAFILTRATES—

The concentration of oxalic acid observed in normal human serum ranged from 0 to 40 μg per 100 ml but the recovery of added oxalic acid varied from 25 to 70 per cent. Ultrafiltrates of serum from the same subjects yielded much higher values. Moreover, the recovery of oxalic acid added to the ultrafiltrates averaged 110 per cent. (Table I).

The lower values obtained with serum were caused by alterations in the recorder base-line. These, in turn, appeared to result from an effect of protein on the formation of aerosols in the gas separator, the aerosol being more completely removed when protein is present.

SERUM OXALATE LEVELS IN MAN AND IN THE HORSE—

Table I shows the concentrations of oxalic acid that were observed in ultrafiltrates of serum from twenty normal human adults and eleven horses. In man, the values ranged from 80 to 148 μg per 100 ml (mean 118 μg per 100 ml). A similar range and mean were observed in horses.

TABLE I

SERUM OXALATE LEVELS IN NORMAL HUMAN ADULTS AND HORSES

Man					Horse	
Subject	Age	Sex	Oxalic acid/ μg per 100 ml*	Recovery, per cent.	Subject	Oxalic acid/ μg per 100 ml*
A.H.	48	M	125	—	1	118
A.Hol.	50	F	132	—	2	105
A.W.	28	F	148	120	3	118
C.G.	30	M	135	107	4	138
D.A.	24	M	90	103	5	159
D.Ma.	33	M	119	—	6	133
D.Mi.	32	M	114	110	7	101
F.K.	54	M	145	109	8	92
H.C.	30	M	118	—	9	170
J.M.	25	F	120	—	10	117
J.T.	21	M	85	—	11	168
M.F.	22	M	80	109		
M.H.	45	F	85	108		
M.O.	23	F	138	—		
P.Bi.	20	M	116	—		
P.Bo.	26	M	95	—		
P.K.	26	M	100	118		
R.W.	27	M	142	103		
V.H.	26	F	134	—		
Y.K.	33	M	140	111		
Mean			118	110		129
Standard deviation			24·1			27·0

* μg of anhydrous oxalic acid per 100 ml of serum ultrafiltrate.

Increased concentrations of oxalate were observed in patients with renal failure and uraemia. The highest values, ranging from 2 to 4 mg per 100 ml, were observed in a patient with renal failure resulting from primary hyperoxaluria. Because of these exceptionally high concentrations it was possible to compare the present results with those obtained by a more conventional colorimetric procedure.[17] The results showed good agreement (Table II).

TABLE II

COMPARISON OF SERUM OXALATE LEVELS DETERMINED BY CHEMICAL AND ENZYMIC METHODS IN A PATIENT WITH PRIMARY HYPEROXALURIA AND TERMINAL RENAL FAILURE

	Oxalic acid/mg per 100 ml*	
Sample	Present method	Chemical method[17]
1	2·5	2·3
2	2·9	2·5
3	3·1	2·7
4	3·2	2·9
5	3·3	3·2
6	3·3	3·6

* mg of anhydrous oxalic acid per 100 ml of serum ultrafiltrate.

DISCUSSION

The mean concentration of oxalic acid in normal human serum was found to be 118 μg per 100 ml. The mean recovery of oxalic acid added to serum (110 per cent.) suggests that this value may be a little too high and that the true mean value is nearer to 110 μg per 100 ml, which compared with an earlier mean value of 146 μg per 100 ml obtained in this laboratory by using a fluorimetric method.[9,10]

The lower values obtained in the present study may result from the greater specificity of the enzymic method compared with existing chemical methods. However, the present values are still higher than most of the values obtained by isotopic methods. The latter values, ranging from 3 to 10 μg per 100 ml[11–13] are based on several assumptions, for example, that the miscible pool of oxalic acid in man (about 5 mg of anhydrous oxalic acid) is distributed throughout the total body water, or at least extracellularly.[11] Such assumptions may not

be justified, however, as oxalic acid is a relatively strong acid ($pK_{a_1} = 1{\cdot}23$; $pK_{a_2} = 4{\cdot}19$) and, in consequence, would not be expected to pass freely through biological membranes. This problem is being studied further.

The presence of raised oxalate levels in renal failure has been observed previously[14,18] and was confirmed in the present study. Oxalic acid in urine is derived partly from the diet and partly as an end-product of the metabolism of amino-acids and other compounds within the body. Elevation of the serum oxalate level in renal failure is presumably caused by the rate of excretion falling below the rate of intestinal absorption or the rate of endogenous production of oxalate, or both. The highest serum oxalate levels were observed in a patient with renal failure resulting from primary hyperoxaluria, a condition in which there is a marked increase in the production of endogenous oxalate.[19] Because of the marked elevation of the oxalate level in this particular patient it was possible to compare the results obtained by the present method with those given by a more conventional chemical method[17] (Table II). The results show good agreement, allowing for the fact that the methods are based on two entirely different principles.

The method was also applied to human urine and gave results that agreed well with a conventional chemical method.[17] Urine was diluted twenty times with citrate buffer (pH 3·0) and treated in the same way as serum ultrafiltrate. The same oxalic acid standards, containing phosphate and sulphate, were used, as for serum. Urine samples from ten different subjects gave a mean value of 1·86 mg per 100 ml (standard deviation 0·44 mg per 100 ml). This compared with a mean value of 2·00 mg per 100 ml (standard deviation 0·74 mg per 100 ml) by the chemical method. The mean recovery of oxalic acid added to the same urine samples (2·0 mg per 100 ml of urine) was 98·2 per cent. (standard deviation 3·7 per cent.).

A completely automated method for determining oxalate in urine was investigated. Untreated urine was sampled automatically in the AutoAnalyzer and diluted twenty times with citrate buffer of twice the previous concentration. To remove carbon dioxide, the mixture was pumped at the rate of 3·75 ml min^{-1}, together with a stream of carbon dioxide free air, through 8 m of polythene tubing that had an internal diameter of 1·77 mm. This tubing was made into a vertical coil with a diameter of 25 mm and the mixture, which was fed in at the top, passed through the coil in 15 s. The liquid was separated from the gas phase in a Technicon C4 joint and returned to the AutoAnalyzer as for serum ultrafiltrate. This system yielded satisfactory results but there was increased interaction between samples because of the greater tube length, and it was therefore necessary to run the apparatus at 15 instead of 30 samples per hour in order to avoid interference between recorder peaks.

In addition to its present application, the apparatus described here provides a highly sensitive method for measuring carbon dioxide in a variety of media. It can be used, for example, to monitor atmospheric carbon dioxide, respiratory changes in plant and animal tissues or the small amounts of carbonate present in bone tissues.

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An Investigation into More Rapid Procedures for Preparing Leaf-tissue Samples for Analysis

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Several rapid ashing procedures for the analysis of leaf-tissue samples to determine their inorganic constituents are described. Various types of sample were extracted with different acid mixtures or ashed for short periods under different conditions and their constituents were then determined by means of atomic absorption. The values obtained by these methods were compared with those obtained by use of standard procedures.

A general procedure for the rapid ashing of plant material, which is suitable for the determination of a number of inorganic constituents, is described in detail.

The decomposition or dissolution of the analytical sample may be an integral part of any chemical analysis and no single method has been found that is equally satisfactory for all types of material. To complicate matters, much conflicting information on methods for decomposing organic samples is reported in the literature. Most of this information, however, applies only to inorganic analysis.

Middleton and Stuckey[1,2] described a general procedure for the preparation of biological material for trace analysis but pointed out numerous modifications for different types of samples. They also presented a critical review of existing procedures for the decomposition of biological material. From their work it is clear that before deciding upon a specific decomposition procedure, consideration should be given to the type of material; the elements to be determined; the required precision and accuracy of results; the size of sample and whether a routine procedure is to be followed; and the time, cost and amount of attention required.

When decomposing biological material it is accepted that unless appropriate precautions are taken many elements are partially or totally lost. Although this subject has been studied in considerable detail the results are inconclusive for losses of inorganic constituents because of the use of a dry-ashing procedure. These losses generally occur because of matrix effects, the ashing temperature and the material of the vessel used for ashing, as described below.

