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Determination of Trace Amounts of Cobalt in Alumina by Atomic-absorption Spectroscopy

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After the dissolution of alumina by hydrochloric acid in a sealed tube at 270° C traces of cobalt are determined by measurement of atomic absorption at 240·7 nm. In one of the two procedures described cobalt, in the range 50 to 250 p.p.m. in alumina, is determined by aspiration directly into an air - acetylene or nitrous oxide - acetylene flame, and in the other procedure cobalt, in the range 10 to 100 p.p.m. in alumina, is determined by co-precipitation on hydrated manganese dioxide, followed by extraction into isobutyl methyl ketone as its 8-hydroxyquinoline complex. The extract is sprayed into an air - propane flame and absorbance measurements are made as before. No interferences were found from the other elements likely to be present in alumina.

The nitrous oxide - acetylene flame is less favourable than air - acetylene for the determination of cobalt because of the unfavourable effect of the strongly reducing cyanogen zone of the flame and because of considerable loss of atomic cobalt caused by ionisation.

SEVERAL resonance lines have been used in the determination of cobalt by atomic-absorption spectroscopy,[1,2,3,4,5] but that at 240·73 nm appears to be the most sensitive, provided that a sufficiently narrow slit is used to isolate the line from others nearby,[6] *e.g.*, those at 240·63 and 240·88 nm. The sensitivity of the 240·73 nm line was confirmed in the present study, as shown in Table I. Generally the measurement is best made in an oxidising air - acetylene flame. Procedures have been described for cobalt in copper alloys,[7] steels and nickel,[5] but there is otherwise a sparsity of information on the interference of other metal ions, particularly those which form refractory oxides, such as aluminium.

TABLE I

CHOICE OF ATOMIC LINE FOR MEASUREMENT
(about 4 p.p.m. aqueous Co^{2+}/air - propane flame)

Spectral-line wavelength, nm	Remarks on absorption
345·3	No absorption observed
353·3	No absorption observed
240·7	0·22 absorbance unit
242·5	0·14 absorbance unit
252·1	0·08 absorbance unit
352·9	No absorption observed
340·5	Insufficient intensity to make measurements possible

The determination of cobalt in nearly pure alumina by atomic-absorption spectroscopy presents problems, such as sample dissolution to produce a medium suitable for nebulisation into a conventional burner and the matrix effect of large amounts of aluminium on the atomisation of small amounts of cobalt. In this communication we propose two analytical methods. The first of these permits the determination to be effected on a simple atomic-absorption apparatus with a relatively cool, air - propane flame. It involves collection of the traces of cobalt by co-precipitation on manganese dioxide and solvent extraction with a hexone solution of 8-hydroxyquinoline. This method is sensitive and precise, but is more time consuming than the alternative procedure, which relies on the use of a relatively hot, air - acetylene flame and more sophisticated apparatus. The latter procedure does not

require chemical pre-manipulation other than sample dissolution, but it is less sensitive. The sensitivity of the latter procedure could be increased to equal that of the former by combination with the collection - extraction process used in the cool-flame method, but only by sacrificing its advantages of rapidity and simplicity.

Experimental

Apparatus—

Techtron AA4 atomic-absorption spectrophotometer, with a lamp and detector modulated at 285 c.p.s. and fitted with an R213 photomultiplier; 10-cm slot burner heads for air - propane (AB42) and air - acetylene (AB41), and 5-cm slot burner head for nitrous oxide - acetylene (AB40); and a Hilger & Watts (FL157) hollow-cathode lamp for cobalt.

Instrumental settings—

Wavelength peaked on 240·7 nm; slit width 50 μ (minimum compatible with signal strength available from lamp); lamp current 29 mA; air pressure 15 lb per sq. inch; nitrous oxide pressure 16 lb per sq. inch; propane pressure sufficient to give a non-luminous flame; acetylene pressure sufficient to give a "rich" slightly luminous flame with air or to give a 15-mm high red feather with nitrous oxide; height of measurement in air - propane flame, 15 mm; in air - acetylene, 13 mm; in nitrous oxide - acetylene, 15 mm; solution uptake rates in air - propane and air - acetylene were 1 ml per 14 seconds for water, 1 ml per 17 seconds for $AlCl_3$ solution, 1 ml per 13·5 seconds for hexone and in nitrous oxide - acetylene, 1 ml per 11·6 seconds for water and 1 ml for 15·2 seconds for $AlCl_3$ solution. ($AlCl_3$ solution equivalent to 0·1 g of Al_2O_3 per 2·5 ml.)

Pressure vessel for dissolution of alumina—

A stainless-steel pressure vessel operating at 3000 lb per sq. inch pressure at 270° C, similar to that described elsewhere,[8] and supplied by A.W.R.E., Aldermaston, was used. The vessel is loaded with the requisite amount of solid carbon dioxide to equalise pressure on both sides of a sealed inner silica tube containing the alumina sample and concentrated hydrochloric acid at 270° C.