Most of the conflicting information regarding the loss of different elements that appears in the literature results from the different types of biological samples investigated. It is obviously essential to treat each type of sample individually, and it is not advisable to accept losses reported for a particular element that have been obtained on significantly different matrices.

A general survey of the literature[3–6] indicates that a maximum ashing temperature of 450 °C produces negligible losses for the majority of elements, exceptions being compounds of arsenic, mercury and the halogens. However, to illustrate the problems involved in maintaining such a temperature, Hamilton, Minski and Cleary[7] showed that a variation of ± 50 °C at an indicated temperature of 450 °C could exist in muffle furnaces, depending on the position of the sample in the furnace.

As regards the material from which the ashing vessel is made, the analysis of residues from platinum dishes usually shows trace amounts of platinum, and unless the dishes have been cleaned thoroughly with hydrochloric acid they will show other contaminations as well. The use of porcelain, Vycor or silica dishes may also lead to problems as samples containing phosphorus etch the surface and some elements form insoluble silicates. It has also been reported[8,9] that some elements, including magnesium, copper and zinc, are lost by absorption on to silica.

The wet decomposition of biological material, on the other hand, receives almost universal application. The use of a single acid for decomposing a biological sample is desirable, but usually not practical. From radiochemical studies conducted by Gorsuch[3] on the recovery

of trace elements following the oxidation of organic matter, it was concluded that a mixture of nitric and perchloric acids forms the basis of the most satisfactory of all oxidation methods.

When deciding upon a specific method for decomposing biological material when a large number of samples are to be analysed on a routine basis, a dry-ashing procedure is invariably preferred. In comparison with a wet-oxidation procedure it requires very little attention and also larger amounts of material can be conveniently used. Further, when use is being made of automated procedures for the determination of the elements, the decomposition stage will determine the rate at which results will become available. The existing decomposition procedures, both dry and wet, are too tedious and slow for this purpose; consequently, an investigation has been carried out to modify existing procedures and to establish more rapid methods for preparing leaf-tissue samples for analysis.

Procedure and results

Sample preparation—

Plant-tissue samples were dried at 65 °C and ground to pass through a 0·8-mm mesh screen. The resulting material was dried at 65 °C for 8 hours and used for all subsequent determinations.

Apparatus—

A Model 303 Perkin-Elmer atomic-absorption spectrophotometer, equipped with a Hitachi QPD 56 recorder, was used for the determination of calcium, magnesium, copper, iron, zinc and manganese, and a Zeiss flame photometer was used for determining sodium and potassium.

Comparison between standard wet and dry-ashing procedures—

In the investigation of different ashing procedures and techniques, citrus leaf-tissue samples were used for all preliminary work; the samples were subsequently extended to include a wide range of different types of leaf-tissue sample.

Citrus leaf-tissue samples were ashed according to the standard methods for wet and dry-ashing procedures as described by the Society for Analytical Chemistry.[10] To minimise the risk of explosion when carrying out a wet digestion with perchloric acid, 1 cm^3 of sulphuric acid was added to the digestion mixture. The results obtained are shown in Table I.

No difference between the results obtained with the standard dry-ashing and the standard wet-ashing procedures is apparent. When performing the wet digestion in the presence of sulphuric acid, however, significant differences between the procedures are observed for all the elements. This is undoubtedly caused by the formation of a bulky calcium sulphate precipitate that carries with it considerable amounts of the elements under determination.

Both the standard dry and wet-oxidation procedures are, however, lengthy and tedious so that the possibility of decreasing the time necessary for the actual ashing stage of the dry-ashing procedure was investigated by attempting to use an ignition process, the elimination of the ashing procedure by direct extraction of finely milled leaf tissue with an acidic solution, and a rapid ashing procedure.

Table I

Comparison between standard dry and wet-oxidation procedures for citrus leaf samples

Sample	Procedure	Cu, p.p.m.	Fe, p.p.m.	Mn, p.p.m.	Zn, p.p.m.	Ca, per cent.	Mg, per cent.
1	Standard dry oxidation	8	62	23	13	1·99	0·67
	Standard wet oxidation—						
	(*a*) without sulphuric acid	8	62	23	14	2·10	0·68
	(*b*) with sulphuric acid	6	48	17	8	0·77	0·60
2	Standard dry oxidation	8	78	28	11	3·72	0·75
	Standard wet oxidation—						
	(*a*) without sulphuric acid	8	80	27	12	3·78	0·75
	(*b*) with sulphuric acid	5	63	19	7	0·67	0·63
3	Standard dry oxidation	24	67	28	23	4·02	0·70
	Standard wet oxidation—						
	(*a*) without sulphuric acid	23	67	28	24	4·19	0·70
	(*b*) with sulphuric acid	16	50	17	9	0·68	0·65

IGNITION PROCESS—

A 2-g sample was weighed into a silica dish, which was placed into a furnace at 450 °C. The sample was ignited and allowed to burn for about 5 minutes with the furnace door open. After the burning had subsided the furnace was closed and the sample ashed at 450 °C. After 2½ hours the sample was removed, cooled, 5 cm³ of concentrated hydrochloric acid were added and the residue evaporated to dryness on a water-bath. It was then dissolved in a mixture of 5 cm³ of 6 M hydrochloric acid and 5 cm³ of 6 M nitric acid, warmed on a water-bath and filtered through a Whatman No. 41 filter-paper into a 100-cm³ calibrated flask. The filter-paper was washed with warm distilled water and the solution diluted to 100 cm³.

Copper, iron, manganese and zinc were determined directly on this solution, while calcium, magnesium and potassium were determined on suitably diluted aliquots of it. The results obtained were compared with those obtained with the standard dry-ashing procedure in which the samples are ashed overnight (see Table II). For most purposes the differences indicated in Table II are negligible in comparison with the advantage gained by the considerably shorter ashing period.

Further, this modified ashing procedure is similar to the widely used method for the determination of metals in petroleum products in which a flame is applied to the surface of a large sample until it ignites[11]; gentle burning is sustained by slightly heating the dish, followed by ashing in the normal manner.

TABLE II

COMPARISON BETWEEN THE STANDARD DRY-ASHING PROCEDURE AND AN IGNITION PROCEDURE FOLLOWED BY ASHING FOR 2½ HOURS

Sample	Procedure	Cu, p.p.m.	Zn, p.p.m.	Fe, p.p.m.	Mn, p.p.m.	Ca, per cent.	Mg, per cent.
1	Standard	6	52	96	88	4·78	0·36
	Ignition	6	50	94	88	4·82	0·36
2	Standard	14	23	150	114	6·69	0·29
	Ignition	12	24	148	115	6·60	0·31
3	Standard	20	12	83	73	7·43	0·47
	Ignition	19	11	80	71	7·49	0·45
4	Standard	8	63	111	140	3·98	0·29
	Ignition	7	60	110	138	4·02	0·27
5	Standard	3	9	65	73	5·67	0·31
	Ignition	3	9	67	74	5·54	0·31

DIRECT EXTRACTION OF FINELY MILLED LEAF TISSUE—

At this stage it was thought that if samples were to be prepared for analysis at an even faster rate, a method other than the conventional ashing procedures would have to be found. Consequently, the direct extraction of finely ground leaf-tissue samples with mineral acids was investigated. Preliminary investigations indicated that higher recoveries could be obtained with solutions of acids than with extraction procedures involving EDTA solutions. Extractions with EDTA not only resulted in lower recoveries but also presented additional difficulties insofar as they caused heavy deposits to accumulate in the atomic-absorption burner slot.

EXTRACTION OF FINELY GROUND LEAF TISSUE WITH 6 M MINERAL ACIDS—

Several 2-g samples were weighed, either into 100-cm³ Pyrex reagent bottles and extracted by shaking with 25 cm³ of 6 M hydrochloric acid at room temperature for 15 minutes, or into 100-cm³ Pyrex beakers and extracted with 25 cm³ of 6 M hydrochloric acid by heating for 15 minutes on a sand-bath.

The resulting solutions were filtered through Whatman No. 41 filter-paper into 100-cm³ calibrated flasks, washed with warm 0·1 M hydrochloric acid and diluted to the calibration mark with more 0·1 M hydrochloric acid. The results obtained on these solutions were compared with those obtained with the standard dry-ashing procedure (Table III).

Although the extraction was not complete, these results indicate that if an extraction with acid is to be used, it is essential to introduce some form of heating. The acid concentration of 6 M also proved to be too high as difficulties such as corrosion and clogging in the atomic-absorption burner head were experienced.

Table III

Comparison between (*a*) the standard dry-ashing procedure and extractions with 25 ml of (*b*) cold hydrochloric acid and (*c*) warm hydrochloric acid

Sample	Procedure	Cu, p.p.m.	Zn, p.p.m.	Fe, p.p.m.	Mn, p.p.m.	Ca, per cent.	Mg, per cent.	K, per cent.
6	(*a*)	9	21	100	54	4·37	0·47	1·00
	(*b*)	6	20	64	48	4·01	0·39	0·92
	(*c*)	8	21	90	50	4·17	0·42	0·91
7	(*a*)	21	67	63	47	5·40	0·41	0·71
	(*b*)	17	58	46	42	5·04	0·31	0·54
	(*c*)	21	60	57	45	5·20	0·35	0·66
8	(*a*)	5	37	63	61	6·02	0·50	1·15
	(*b*)	3	30	51	49	5·12	0·39	0·91
	(*c*)	5	38	55	57	5·83	0·46	0·99

Extraction with 3 M hydrochloric and nitric acids on a sand-bath—

To eliminate the above difficulties, the leaf-tissue samples were then extracted, either with increasing amounts of 3 M hydrochloric acid, or with a solution 3 M with respect to hydrochloric and nitric acids, by heating on a sand-bath for 15 minutes. The results obtained are shown in Table IV. Magnesium and potassium appeared to be completely extracted over the whole range of volumes of 3 M acid used although lower recoveries were obtained for calcium at the lowest and highest volumes of acid. Extractions of copper, manganese and zinc were satisfactory for most purposes; iron, on the other hand, was incompletely extracted.

Table IV

Comparison between (*a*) the standard dry-ashing procedure and (*b*) extraction with increasing amounts of 3 M hydrochloric acid and 3 M hydrochloric acid - nitric acid solutions

Sample	Procedure	3 M HCl/ ml	3 M HCl–HNO_3/ml	Cu, p.p.m.	Fe, p.p.m.	Mn, p.p.m.	Zn, p.p.m.	Ca, per cent.	Mg, per cent.	K, per cent.
9	(*a*)	—	—	24	115	66	45	5·66	0·44	1·04
	(*b*)	4	—	20	73	65	45	3·99	0·44	1·03
	(*b*)	8	—	22	88	67	46	5·13	0·43	1·02
	(*b*)	12	—	23	90	67	46	5·63	0·44	1·04
	(*b*)	20	—	22	94	67	45	5·64	0·44	1·04
	(*b*)	—	4	18	64	64	43	3·47	0·47	1·04
	(*b*)	—	8	23	79	64	42	5·58	0·47	1·04
	(*b*)	—	12	23	73	63	43	5·53	0·46	1·04
	(*b*)	—	20	20	75	60	42	5·27	0·46	1·03

Extraction with 6 M hydrochloric and nitric acids on a sand-bath—

In an attempt to obtain a higher recovery for iron, higher concentrations of acids were used. The results obtained are shown in Table V.

A distinct decrease in the values obtained for all the elements tested is apparent from the results. Upon standing the solution after filtration, a colloidal suspension (which increased with increasing acid concentration) was formed. The decrease in values obtained with an increase in acid concentration could, therefore, be attributed to differences in the viscosities of the different samples. If the extracted samples are allowed to cool completely and are then filtered, clear solutions are obtained. As before, high acid concentrations should be avoided because of unnecessary corrosion and clogging of the atomic-absorption burner head.

For the purposes of determining calcium, magnesium, potassium, copper, zinc and manganese in citrus leaf tissue, extraction of the finely ground sample with 15 cm^3 of 3 M

hydrochloric acid gives satisfactory results, comparable with those obtained by the rather tedious and slow standard procedures.

The element that appears to be the least successfully extracted is iron. This is in accordance with the results obtained by Baker and Greweling,[12] as well as by Knezek and Maier,[11] who indicated the possibility of iron being bound in some partly unextractable form. To investigate the possibility that only Fe^{2+} and not Fe^{3+} ions are extracted, various reducing and complexing reagents were tried, but without success.

TABLE V

COMPARISON BETWEEN (*a*) THE STANDARD DRY-ASHING PROCEDURE AND (*b*) EXTRACTION WITH INCREASING AMOUNTS OF 6 M HYDROCHLORIC ACID AND 6 M HYDROCHLORIC ACID — NITRIC ACID SOLUTIONS

Sample	Procedure	6 M HCl/ml	6 M HCl–HNO_3/ml	Cu, p.p.m.	Fe, p.p.m.	Mn, p.p.m.	Zn, p.p.m.	Ca, per cent.	Mg, per cent.
10	(*a*)	—	—	23	125	58	38	5·33	0·40
	(*b*)	8	—	23	113	59	40	5·37	0·40
	(*b*)	12	—	21	108	58	38	5·33	0·41
	(*b*)	20	—	18	98	50	32	4·85	0·37
	(*b*)	25	—	16	83	50	30	4·67	0·33
	(*b*)	—	8	23	108	57	39	5·27	0·40
	(*b*)	—	12	23	107	53	38	5·36	0·40
	(*b*)	—	20	20	98	50	37	5·17	0·38
	(*b*)	—	25	17	74	44	28	3·80	0·26

RAPID ASHING PROCEDURES—

Because most of the elements can readily be extracted it was thought that if a procedure could be followed in which most of the organic constituents of the sample were destroyed by a very short dry-ashing procedure, followed by extraction of the residue with acid, then iron could be more completely recovered.

A 2-g sample was placed into a furnace at 490 °C, ignited as previously described, the furnace door closed and the sample left at this temperature for 10 minutes. It was then taken out and allowed to cool, after which 10 cm^3 of 4 M hydrochloric acid were added and the dish was placed in a water-bath for 20 minutes. The resulting solution was filtered through a Whatman No. 41 filter-paper and the residue washed with hot 0·1 M hydrochloric acid.

TABLE VI

COMPARISON BETWEEN (*a*) THE STANDARD DRY-ASHING PROCEDURE AND (*b*) A RAPID PROCEDURE INVOLVING THE EXTRACTION OF THE RESIDUE WITH 4 M HYDROCHLORIC ACID

Sample	Procedure	Cu, p.p.m.	Zn, p.p.m.	Fe, p.p.m.	Mn, p.p.m.	Ca, per cent.	Mg, per cent.	K, per cent.
11	(*a*)	81	83	105	52	6·74	0·98	1·06
	(*b*)	61	82	100	52	6·76	0·99	1·06
12	(*a*)	14	78	114	71	4·26	0·51	0·98
	(*b*)	8	76	110	73	4·22	0·48	0·97
13	(*a*)	24	73	103	85	5·64	0·47	1·32
	(*b*)	15	72	99	87	5·70	0·47	1·34
14	(*a*)	45	20	101	52	4·85	0·39	1·82
	(*b*)	33	21	96	55	4·80	0·40	1·86
15	(*a*)	27	16	99	35	5·26	0·63	0·74
	(*b*)	14	17	92	35	5·28	0·61	0·71
16	(*a*)	118	64	71	68	6·24	0·88	0·69
	(*b*)	95	61	66	69	6·20	0·89	0·68
17	(*a*)	27	23	82	47	3·98	1·21	0·98
	(*b*)	14	23	80	47	3·84	1·23	0·99
18	(*a*)	34	17	110	62	5·10	0·46	1·08
	(*b*)	27	20	104	64	5·23	0·46	1·04
19	(*a*)	50	73	80	36	4·64	0·52	0·94
	(*b*)	34	71	76	37	4·66	0·51	0·93
20	(*a*)	14	10	104	66	6·84	0·63	1·38
	(*b*)	6	11	100	68	6·79	0·61	1·39

The actual ashing temperature in this instance is obviously not 490 °C, because higher temperatures are produced during localised and uncontrolled burning of the sample, and although losses of inorganic constituents due to volatilisation could have been expected, none were observed for citrus leaves. This phenomenon can, perhaps, be attributed to the high calcium content of citrus leaf samples. Table VI shows the results obtained by use of this method.