Silica digestion tubes—

These were made from 2 to 2·5-mm wall silica tubing with an inner bore of 5 mm, about 23 cm long, sealed at one end. The tubing was obtained from Messrs. Hanovia Limited.

Reagents—

All reagents were of analytical-reagent grade, except where stated.

Stock cobalt solutions, 1000 and 100 p.p.m.—Prepare by dissolving 4·0372 g and 0·4037 g of $CoCl_2.6H_2O$, respectively, in water and diluting to 1 litre. Lower concentrations were prepared by dilution immediately before use.

Sodium hydroxide solution, 4 M.

Hydrochloric acid, concentrated (about 35 per cent.).

8-Hydroxyquinoline, 5 per cent. (0·35 M) in 2 M acetic acid.

Potassium aluminium sulphate, saturated solution—About 11·4 g of $KAl(SO_4)_2.12H_2O$ were dissolved in 100 g of water.

Aluminium chloride solution—Aluminium foil, 50 g, was dissolved in excess of hydrochloric acid (1 + 1) and diluted to 1 litre with distilled water.

Sodium acetate trihydrate, solid.

Hydroxylammonium chloride, solid.

Analytical procedures

(1) Dissolution of alumina sample—

Weigh accurately about 0·1 g of alumina into the bottom of a silica tube of wall thickness 2·0 to 2·5 mm and bore 5 mm, add sufficient concentrated hydrochloric acid to fill a 9 to 10-cm length. Draw out the upper part of the tube, cool and seal off quickly, keeping an even wall thickness and avoiding the formation of a vacuum in the tube (a vacuum could cause loss of contents on opening the tube). The final length should be about 19 cm.

The tubes should be identified by lightly marking with a diamond and placed in the inner container of the pressure vessel. Add sufficient solid carbon dioxide to equalise the pressure at 270° C (weights calculated from a formula given elsewhere[8]). Screw down the pressure block and tighten the release valve, and then the compression bolts. Heat the vessel in a hot-air oven at 270° C and leave overnight (about 15 hours).

After this period remove from the oven and cool the vessel to room temperature; allow the carbon dioxide to escape by cautiously opening the release valve. Loosen the compression bolts and unscrew the head. Remove the tubes, ensure that they are all at room temperature and break open at a scratch made near the top. Transfer the solution into a 25-ml beaker and dissolve any crystals in a little water. The solutions obtained can be treated by the analytical methods described below.

(2) CALIBRATION CURVE FOR COBALT IN ALUMINIUM SALTS AND DETERMINATION OF COBALT IN SOLID ALUMINA IN THE AIR - PROPANE FLAME—

Calibration curve—Pipette 8 ml of the potassium aluminium sulphate solution into each of a series of 100-ml beakers and add 0·5 to 9·0-ml aliquots of standard 2 μg per ml cobalt solution. Add 5 ml of 4 M sodium hydroxide solution and 1·0 ml of 0·01 M potassium permanganate solution to each. Bubble sulphur dioxide through the solutions until the purple colour has been discharged and all the permanganate has been converted into manganese dioxide. Filter immediately on pulp pads and wash the pads with about 20 ml of distilled water and allow to drain. Place a clean 100-ml beaker under each funnel and dissolve the manganese dioxide on each pad with about 2 ml of concentrated hydrochloric acid. Wash the pads with about 20 ml of water and add a few crystals of hydroxylammonium chloride (about 10 mg) to each beaker in order to ensure complete reduction of manganese to the bivalent state. Render the solutions nearly neutral (about pH 6) by the addition of solid sodium acetate trihydrate, by using test-papers or a pH meter. Transfer the solutions to 100-ml separating funnels via a 25-ml measuring cylinder. Make each solution (with washings) up to 35 ml. Pipette 2·0 ml of 5 per cent. 8-hydroxyquinoline reagent into each funnel and add 10 ml of isobutyl methyl ketone. Shake the funnels vigorously for 1 minute in order to effect the extraction, discard the aqueous layer in each funnel and spray the organic extracts into the air - propane flame. Measure the absorbance values *versus* the pure solvent with the instrumental settings given above. The calibration curve is linear from 1 to 10 μg of cobalt.

Alumina in the form of 0·1-g samples can be treated in the same way after dissolution in the pressure vessel, *cf.* (1).

(3) CALIBRATION CURVES FOR COBALT IN ALUMINIUM SALTS AND DETERMINATION OF COBALT IN SOLID ALUMINA IN AIR - ACETYLENE AND NITROUS OXIDE - ACETYLENE FLAMES—

Transfer by pipette 11·0 ml of the aluminium chloride solution into each of a series of 25-ml graduated flasks and add 0·5 to 10·0-ml aliquots of standard 100 μg per ml cobalt solution. Adjust the volume to 25 ml. These solutions contain the equivalent of a 0·1-g sample of alumina per 2·5 ml of solution. The solutions can be sprayed directly into the air - acetylene and nitrous oxide - acetylene flames. The absorbance is measured against a blank that contains no added cobalt.

Alumina samples can be determined in a similar manner, following dissolution in the pressure vessel, by adjusting the volume of the hydrochloric acid solution to 2·5 ml with distilled water followed by direct spraying.

ANALYSIS WITH AN AIR - PROPANE FLAME—

In the exploratory experiments cobalt was determined by measurement of its atomic absorption in an air - propane flame at 240·73 nm in the presence of increasing amounts of added potassium aluminium sulphate. It soon became apparent that, at the temperature in the mantle of the air - propane flame, aluminium oxide clotlets, formed by degradation of the aluminium salt in the strongly oxidising primary cone, were not sufficiently dissociated to release the cobalt atoms into the flame. The addition of releasing agents such as strontium and lanthanum salts was of little avail, and for small amounts of cobalt in a medium corresponding to that which would be obtained on dissolution of 0·1 g of alumina no reproducible

signals could be obtained. It was, therefore, found necessary to separate the cobalt from the matrix element by using a procedure similar to that devised by Marshall and West[9] for traces of nickel and iron in alumina.

As atomic-absorption measurements provide a chemically specific analytical procedure, selectivity of extraction is unnecessary, except that the traces of cobalt must be separated from the bulk of the matrix element, aluminium. For this reason, 8-hydroxyquinoline was selected as the ligand and isobutyl methyl ketone(hexone) as the solvent. Cobalt(II), having an electrovalence of two and a co-ordination number of six, forms a co-ordination unsaturated $Co.Ox_2$ complex and it is, therefore, necessary to select a solvent that will displace the two co-ordinated water molecules remaining in the complex by two oxygen-donating solvent molecules. A systematic examination of various solvents showed that hexone offered optimal conditions, both for ease of quantitative extraction and efficiency of nebulisation and atomisation.

Quantitative extraction of the cobalt could only be obtained at pH 6 or greater than 6, in which region aluminium was also sufficiently well extracted to cause serious consumption of reagent. It was also found impossible to keep such large amounts of aluminium 8-hydroxyquinolinate in solution. Furthermore, the amounts of aluminium remaining in the extract interfered with the atomic-absorption measurements. The masking of such large amounts of aluminium to permit extractive concentration of the cobalt(II) did not prove feasible and the alternative of extracting aluminium to leave the cobalt behind in solution did not appear to be attractive or practicable. Consequently a collection technique was used to preconcentrate and separate the cobalt from the aluminium. A survey of the over-all experimental design showed that hydrated manganese dioxide would be an excellent collector in view of the subsequent use of 8-hydroxyquinoline as an extractant. Although manganese dioxide has been used previously for iron and nickel[9] we could find no previous record of the collection of cobalt(II) hydroxide in this way.[10] The manganese dioxide was generated *in situ* by the action of sulphur dioxide on permanganate in a strongly alkaline solution. At this pH the aluminium is held in solution quantitatively as sodium aluminate. It was subsequently proved that from 0·5 to 50 μg of cobalt could be collected quantitatively on 1 mg of manganese dioxide in this way from 80 ml of solution containing the equivalent of 0·1 g of alumina.

The hydrated manganese dioxide is easily filtered on a pulp pad and, after washing briefly, can be dissolved in a small amount of concentrated hydrochloric acid washed through the pad. The addition of a small amount of hydroxylammonium chloride to reduce manganese(IV) is beneficial. Direct aspiration of the dissolved manganese dioxide sample into the air - propane flame yielded cobalt signals, but at a much lower sensitivity than that which could be obtained by subsequent extraction with 8-hydroxyquinoline in hexone at pH 6. Manganese (II) is not extracted at this pH and consequently does not interfere in any way with the determination of cobalt. Aluminium does not pass through the collection - extraction procedures in detectable amounts. Interference studies were carried out on a range of other metals likely to be present in nearly pure alumina or aluminium salts. This was done by adding a 100-fold excess of Ca^{2+}, Cr^{3+}, Fe^{3+}, Mg^{2+} or Ni^{2+} to 5 μg of Co^{2+} in a solution containing the equivalent of 0·1 g of Al_2O_3. No interference was found. All these measurements were made against a blank carried out on the aluminium solution without addition of cobalt or the other ions. In all cases these blanks themselves measured against pure solvent revealed only negligible amounts of cobalt present in the aluminium salt.