Calcium, magnesium, potassium, zinc and manganese appear to be extracted quantitatively into 4 M hydrochloric acid after destruction of most of the organic material. A definite increase in the amount of iron extracted was also observed. Copper, however, is not quantitatively extracted by this method. In this regard it has been shown[9] that retention of copper on silica basins could be the cause of losses at an ashing temperature of approximately 500 °C. It has further been claimed that this material can be recovered by extraction with a mixture of hydrochloric and nitric acids. In an attempt to verify these findings samples were treated as follows. Some samples were dry ashed according to standard procedure, others were dry ashed according to the rapid procedure and extracted with 4 M hydrochloric acid on a water-bath for 20 minutes, while a third group was dry ashed according to the rapid ashing procedure and extracted with equal volumes of 4 M hydrochloric acid and 4 M nitric acid on a water-bath for 20 minutes.

No difference in the results obtained for calcium, magnesium, potassium, iron, manganese and zinc was observed. The results obtained for copper are shown in Table VII.

TABLE VII

COMPARISON BETWEEN (*c*) STANDARD DRY-ASHING PROCEDURE AND A RAPID DRY-ASHING PROCEDURE FOLLOWED BY EITHER (*d*), EXTRACTION WITH 4 M HYDROCHLORIC ACID, OR (*e*), EXTRACTION WITH A MIXTURE OF EQUAL VOLUMES OF 4 M HYDROCHLORIC AND 4 M NITRIC ACIDS, FOR COPPER

Sample	Procedure	Cu, p.p.m.
21	(*c*)	64
	(*d*)	50
	(*e*)	63
22	(*c*)	87
	(*d*)	75
	(*e*)	87
23	(*c*)	32
	(*d*)	26
	(*e*)	32
24	(*c*)	24
	(*d*)	17
	(*e*)	23
25	(*c*)	48
	(*d*)	31
	(*e*)	47
26	(*c*)	122
	(*d*)	101
	(*e*)	122

It is clear that when a mixture of nitric and hydrochloric acids is used to extract the residue, a very good correlation exists between the standard dry-ashing procedure and the suggested rapid ashing procedure; this makes the rapid ashing procedure very attractive for application when samples are to be prepared quickly for analysis, particularly for routine analysis in which automated procedures are used and congestion usually occurs at the sample preparation stage. If this procedure were followed, one technician could handle a series of 200 samples daily, compared with approximately 80 per day with a standard dry-ashing procedure and with the same facilities.

APPLICATION TO OTHER TYPES OF LEAF-TISSUE SAMPLES—

Citrus leaf-tissue samples were used in the basic investigation to devise rapid procedures for preparing leaf-tissue samples for analysis because of their favourable composition, *i.e.*, a

high calcium content. To test the application of the rapid procedures to a wider field of leaf-tissue analysis, the following leaf-tissue samples, grape, rice, deciduous wheat, pear, pineapple, oil palm, tea and pine needles, were analysed according to the suggested procedures. The results obtained are shown in Table VIII.

When following the acid-extraction procedure it was again found that iron was incompletely extracted; this was subsequently confirmed for all the leaf-tissue samples analysed. The rapid ashing procedure greatly improved the results.

For tea, pineapple and wheat-leaf samples very small differences between the calcium, potassium, magnesium, copper, zinc and manganese contents are observed with the different procedures. For the rice samples, however, considerable differences between the results given by the procedures for copper, manganese and zinc are apparent. A good correlation appears to exist between the results given by the wet-digestion and acid-extraction procedures for

TABLE VIII

COMPARISON OF STANDARD WET AND DRY-ASHING PROCEDURES WITH RAPID ASHING AND ACID-EXTRACTION PROCEDURES

Sample	Procedure	Cu, p.p.m.	Zn, p.p.m.	Fe, p.p.m.	Mn, p.p.m.	Ca, per cent.	Mg, per cent.	K, per cent.
Tea—								
27	W*	9	17	50	297	0·74	0·65	2·02
	S†	9	16	46	302	0·73	0·65	2·01
	R‡	8	17	40	302	0·74	0·65	2·01
	E§	9	19	34	303	0·73	0·65	2·01
28	W	12	26	49	252	0·62	0·58	2·39
	S	12	24	46	252	0·63	0·58	2·39
	R	12	26	40	253	0·62	0·58	2·38
	E	12	25	31	252	0·62	0·57	2·38
29	W	14	24	51	304	0·54	0·73	1·98
	S	14	25	52	303	0·54	0·72	1·98
	R	14	24	44	304	0·55	0·73	1·98
	E	14	24	41	304	0·53	0·73	1·98
Pineapple—								
30	W	11·5	33	81	188	0·27	0·32	3·49
	S	11·5	34	66	193	0·28	0·32	3·50
	R	10·2	35	70	188	0·27	0·32	3·50
	E	10·0	34	61	188	0·27	0·32	3·50
31	W	7·6	33	60	152	0·31	0·45	3·02
	S	6·8	33	58	152	0·30	0·45	3·02
	R	7·5	33	54	149	0·30	0·44	3·02
	E	7·6	33	34	149	0·31	0·45	3·02
Rice—								
32	W	10·7	10	162	126	0·64	0·40	3·40
	S	4·4	8	81	77	0·68	0·41	3·43
	R	6·8	8	84	85	0·67	0·41	3·42
	E	11·3	14	123	130	0·68	0·42	3·43
33	W	9·4	13	222	105	0·73	0·38	3·16
	S	4·6	7	113	66	0·74	0·38	3·16
	R	9·0	9	155	73	0·72	0·39	3·16
	E	11·7	15	162	100	0·73	0·39	3·17
34	W	13·0	19	217	102	0·61	0·45	2·98
	S	6·8	16	92	75	0·61	0·46	2·98
	R	8·3	17	93	76	0·61	0·46	2·99
	E	15·0	31	181	101	0·61	0·45	2·98
Wheat—								
35	W	5·8	24	212	58	1·23	0·16	2·39
	S	5·6	25	213	58	1·22	0·16	2·39
	R	5·7	25	212	58	1·23	0·16	2·38
	E	5·8	26	189	58	1·22	0·16	2·39
36	W	7·6	33	182	66	0·87	0·14	2·14
	S	7·7	30	184	64	0·86	0·13	2·14
	R	7·6	32	183	64	0·87	0·14	2·14
	E	7·5	32	147	65	0·87	0·14	2·14

*W Standard wet-digestion procedure.
† S Standard dry-ashing procedure.
‡ R Rapid ashing procedure.
§ E Acid-extraction procedure.

these elements in rice. The standard and rapid dry-ashing procedures, however, give results considerably lower than those obtained with the above procedures. This could, perhaps, be explained by the relatively high chloride content of rice samples (about 0·9 per cent.). The loss of different elements under these conditions by volatilisation as the chloride has been postulated. The most common mechanism of this type put forward has been the formation of a volatile chloride by interaction between the oxide and sodium chloride.[13] A recent paper,[5] however, indicated that such reactions do not occur with several oxides and, further, that they are not thermodynamically possible.

If zinc is present as zinc chloride, losses can be expected to occur when the sample is heated. If, on the other hand, the oxide is present, interaction with chloride-containing material may give rise to the volatile chloride. It has been shown[14] that although heating zinc oxide with sodium chloride does not cause volatilisation, it does cause very significant interaction between the zinc and the silica reaction vessel. Retention of zinc on the material of the reaction vessel has been demonstrated by radiotracer experiments.[3,15]

For copper, all the reports of errors arising during dry ashing, when they indicate the source at all, implicate interaction between the copper and the solid material in the system during the process.