In the air - propane flame the calibration curve for pure aqueous solutions was a near straight line through the origin giving absorbance readings of 0·05 and 0·425 for 1 and 10 p.p.m. of cobalt, respectively, in a 10-cm path length. Following extraction into hexone - 8-hydroxyquinoline, a near linear calibration curve was obtained with absorbances of 0·012 and 0·125 corresponding to 0·1 p.p.m. and 1·2 p.p.m. of Co^{2+} in the organic phase. The limit of detection (0·005 absorbance unit) for the entire analytical procedure (collection in the presence of alumina, extraction and measurement) was 0·04 μg of cobalt per ml of hexone and the analytical range was 0·1 to 1·0 μg of cobalt per ml of hexone (equivalent to 10 to 100 μg of cobalt per g of Al_2O_3). At the level of 0·5 μg of cobalt per ml of hexone the relative standard deviation of the entire analytical procedure was found to be $\pm 2{\cdot}13$ per cent. The time required for a complete analysis following dissolution of the sample was about 1 hour.

ANALYSIS WITH AN AIR - ACETYLENE FLAME—

As the air - propane flame was insufficiently hot to dissociate the alumina clotlets efficiently, attention was turned to the air - acetylene flame as an atom reservoir for cobalt in the presence of large amounts of aluminium. The oxy-acetylene flame might be still more favourable, but its high burning velocity makes it unsuitable for support at a long-slot burner of the atomic-absorption type. However, the air - acetylene flame has been used in its oxidising mode for the determination of cobalt and in its fuel-rich (luminous) form for aluminium. Experiments were, therefore, made on a solution containing aluminium chloride equivalent to 0·1 g of Al_2O_3 per 2·5 ml of solution and varying amounts of cobalt, on a 10-cm long, air - acetylene slot burner. Satisfactory results were obtained in this way, but at a sensitivity approximately only one half of that obtained in the air - acetylene flame in the absence of aluminium. There is little doubt that this is partly caused by the increased viscosity of the solutions containing high concentrations of dissolved salts, because there was a 20 per cent. decrease in the rate of uptake of the solution. A further factor may arise from the formation within the nebulising chamber of larger droplets with a subsequent smaller specific surface in the flame. However, the fact that the best cobalt signals could only be obtained for aluminium-containing solutions in a luminous reducing flame is also significant. Experiments showed that these are the conditions that most favour the atomisation of aluminium rather than cobalt. The best signals for pure cobalt solutions were obtained in a *lean* air - acetylene flame. Obviously minimisation of the partial pressure of atomic oxygen in the flame is necessary to induce increased thermal dissociation of the Al_2O_3 clotlets. These conditions are, however, not too favourable for efficient atomisation of cobalt. This balance of flame conditions is revealed in the criticality of air - acetylene ratios and of the position of measurement in the flame (about 1·3 cm above the burner head), as shown in Fig. 1.

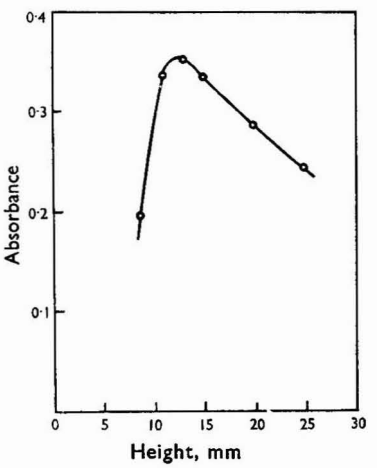

Fig. 1. Graph of absorbance against height of measurement above burner head for cobalt in aluminium chloride solutions, with an air - acetylene flame

Best results were obtained at maximum lamp current and minimum slit width (see Fig. 2).

No interference was observed from 50-fold excesses of the same ions used in the air - propane flame work. In this instance, in the presence of 0·1 g of Al_2O_3 the limit of detection (0·005 absorbance unit) was 0·22 μg of cobalt per ml, with an analytical range of 2 to 10 μg per ml (equivalent to 50 to 250 μg of cobalt per g of Al_2O_3). At the level of 4 μg of cobalt per ml the relative standard deviation of the method was $\pm$1·4 per cent. The time required for a complete analysis following dissolution of the sample was only a few minutes.

ANALYSIS WITH A NITROUS OXIDE - ACETYLENE FLAME—

Recent work[11] has shown that the temperature of the type of nitrous oxide - acetylene flame used for atomic-absorption measurements is 2780° $\pm$ 50° C. This compares with a value

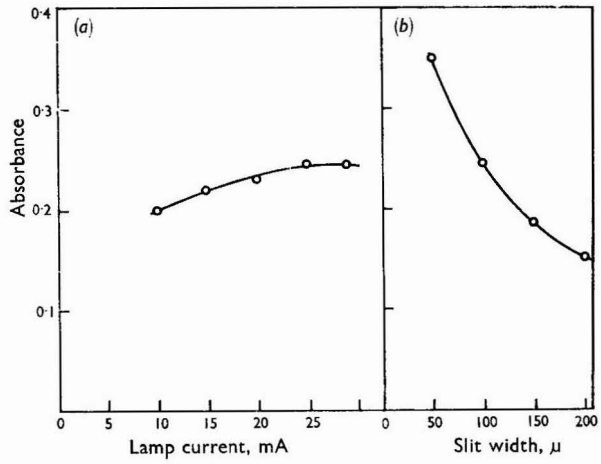

Fig. 2. Effect of lamp currents and slit width on cobalt signal: (*a*), absorbance *versus* lamp current; (*b*), absorbance *versus* slit width. Both sets of measurements were made on aqueous solutions in the air - acetylene flame, under optimised conditions