Losses of iron due to the volatilisation of iron(III) chloride have also been postulated.[13] For zinc, however, Gorsuch[5] indicated that such volatilisation does not occur and that, at least as far as the oxide is concerned, it is not thermodynamically possible. Iron is more likely to be firmly fixed to the silica crucibles.

Losses of manganese have been reported after ashing it in both platinum and porcelain. Reasons given for these losses were the formation of insoluble residues and the embedding of manganese in the surface.[15]

Lucerne leaf tissue also contains a fairly large amount of chloride and the same tendency to interact with copper, iron, zinc and manganese, although not as pronounced as that for rice, is observed.

Conclusion

The suggested rapid dry-ashing procedure gives results comparable with the standard wet and dry-oxidation procedures, except in the instance of iron, for which low values are obtained. Samples with a high chloride content, however, should not be ashed in either this manner or by a standard dry-ashing procedure without taking precautions to avoid elemental losses. The acid-extraction procedure gives equally good results for the elements mentioned except iron.

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The Shape Factors of China Clay - Polyacrylamide Suspensions

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The average floc radii of china clay - polyacrylamide suspensions were calculated from sedimentation rates by using the standard hindered settling equations.

The shape factors of the flow spaces of the suspensions have been calculated and their effect on the sedimentation of the suspensions is discussed. The shape factors are found to be constant for a given clay suspension concentration and to be independent of the ionic nature of the polyacrylamides used to flocculate the clay.

The average floc radii have been determined by using the calculated shape factors and the results are compared with the floc radii as calculated by using the standard hindered settling equations.

A method is proposed for calculating the floc radii from one sedimentation experiment and its reproducibility is discussed.

AT Reynold's numbers of less than 0·2 a solid sphere in an infinite expanse of fluid falls at a uniform velocity, which is given by the Stokes equation—

$$v_s = \frac{2g\,(l_s - l_g)\,r^2}{9\eta} \qquad (1)$$

where g is the acceleration due to gravity, l_s and l_g are the densities of the solid and fluid, respectively, r is the particle radius and η the viscosity of the fluid. The particle radius can thus be determined from one experiment.

Steinour[1] studied the sedimentation of concentrated suspensions and derived the following equation—

$$Q = \frac{v_s \epsilon^3}{1 - \epsilon}\,\theta(\epsilon) \qquad (2)$$

where Q is the rate of fall of the concentrated suspensions, ϵ the part of the total volume of the suspension occupied by fluid and $\theta(\epsilon)$ a function of ϵ, which is determined by the size and shape of the flow spaces of the suspension and is termed the shape factor.

Powers[2] had reported that part of the fluid in a sedimenting suspension was adsorbed on to the solid particles and by assuming that the amount of adsorbed liquid was proportional to the amount of solid in the suspension Steinour[1] modified equation (2) to

$$Q = \frac{\theta(\epsilon) v_s\,(\epsilon - W_1)^3}{(1 - W_1)^2\,(1 - \epsilon)} \qquad (3)$$

where $W_1 = \dfrac{\alpha}{1 + \alpha}$, in which α is the amount of adsorbed liquid per unit volume of solid.

Therefore, assuming that $\theta(\epsilon)$ is constant, a graph of $[Q\,(1 - \epsilon)]^{\frac{1}{3}}$ *versus* ϵ will be a straight line of slope $\left[\dfrac{\theta(\epsilon) v_s}{(1 - W_1)^2}\right]^{\frac{1}{3}}$ and an intercept of W_1 on the ϵ axis. Hence, if $\theta(\epsilon)$ is known, v_s can be determined and thus r calculated from equation (1). Steinour[1] found experimentally for the system that he was investigating that $\theta(\epsilon)$ was constant at 0·123 for the ϵ range 0·3 to 0·7 and derived an empirical equation for its calculation, *viz.*,

$$\theta(\epsilon) = \frac{(1 - \epsilon)}{\epsilon}\,10^{-1.82(1 - \epsilon)} \qquad (4)$$

and substituting for $\theta(\epsilon)$ in equation (2) gives

$$Q = v_s \epsilon^2 \, 10^{-1.82(1-\epsilon)} \quad .. \quad .. \quad .. \quad .. \quad (5)$$

A graph of log Q/ϵ^2 *versus* ϵ will then be a straight line of slope 1·82, and if the graph is extrapolated to unit porosity, *i.e.*, infinite dilution, v_s can be equated to Q and the average particle size calculated from equation (1).

Several workers[3–7] have used Steinour's equations to calculate particle size. However, all of these workers have obtained results for ϵ values some or all of which were greater than 0·7, even though Steinour[1] concluded that the shape factor was constant only for suspensions in the ϵ range 0·3 to 0·7. This is true when using equation (5) but not when using equation (3). Owens[8] has recalculated several sets of published data and shown that the slope of a graph of log Q/ϵ^2 *versus* ϵ is not necessarily 1·82, as reported by Steinour, and hence the shape factor when calculated by using equation (4) with the experimental slope in place of 1·82 is not 0·123 and may not be constant over a range of ϵ values.

Empirical equations for the calculation of particle radii from sedimentation results have been reported by several workers. The most extensively used equations are those by Richardson and Zaki[9] [equation (6)] and Dollimore and McBride[10] [equation (7)]—

$$\log Q = n \log \epsilon + \log v_s \quad .. \quad .. \quad .. \quad .. \quad (6)$$

$$\log Q = n \, W + \log v_s \quad .. \quad .. \quad .. \quad .. \quad (7)$$

where n is a constant and W is the weight of solid in a fixed volume of the suspension.

The floc sizes of china clay flocculated by polyacrylamide and calculated by using equations (5) to (7) have been reported.[11] In this paper the investigation has been extended to include more polyacrylamides, and the shape factors of the suspensions have been determined and the floc radii calculated from them.

Methods

Materials—

The clay used in these experiments was a china clay supplied by English Clays, Lovering, Pochin and Co. Ltd. and was designated SPS; a chemical analysis and particle-size distribution for the clay is given in Table I. The anionic and cationic polyacrylamides (A and C series, respectively) were supplied by B.T.I. Chemicals Ltd., Bradford. The molecular weights are about 3×10^6 and the order of anionic character is C110 < C100 < A100 < A150. The non-ionic polyacrylamide S20 was supplied by Cyanamid Inc. and has a molecular weight of 3×10^6 to 4×10^6.

Table I

Chemical analysis and particle-size distribution of clays (SPS)

Chemical analysis, per cent.	SiO_2 46·2	Al_2O_3 38·7	Fe_2O_3 0·56	TiO_2 0·09	CaO 0·20	MgO 0·20	K_2O 1·01	Na_2O 0·07	Loss on ignition 13·14
Particle-size distribution, per cent.	Below 2 μm, 80·0			Above 10 μm, 0·2		300-mesh residue, 0·01			
Cation-exchange capacity/m-equiv per 100 g	3·1								

Sedimentations—

All sedimentations were carried out in 250-ml stoppered measuring cylinders with 150 ml of total suspension at 293 °K. All polymer solutions were freshly prepared to avoid loss of flocculating activity on ageing.[12]

Floc size can be significantly affected by varying the method of agitation of the suspension and of addition of the flocculant.[12] To achieve reproducible results, it therefore became necessary to carry out a detailed systematic study on the system, when the following standard procedure was evolved.

A 100-ml suspension of the required amount of clay was allowed to equilibrate in a stoppered measuring cylinder for 24 hours at 293 °K. A stock solution of the polyacrylamide was prepared and the required amount of this solution was transferred by pipette into a 50-ml calibrated flask, which was made up to the mark with distilled water.

The clay suspension was then dispersed by inverting the cylinder several times and the polyacrylamide solution immediately added to the suspension in the cylinder. The resulting 150-ml suspension was then mixed by stoppering the cylinder and inverting it once per second for 60 s. The rate of fall of the suspension was then determined and, when constant, the settled volume was recorded.

Checks were periodically made on the suspensions to ensure that the pH was constant. There was no need to adjust it as it did not vary from 4·9 by more than half a pH unit.