of about 2300° C for the air - acetylene flame. This flame was, therefore, investigated in a similar manner. In this instance aqueous solutions of cobalt gave only one quarter of the signal obtained in the air - acetylene flame. This is only partly accounted for by the shorter (5 cm) path length of the flame. It would appear, therefore, that the higher temperature of the flame is not a decisive factor in dissociating the Al_2O_3. This is borne out by the lower atomic-absorption signals that are obtained in a stoicheiometric (and therefore hotter) nitrous oxide - acetylene flame, as opposed to a fuel-rich flame.

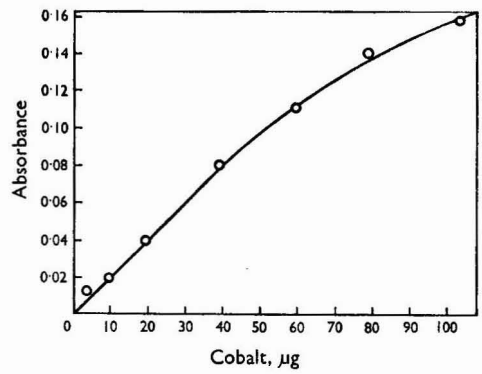

Fig. 3. Calibration curve at 240·7 nm for cobalt in aluminium solutions in the nitrous oxide - acetylene flame. Net absorbance against μg of cobalt per 0·1 g of alumina

It was felt that the higher temperature of the nitrous oxide flame might cause loss of atomic cobalt by ionisation, and this was borne out to a certain extent by the increase in signal (about 30 per cent.) obtained by addition of an excess of potassium ions. Because of the high ionisation potential of cobalt, 7·8 eV, it is not clear, however, if this effect is caused entirely by suppression of ionisation. In the absence of aluminium, cobalt yields a higher signal in a stoicheiometric nitrous oxide - acetylene flame than in a fuel-rich flame. It is apparent, therefore, that the strongly reducing cyanogen zone of the atomic-absorption type of nitrous oxide - acetylene flame has an adverse effect on the atomisation of cobalt, but is beneficial to a limited extent in dissociating alumina clotlets. Because of both of these

effects, however, this flame is inferior to the air - acetylene flame as an atom reservoir for cobalt.

In the slightly fuel-rich (red feather) nitrous oxide - acetylene flame, in the presence of 0·1 g of Al_2O_3, a detection limit (0·005 absorbance unit) of 1 μg of cobalt per ml was obtained, with an analytical range of 2 to 20 μg of cobalt per ml (equivalent to 50 to 500 μg of cobalt per g of Al_2O_3). A relative standard deviation of $\pm$3·0 per cent. was obtained at the 8 μg of cobalt per ml level. Fig. 3 shows the nature of the analytical curve obtained for cobalt in the presence of 0·1 g of alumina.

Discussion and conclusions

Two analytical procedures have been developed for the determination of trace amounts of cobalt in alumina or aluminium salts. The collection - extraction procedure offers a sensitive but more time-consuming procedure that can be applied with simple equipment, while the direct procedure, in which an air - acetylene or nitrous oxide - acetylene flame is used, offers manipulative simplicity and rapidity.

In order to determine cobalt in the presence of aluminium the use of hot flames is necessary to produce dissociation of alumina clotlets. Despite its unfavourable effect on the determination of cobalt (alone) it is best to use a fuel-rich flame in order to establish conditions where a compromise is struck between reasonably effective liberation of cobalt from the matrix and the unfavourable effect of a reducing medium on maintaining a population of cobalt atoms. The nitrous oxide - acetylene flame produces considerable ionisation of cobalt, and its strongly reducing cyanogen zone, which appears in the hottest part of the mantle above the primary zone, has a strongly adverse effect on atomic-absorption measurements of cobalt atoms.

Table II

Determination of known amounts of cobalt in the presence of aluminium ($\equiv$ 0·1 g of Al_2O_3) by atomic-absorption procedures
All amounts are stated in μg

Flame medium					
Air - propane*		Air - acetylene		Nitrous oxide - acetylene	
Taken	Found	Taken	Found	Taken	Found
3·0	3·2	9·0	9·2	7·5	7·8
3·0	3·0	9·0	8·9	7·5	7·5
3·0	3·1	9·0	9·0	7·5	8·0
4·9	5·0	20·0	20·0	30·0	30·5
4·9	5·1	20·0	20·1	30·0	28·5
4·9	4·9	20·0	20·5	30·0	28·5
4·9	4·8	20·0	19·8	30·0	29·0
4·9	4·7	47·5	46·5	47·5	47·5
4·9	4·9	47·5	47·0	47·5	48·5
7·5	7·6	47·5	47·0	47·5	48·7
7·5	7·8	—	—	47·5	48·0
7·5	7·5	—	—	—	—
7·5	7·4	—	—	—	—

* The following extractive separation was as given under Experimental.