Results and discussion

The rates of fall of flocculated concentrated suspensions have been shown by Kitchener and Slater[13] to be dependent on the concentration of both the flocculant and the mineral to be flocculated. Sedimentations were carried out for suspensions containing 10 to 20 g of clay and various polyacrylamide concentrations. The results given in Table II are the maximum rates of fall found experimentally for each particular clay - polyacrylamide suspension. From these results the average floc radii were calculated by using equations (5) to (7) and recorded graphically. The graphs were analysed on a KDF9 computer by the method of least squares and the average floc radii calculated from the intercepts given by this analysis. All the percentage errors calculated from the standard deviations were between 3 and 5 per cent.

Table II

Results for sedimentation experiments

Weight of clay in 150 ml of suspension/g	Voidage (ϵ)	Rate of fall of interface/cm s^{-1}				
		C110	C100	A100	A150	S20
10·0	0·9733	0·677	0·620	0·575	0·240	0·357
12·5	0·9666	0·492	0·437	0·422	0·173	0·254
15·0	0·9600	0·335	0·316	0·306	0·126	0·182
17·5	0·9533	0·251	0·229	0·224	0·091	0·132
20·0	0·9466	0·187	0·167	0·162	0·061	0·093

Calculation of the shape factors—

The slopes of the graphs obtained with each equation are given in Table III. The equation of interest for the calculation of the shape factors is equation (5) and the average slope of the graphs of log Q/ϵ^2 *versus* ϵ was 20·20 $\pm$ 0·5. This result is in disagreement with the value of 1·82 determined by Steinour,[1] which is to be expected as Steinour's value of 1·82 is valid only for the range $0{\cdot}3 < \epsilon < 0{\cdot}7$. If, however, equation (4) is amended by the replacement of 1·82 by 20·20, then

$$\theta(\epsilon) = \frac{(1-\epsilon)\,10^{-20{\cdot}20(1-\epsilon)}}{\epsilon} \quad .. \quad .. \quad .. \quad .. \quad (8)$$

and the shape factors at the experimental voidages can be calculated.

Table III

Results by the three methods of calculating average floc size

Method .. Polymer	Floc radius/μm			Average floc radius/μm	Error, per cent.	Slope of graph		
	a	b	c			a	b	c
C110	84·6	85·3	86·6	85·5	1·3	20·23	0·056	46·8
C100	81·3	82·6	82·7	82·2	1·1	20·48	0·056	48·4
A100	79·0	78·4	79·1	78·8	0·5	19·71	0·055	45·7
A150	51·1	51·9	51·1	51·4	1·6	19·92	0·057	46·6
S20	63·6	63·9	64·9	64·0	1·3	20·66	0·058	48·4
					Average slope ..	20·20	0·056	47·2
					Error, per cent. ..	2·5	1·8	3·6

a. Ramakrishna and Rao,[4] *viz.*, equation (5).
b. Dollimore and McBride,[10] *viz.*, equation (7).
c. Richardson and Zaki,[9] *viz.*, equation (6).

The shape factors calculated for each experimental voidage are given in Table IV. The floc radii were then calculated from equations (1) and (2) and the results are given in Table V.

TABLE IV

SHAPE FACTOR FOR CLAYS FLOCCULATED BY POLYACRYLAMIDE FOR EXPERIMENTAL VOIDAGES

Weight of clay in 150 ml of suspension/g	Voidage (ϵ)	Shape factor, $\theta(\epsilon) \times 10^3$
10	0·9733	7·92
12·5	0·9666	7·31
15·0	0·9600	6·47
17·5	0·9533	5·59
20·0	0·9466	4·71

COMPARISON OF FLOC RADII—

The results given in Table V show that floc radii calculated by using the shape factor are in good agreement with the floc radii calculated by using the empirical equations (5) to (7). The original method of Steinour,[1] *i.e.*, a graph of $[Q\ (1 - \epsilon)]^{\frac{1}{3}}$ *versus* ϵ and the calculation of V_s from equation (3), has not been used. This method depends on the shape factor being constant and the results show this to be an erroneous assumption for clay - polyacrylamide suspensions.

TABLE V

FLOC SIZE CALCULATED FROM SHAPE FACTOR AND RATE OF FALL

Weight of clay in 150 ml of suspension/g	Floc radius/μm				
	C110	C100	A100	A150	S20
10	87·0	83·2	80·2	51·8	63·3
12·5	88·7	82·4	80·8	51·7	62·7
15	84·4	82·3	80·7	51·9	62·3
17·5	86·0	82·2	81·3	51·9	62·4
20	87·4	82·6	82·2	51·9	62·4
Average floc radius/μm	86·6	82·7	81·0	51·8	62·6
Error, per cent.	1·3	0·7	12·7	0·2	2·2

IONIC NATURE OF POLYACRYLAMIDE—

The slopes of the graph of $\log Q/\epsilon^2$ *versus* ϵ and hence the shape factors are for a particular clay concentration and are independent of the ionic nature of the polyacrylamide at the optimum polyacrylamide concentration for that particular clay - polyacrylamide suspension. The sedimentation rates, however, and hence the floc radii tend to increase as the cationic nature of the polyacrylamide increases. This agrees with the previous results[11] and is expected because china clays carry an over-all ionic charge that results in attractive forces between the clay and cationic polyacrylamides and repulsive forces between the clay and anionic polyacrylamides.

CONCLUSION

The shape factor for the china clay - polyacrylamide suspensions was found to be independent of the ionic nature of the polyacrylamide but to be dependent on the voidage, ϵ. It is possible, therefore, to calculate the average floc radii of a suspension of known concentration if the shape factor at this concentration has been previously determined. Average floc radii can thus be calculated from a single sedimentation instead of the several sedimentations required by the graphical analyses.

One of us (T.A.H.) thanks English Clays, Lovering, Pochin and Co. Ltd., and the Science Research Council for the provision of a maintenance grant.

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Book Reviews

GAS CHROMATOGRAPHY. Second Edition. By D. AMBROSE. Pp. xii + 321. London: Butterworths. 1971. Price (Cased) £4; (Limp) £2·50.

Most authors would agree that the production of the second edition of a scientific text is sheer drudgery. The novelty of piecing the information together has gone, and the thrill of seeing one's name along the spine of a new volume no longer compensates for the tedium of reading, re-reading and reading yet again the well thumbed text. For this reason, Dr. Ambrose is to be congratulated on his long overdue revision of the first edition of Gas Chromatography; he has produced an eminently readable account of the development, practice and use of this technique.

After a general survey, four chapters are devoted to the apparatus used in gas chromatography. These are followed by comprehensive chapters on retention volumes, column performance and separations and selective solubility, which, taken together, give the best account of the chromatographic process that the reviewer has yet read. The remaining chapters are devoted to the use of the technique. A very minor criticism is that the reviewer would have preferred to see a little more of the text devoted to the subject of errors, precision and accuracy of the chromatographic method.

This book provides a sound theoretical and practical introduction for the beginner in gas chromatography and much of interest and value for those who have spent years using it. It is a book that should be on every chromatographer's bookshelf, and, at £4 with hard covers and £2·50 with limp covers, certainly deserves to be. P. G. JEFFERY

ANALYTICAL CHEMISTRY OF MOLYBDENUM AND TUNGSTEN (INCLUDING THE ANALYSIS OF THE METALS AND THEIR ALLOYS). By W. T. ELWELL and D. F. WOOD. Pp. xii + 277. Oxford, New York, Toronto, Sydney and Braunschweig: Pergamon Press. 1971. Price £8.

The increasing use and distribution of molybdenum and tungsten throughout the many and varied aspects of modern technology continue to stimulate interest in the analytical chemistry of these two important elements, not only with respect to new techniques but also towards re-appraisals of well established methods of analysis.

In this publication, which represents Volume 47 in the well known series of monographs published by Pergamon Press on Topics in Analytical Chemistry, the authors have reviewed, assessed and summarised a vast amount of information within the compass of a single volume and the outcome is a work of genuine utility and considerable authority.

Chapters 1, 2, 3 and 4 deal briefly but succinctly with historical background, physical and chemical properties, sampling and decomposition and qualitative detection, respectively. The case for summarising general physical and chemical properties (Chapter 2) is sound in principle as it provides an essential foundation for the detailed analytical chemistry of the two elements concerned. This chapter of only six pages is remarkably concise, one wonders if not a little too concise, when judged against the background of the book as a whole.

Chapter 5 describes various procedures for separating molybdenum and/or tungsten from a wide variety of other elements and forms a natural introduction to the various gravimetric and titrimetric procedures presented in the following two chapters, 6 and 7, respectively.

The emphasis in Chapters 6 and 7 is orientated towards well established classical and semi-classical procedures.

Chapter 8, comprising 59 pages, deals with various colorimetric methods. Not surprisingly, the thiocyanate and dithiol procedures are considered in depth and the authoritative discourse concerning their various applications gives the reader a clear understanding of the relative merits, disadvantages and limitations of these two reagents.

Chapters 9 to 13 deal comprehensively with the applications of various instrumental techniques, with individual chapters devoted to electroanalytical techniques, emission spectroscopy, atomic-absorption spectroscopy, X-ray spectrometry and radiochemical and mass-spectrometric techniques.

Chapter 14 briefly discusses other methods that are less widely used and accepted.

The final chapter, 15, reviews procedures reported for the determination of other elements in molybdenum and tungsten both at impurity and alloying concentrations.

The book is competently written and presented and contains a wealth of information of value both to academic and industrial interests.

Applications are described for disciplines as diverse as plants, soils, lubricants, rocks, ores and medical pathology, in addition to metallurgical materials and products.

Specific methods are presented with precise working details and instrumental techniques are well documented with recommended operating parameters.

With more than 1700 references to original works, the book combines the advantages of a comprehensive review, with a compendium of working methods of analysis at a price which, having regard to current trends, must be considered reasonable and competitive.

B. BAGSHAWE

PROGRESS IN NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY. Volume 7. Edited by J. W. EMSLEY, J. FEENEY and L. H. SUTCLIFFE. Pp. viii + 529. Oxford, New York, Toronto, Sydney and Braunschweig: Pergamon Press. 1971. Price £9.

This seventh volume departs somewhat from the others in the series in that the whole volume is devoted to a single article, entitled "Fluorine Chemical Shifts," by J. W. Emsley and L. Phillips.

Over half of this article comprises a set of tables containing more than 3500 values of fluorine chemical shifts arranged by chemical structure. These shifts are mostly for organic compounds and are associated with their literature reference and the shift corrected to a δ-scale based on fluorotrichloromethane by the present compilers. This is a great time saver for those in need of this information. It is a pity that a compilation that closed in January, 1968, was not published before 1971.

The true text amounts to less than one quarter of the whole and presents the empirical rules, which have been proposed by various authors, connecting the fluorine shifts with variables such as solvent, electronegativity of substituents, π-electron density in aromatic compounds, molecular flexibility, steric hindrance, linear and quadratic powers of the local electric field, and so forth. The authors point out the merits and demerits of the proposed relations; nevertheless, to the non-specialist reader the over-all impression is of a complicated story provided by na'ure herself which is honestly re-told here but not greatly clarified.

While the volume is a useful handbook for those concerned with fluorine nuclear magnetic resonance spectra, some may regret that even with this space it has not been possible to match the shift data with a related tabulation of spin - spin couplings involving fluorine. Those whose interest is in other areas of science can omit this specialist volume. D. H. WHIFFEN

MODERN FOOD ANALYSIS. By F. LESLIE HART and HARRY JOHNSTONE FISHER. Pp. xii + 519. Berlin, Heidelberg and New York: Springer-Verlag. 1971. Price DM117.10; $32.

This book is written by two food scientists, each of whom has retired from a full career in government service in the United States. It is in some ways an extended version of the "A.O.A.C. Methods Book" without the "reference" character associated with that publication: it is also similar in shape and size. The text is well written and presented and is free from the telegraphese style of the A.O.A.C. It includes selected A.O.A.C. methods but also others from such methods books as those of the American Public Health Association and the American Association of Cereal Chemists, all of which are specifically acknowledged. The methods are basically selected with the U.S. Food and Drug Administration or Defense Department in mind, but are for the most part also relevant to British practice. The detail is presented in twenty commodity-oriented chapters, ranging alphabetically from Alcoholic Beverages to Vegetables and Vegetable Products. There are separate chapters for Colours, Pesticide Residues and Vitamins, but the last two are dealt with only briefly and mainly by way of literature reference lists. The chapter on Fruits and Fruit Products is supported by extensive composition tables and that on Spices and Condiments includes chemical methods for distinguishing the geographical origin of certain nutmegs and *Cinnamomum* spp.

There is a useful summary chapter on special instrumental methods that includes some practical advice on the selection of commercial instruments from the American market, and a final brief chapter on Standards and Specifications in which government and trade standards for food products in North America are reviewed.

This is clearly a reliable, if not "official," work, and of great value to those for whom North American markets are of interest. As with all such books today it is expensive; it is also both readable and authoritative. H. EGAN

FOR SALE

LECTURES AND COURSES

APPOINTMENTS VACANT

The Determination of Tin in Steels by Solvent Extraction Followed by Atomic-absorption Spectrophotometry

A method is described for the determination of 0·001 to 0·25 per cent. of tin in irons and steels. The tin from a 1-g sample of metal is extracted from an aqueous solution, which is 0·5 M in both hydrochloric acid and thiocyanate and 8 per cent. w/v in ascorbic acid, into isobutyl methyl ketone. The organic phase is concentrated to a small volume by evaporation and diluted to 10 ml. The tin content of this solution is determined by atomic-absorption spectrophotometry with a nitrous oxide - acetylene flame. Good results were obtained for the determination of tin in twelve B.C.S. irons and steels. The limit of detection was 0·001 per cent. of tin.

J. B. HEADRIDGE and ALAN SOWERBUTTS

Department of Chemistry, The University, Sheffield, S3 7HF.

Analyst, 1972, **97**, 442–446.

The Determination of Zinc in the Feed-water to High Pressure Boilers by Atomic-fluorescence Spectroscopy

A rapid and sensitive atomic-fluorescence method is described for the determination of zinc in feed-water to high pressure boilers within the range 0·0004 to 0·01 p.p.m. A microwave-excited electrodeless discharge tube is used as the excitation source to stimulate zinc fluorescence at 213·9 nm from a sample solution sprayed into the air - hydrogen flame.

The limit of detection of the final method was 0·0004 p.p.m. of zinc and within-batch coefficients of variation were 1·2 per cent. at the 0·01 p.p.m. level and 3·4 per cent. at the 0·005 p.p.m. level.

Impurities and additives that are likely to be present in the feed-water were examined and it was shown that none interfered.

The method was compared with a solvent-extraction - atomic-absorption method.

G. B. MARSHALL and A. C. SMITH

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

Analyst, 1972, **97**, 447–452.

Visual Estimation and Spectrophotometric Determination of Zinc in Potable Waters with 4-(2′-Thiazolylazo)resorcinol

A rapid method is described for the visual estimation and spectrophotometric determination of zinc in potable waters by using 4-(2′-thiazolylazo)-resorcinol. A single addition of a reagent mixture that incorporates buffer, chelating reagent and masking agent is possible. The method is more rapid than existing methods and satisfactory agreement with values from atomic-absorption spectrophotometry is obtained for the range of values normally encountered in potable waters.

W. H. EVANS and G. S. SAYERS

Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, SE1 9NQ.

Analyst, 1972, **97**, 453-456.

The Determination of Calcium in Rain Waters by Using High-temperature Flame-emission Spectroscopy

A high-temperature flame-emission method involving the use of the nitrous oxide - acetylene flame is described for determining calcium in rain waters. The method described is compared with the di(2-hydroxyphenylimino)ethane [glyoxalbis(2-hydroxyanil)] colorimetric method over the 0 to 2 μg ml^{-1} calcium concentration range and is shown to be more accurate and less susceptible to contamination.

P. L. SEARLE and GAIL KENNEDY

Soil Bureau, Department of Scientific and Industrial Research, Lower Hutt, New Zealand.

Analyst, 1972, **97**, 457–460.