Table III

Determination of approximately known amounts of cobalt, μg, in samples of Al_2O_3, and comparison with spectrographic results that were obtained by a semi-quantitative method with a relative precision of $\pm$50 per cent.

Alumina sample No.	Analytical method	Cobalt found in 0·1 g of Al_2O_3, μg	Comparative spectrographic result
1	Air - acetylene	12·7	10·0
1	Nitrous oxide - acetylene	13·5	10·0
2	Air - propane	1·0	0·5
3	Air - propane	None detected	$<$0·5

The analytical range for both the acetylene flames was found to be closely similar. The slope of the calibration curve in the air-supported flame was considerably steeper. Although the method described here is devised for trace amounts of cobalt in alumina it is equally applicable to aluminium salts, as shown in Tables II and III.

We thank A.W.R.E., Aldermaston, for financial support in aid of this work, and for the loan of the pressure vessel used to digest the samples of alumina. We also thank the Courtauld Research Foundation for the award of a grant for the purchase of the Techtron AA4 flame spectrophotometer.

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The Determination of Silicon by Atomic-absorption Spectrophotometry, with Particular Reference to Steel, Cast Iron, Aluminium Alloys and Cement

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The determination of silicon by atomic-absorption spectrophotometry in silicon-containing and siliceous materials has been investigated. Means are outlined by which many types of sample can be completely dissolved without loss of silicon, thus avoiding the need for fusion. Full operating details are given, and the results, accuracy and sensitivity of the method are discussed.

INTRODUCTION of the nitrous oxide - acetylene flame[1,2] made possible the determination, by atomic-absorption spectrophotometry, of about twenty-five more elements than had previously been possible when only air - propane and air - acetylene gas mixtures were available. These elements include aluminium, barium, silicon, titanium and vanadium. Bowman and Willis[3] investigated the application of the nitrous oxide - acetylene flame, and have used it for the determination of, amongst others, vanadium in steel and fuel oil, and aluminium, silicon and titanium in bauxite. These authors also discussed problems of ionisation at the high temperature reached by this flame, investigated certain interferences and give results for the analysis of standard samples of the materials mentioned.

Two other papers discuss the determination of silicon by atomic-absorption spectrophotometry. In one method[4] the authors precipitate silica (from cement samples) and collect the precipitate, which is then fused with sodium carbonate. The melt is dissolved in water, and silicon determined in the resulting solution. However, the introduction of a fusion technique clearly detracts from the convenience of the atomic-absorption method. In the other method[5] silicon was determined in steels by using chemically analysed (N.B.S.) samples as standards. It was also noted that with the preparation procedure given, the samples and standards were stable for only 1 or 2 hours.

The present paper describes the direct determination of silicon, by atomic absorption, in steel, cast iron, aluminium alloys and cement, without the need either for precipitation of the silica or for an alkaline fusion technique. An independent calibration is prepared from pure chemicals.

EXPERIMENTAL

APPARATUS AND REAGENTS—

The work was performed with a Unicam SP90 atomic absorption spectrophotometer fitted with an SP91 multiple lamp turret and SP94 nitrous oxide system, and used in conjunction with an SP93 air compressor unit. A high spectral output silicon lamp was used with a lamp holder specially designed to facilitate lining up the smaller cathode characteristic of this type of lamp. Scale expansion up to three times was used when necessary for the determination of low concentrations of silicon in steels.

Silicon stock solution, about 2000 *p.p.m. of silicon*—This was prepared from pure sodium silicate and standardised gravimetrically. Alternatively, it may be prepared from pure silica dried by heating to 500° C, the correct weight taken, fused in the minimum amount of sodium carbonate, and made up to volume in water.

Vanadium stock solution, 2·5 per cent.—A 12·5-g sample of pure vanadium trichloride was dissolved in about 200 ml of water and 10 ml of hydrochloric acid (sp.gr. 1·16); the solution was then filtered and diluted to 500 ml.

Iron stock solution, 5 per cent. Fe^{3+}—A 50-g sample of high purity iron (B.C.S. 260/2) was weighed into a 1-litre beaker, 200 ml of water, together with 500 ml of hydrochloric acid, were added, and the beaker heated on a hot-plate. About 100 ml of hydrogen peroxide (50 vol.) were added carefully in small portions. After the reaction had stopped, the solution was boiled for a few minutes to decompose remaining traces of hydrogen peroxide and, after cooling, the solution diluted to 1 litre in a calibrated flask.

Aluminium stock solution, 2 per cent.—High purity silicon-free aluminium foil (*e.g.*, Specpure or similar grade), 20 g, was dissolved in 200 ml of hydrochloric acid and diluted to 1 litre in a calibrated flask.