The Analysis of Titanium Dioxide Pigments by Automatic Simultaneous X-ray Fluorescence Spectrometry

The use of an automatic fourteen-channel simultaneous X-ray spectrometer for the analysis of titanium dioxide pigments is described. The selection of channel parameters for the determination of calcium, aluminium, silicon, phosphorus, tin, iron, sulphur, chlorine, potassium, zinc, zirconium, niobium, antimony and lead is discussed, and statistical data are given on the accuracy of the determination of these elements.

C. L. DENTON, G. HIMSWORTH and J. WHITEHEAD
Tioxide International Limited, Billingham, Teesside.

Analyst, 1972, **97**, 461–465.

The Determination of the Iodine Content of Organic Compounds as Soluble Iodides with *N*-Bromosuccinimide

A method is proposed for the determination of iodine in organic compounds as soluble iodides with *N*-bromosuccinimide. Details of the procedure are given for the determination of the iodine content of thyroxine sodium, diiodohydroxyquinoline, iodochlorohydroxyquinoline and iodoform. The experimental error does not exceed ±2 per cent. The application of the proposed method to the determination of the iodine content of pharmaceutical preparations such as tablets is also described.

M. Z. BARAKAT, M. BASSIONI and MAMDOUH EL-WAKIL
Biochemistry Department, Faculty of Medicine, Azhar University, Madina Nasr, Cairo, Egypt.

Analyst, 1972, **97**, 466–469.

The Determination of Some Aromatic Phenols with *N*-Bromosuccinimide

A method for the determination of phenol, vanillin and thymol by titration with standard *N*-bromosuccinimide solution is described. The method is sufficiently sensitive to enable amounts ranging from 0·1 to 10 mg of the phenols to be determined. The experimental error does not exceed ±2 per cent. Results for determinations by the proposed method and by previously recognised methods are compared.

M. Z. BARAKAT, A. S. FAYZALLA and S. T. EL-AASSAR
Biochemistry Department, Faculty of Medicine, Azhar University, Madina Nasr, Cairo, Egypt.

Analyst, 1972, **97**, 470–473.

Automated Enzymic Determination of Oxalic Acid in Human Serum

An automated enzymic method is described for the determination of microgram amounts of oxalic acid. The acid is decarboxylated by a specific enzyme, oxalate decarboxylase (E.C.4.1.1.2), and the carbon dioxide evolved is measured colorimetrically in a modified Technicon AutoAnalyzer. The minimum detectable concentration of oxalic acid was about 5 μg per 100 ml. For serum it was necessary to remove protein first by ultrafiltration as it interfered with the recorder base-line readings. Phosphate and sulphate ions inhibited enzymic activity, which was allowed for by adding appropriate concentrations of these ions to the standard oxalate solutions used for calibration. The concentration of oxalic acid in normal human serum ultrafiltrates ranged from 80 to 140 μg per 100 ml (mean 118 μg per 100 ml). Similar values were observed for horse serum. The recovery of microgram amounts of oxalic acid added to serum averaged 110 per cent. The coefficient of variation of replicate determinations of oxalic acid was ±2 per cent. for aqueous solutions and ±5 per cent. for serum ultrafiltrates.

C. F. KNOWLES and A. HODGKINSON
Medical Research Council Mineral Metabolism Unit, The General Infirmary, Leeds, Yorkshire, LS1 3EX.

Analyst, 1972, **97**, 474–481.

An Investigation into More Rapid Procedures for Preparing Leaf-tissue Samples for Analysis

Several rapid ashing procedures for the analysis of leaf-tissue samples to determine their inorganic constituents are described. Various types of sample were extracted with different acid mixtures or ashed for short periods under different conditions and their constituents were then determined by means of atomic absorption. The values obtained by these methods were compared with those obtained by use of standard procedures.

A general procedure for the rapid ashing of plant material, which is suitable for the determination of a number of inorganic constituents, is described in detail.

W. D. BASSON and R. G. BÖHMER

Department of Inorganic and Analytical Chemistry, University of Pretoria, Pretoria, South Africa.

Analyst, 1972, **97**, 482-489.

The Shape Factors of China Clay - Polyacrylamide Suspensions

The average floc radii of china clay - polyacrylamide suspensions were calculated from sedimentation rates by using the standard hindered settling equations.

The shape factors of the flow spaces of the suspensions have been calculated and their effect on the sedimentation of the suspensions is discussed. The shape factors are found to be constant for a given clay suspension concentration and to be independent of the ionic nature of the polyacrylamides used to flocculate the clay.

The average floc radii have been determined by using the calculated shape factors and the results are compared with the floc radii as calculated by using the standard hindered settling equations.

A method is proposed for calculating the floc radii from one sedimentation experiment and its reproducibility is discussed.

D. DOLLIMORE, T. A. HORRIDGE

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

and N. F. OWENS

Unilever Research, Port Sunlight Laboratory, Port Sunlight, Cheshire.

Analyst, 1972, **97**, 490–494.

Reprints of Review Papers

REPRINTS of the following Review Papers published in *The Analyst* since January, 1963, are available from The Society for Analytical Chemistry, Book Department, 9/10 Savile Row, London, W1X 1AF (not through Trade Agents). Orders MUST be accompanied by a remittance for the correct amount made out to "Society for Analytical Chemistry."

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"Methods of Separation of Long-chain Unsaturated Fatty Acids," by A. T. James (August, 1963). Price 25p.

"Beer's Law and its Use in Analysis," by G. F. Lothian (September, 1963). Price 25p.

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

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Report of the Prophylactics Panel: The Determination of Nitrofurazone in Compound Feeding Stuffs.
Report of the Vitamins (Water-soluble) Panel: The Determination of Water-soluble Vitamins in Compound Feeding Stuffs.
Report of the Vitamins (Fat-soluble) Panel: The Determination of Fat-soluble Vitamins in Diet Supplements and Compound Feeding Stuffs.
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Report of the Prophylactics in Animal Feeds Sub-Committee: The Determination of Amprolium, Sulphaquinoxaline and Ethopabate when Present Together in Animal Feeds.
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Application of Gas - Liquid Chromatography to Essential-oil Analysis: Interim Report on the Determination of Citronellol in Admixture with Geraniol.
Spectral Characteristics of Eugenol.
Application of Gas - Liquid Chromatography to the Analysis of Essential Oils. Part 1: Determination of Cedrol.

Fish Products Sub-Committee:

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Nitrogen Factor for Coal Fish.

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

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The Determination of Small Amounts of Zinc in Organic Matter.
The Use of 50 per cent. Hydrogen Peroxide for the Destruction of Organic Matter.
The Determination of Small Amounts of Tin in Organic Matter. Part 2. Amounts of Tin from 30 to 150 μg.
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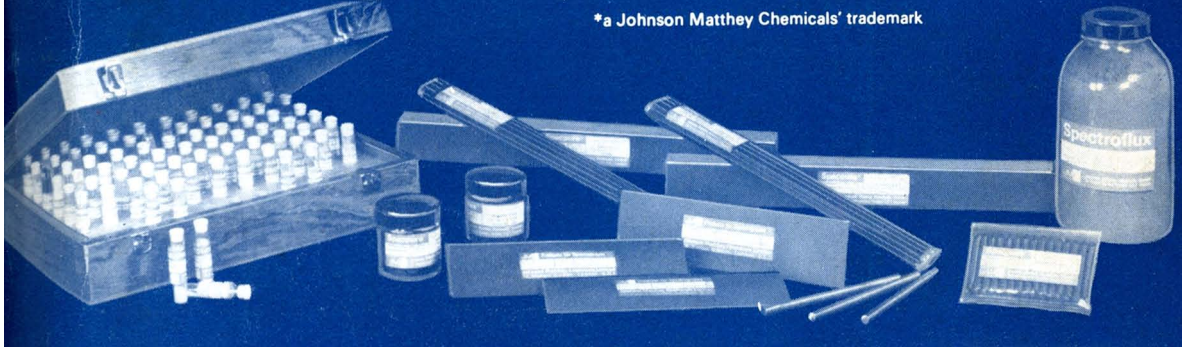

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CONTENTS

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