Stock solutions of calcium, phosphorus and sodium (5000 p.p.m.) were also prepared. All solutions except aluminium (*q.v.*) were made with analytical-reagent grade chemicals and de-ionised water, and stored in polythene bottles. All acids used were of analytical-reagent grade, stock concentrated.

Investigation of optimum instrumental conditions—

As silicon is one of the less sensitive elements that can be determined by atomic-absorption spectrophotometry, it was considered necessary to establish the instrumental conditions giving the greatest sensitivity for silicon. Consequently, "optimum conditions" refer here to those instrument settings that result in the greatest silicon sensitivity.

Two solutions were prepared containing 200 and 400 p.p.m. of silicon. With a monochromator slit of 0·1 mm the absorption of each solution at 251·6 nm was measured at different acetylene flow-rates between 3·5 and 5·0 litres per minute and different heights between 0·5 and 2·0 cm of the light path above the burner top ("observation height"). The nitrous oxide flow-rate was fixed at 5·0 litres per minute. The best sensitivity was obtained with an acetylene flow-rate of 4·2 litres per minute and observation height of 1·0 cm. The effect of slit widths between 0·05 mm and 0·20 mm was also investigated when using the conditions of best sensitivity. As expected, best sensitivity was obtained with slit widths between 0·05 mm and 0·1 mm, and sensitivity fell off slightly with wider slits.

Under the chosen conditions, summarised in Table I, a sensitivity (*i.e.*, that concentration of the element, in p.p.m., causing an absorption of 1 per cent.) of 8 to 10 p.p.m. was obtained for silicon, and a reproducibility at the 400 p.p.m. level of ± 3 p.p.m. These conditions proved also to give the highest signal-to-noise ratio.

TABLE I

RECOMMENDED CONDITIONS FOR THE DETERMINATION OF SILICON

Wavelength	251·6 nm
Slit width	0·1 mm (equivalent to a spectral band width of 0·6 nm)
Observation height	1·0 to 1·3 cm*
Nitrous oxide flow-rate	5·0 litres per minute
Acetylene flow-rate	4·0 to 4·5 litres per minute*

* These were found to vary slightly from instrument to instrument.

Effect of foreign ions—

To investigate the effect of other ions on the silicon absorption, several series of solutions were prepared containing 200 p.p.m. of silicon and different concentrations (0 to 3000 p.p.m.) of aluminium, calcium, iron, phosphorus and sodium. Of these elements, aluminium, calcium, iron and sodium enhanced the silicon absorption; phosphate ion exhibited a suppressive effect. From the results (Fig. 1) it can be seen that the enhancement effect of the interfering ions does not increase linearly as the concentration of ion increases, but levels off if a sufficient amount of interfering ion is present. If, therefore, a sufficient amount of interfering ion is added both to the sample solutions and to the standards, the interference effect will be compensated for. Furthermore, the combined enhancing effect of two or more interfering ions flattens out similarly to give the same over-all enhancement.

Vanadium added as vanadium trichloride was found to have an effect similar to that of aluminium and iron; in the presence of at least 3000 p.p.m. of aluminium, vanadium or iron,

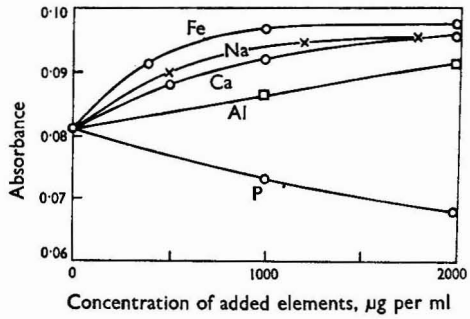

Fig. 1. Effect of interfering ions on silicon absorption. Silicon concentration is 200 µg per ml

the addition of calcium, iron, magnesium, aluminium, manganese, zinc or copper up to 1000 p.p.m. was found to have no further effect on the silicon absorption (Figs. 2 and 3). Moderate amounts of sodium (less than 500 p.p.m.) were found to cause slight enhancement of silicon, even in the presence of aluminium or vanadium.

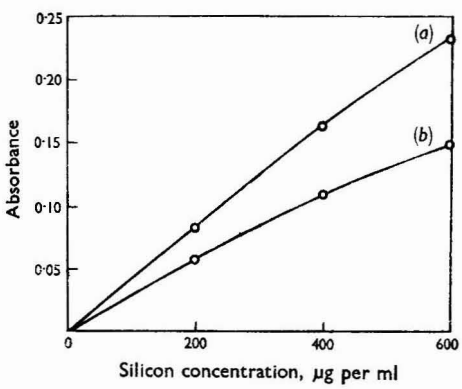

Fig. 2. Effect of added aluminium (1000 µg per ml) on silicon absorption: *a*, silicon *plus* aluminium with and without added interfering ions; *b*, silicon alone

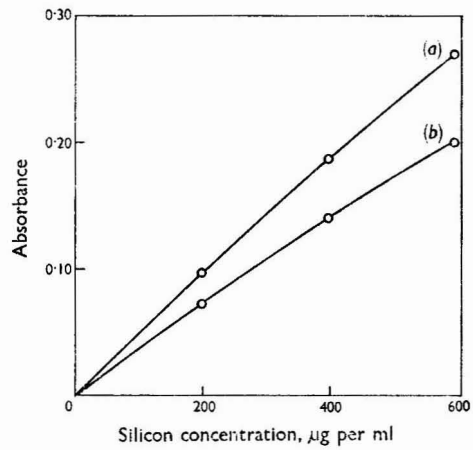

Fig. 3. Effect of added vanadium (5000 p.p.m. of vanadium trichloride) on silicon absorption: *a*, silicon *plus* vanadium with and without added ions (as stated in text); *b*, silicon alone

SAMPLE PREPARATION—

The separation of silicon as silica and subsequent fusion of the precipitate with, *e.g.*, sodium hydroxide, are superfluous steps if the silicon can be brought directly into solution. Clarke[6] has described a rapid method for the dissolution of steels and cast irons that does not involve precipitation of silica; hydrochloric acid attack, followed by treatment with hydrogen peroxide, results in the complete dissolution (except for carbon) of samples containing less than about 1 per cent. of silicon.

The possibility of using hydrofluoric acid to dissolve samples containing more than 1 per cent. of silicon was investigated. Langmyhr and Graff[7] found that hydrofluoric acid was a suitable reagent for the dissolution of siliceous material, and showed that no silicon was lost by volatilisation as silicon tetrafluoride when such material was dissolved in an excess of hydrofluoric acid, provided that the solutions were not heated. This was confirmed during

the present work, and was also found to hold true for silicon dissolved in mixtures of hydrofluoric and hydrochloric acids. However, as expected, the hydrofluoric acid was found to attack glassware with the dissolution of significant amounts of silicon (Fig. 4); polythene apparatus was, therefore, used whenever hydrofluoric acid was present in the solution.

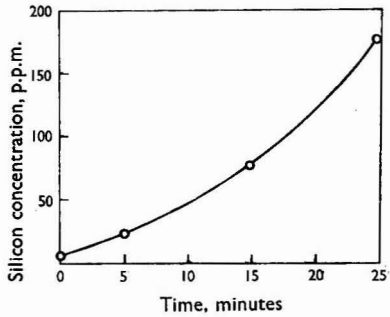

Fig. 4. Dissolution of silicon glassware by 1 ml of hydrofluoric acid *plus* 6 ml of hydrochloric acid diluted to 100 ml

Because of the slight enhancement by sodium of the silicon absorption, and because silicon calibration graphs were prepared from sodium silicate solutions, it was necessary to add sodium to sample solutions containing more than 100 p.p.m. of silicon (corresponding to about 200 p.p.m. of sodium in the sodium silicate solutions). The following dissolution procedures were eventually adopted.

CEMENT—

Weigh out 0·4 g of the sample into a polythene beaker and add 10 ml of water. Stir the mixture with a polythene rod and add 6 ml of hydrochloric acid (sp.gr. 1·16) and 0·5 ml of hydrofluoric acid (40 per cent. w/w) (addition of more hydrofluoric acid may result in the precipitation of calcium fluoride). When dissolution is complete, add 20 ml of vanadium solution and 10 ml of sodium stock solution, and dilute to 100 ml. Prepare a silicon calibration graph (0 to 600 p.p.m.) by diluting suitable volumes of silicon stock solution, 20 ml of vanadium stock solution and 6 ml of hydrochloric acid to 100 ml.

CAST IRON—

Weigh out 1 g of sample into a polythene beaker and add 8 ml and 2 ml of hydrochloric acid and nitric acid (sp.gr. 1·42), respectively. Cover the beaker with a clock-glass and heat on an asbestos mat on a hot-plate until the reaction has stopped. Cool, wash the clock-glass and add 2 ml of hydrofluoric acid. Filter the solution if necessary, add 5 ml of sodium stock solution and dilute to 100 ml. Prepare the calibration graph for silicon (0 to 400 p.p.m. of silicon) by diluting suitable volumes of silicon stock solution and 20 ml of iron solution to 100 ml. It is unnecessary to add vanadium as the large excess of iron present in both standard solutions and sample will overcome any possible interference from other constituents of the sample.

STEEL—

Weigh out 1 g of the material into a glass beaker and add 5 to 10 ml of water followed by 10 ml of hydrochloric acid. Heat the solution and add, carefully and in small portions, 15 ml of hydrogen peroxide (50 vol). Boil the solution, allow to cool and filter into a 100-ml calibrated flask. Dilute to the mark with water. The calibration graph (0 to 100 p.p.m. of silicon) is prepared as for cast iron.

Alternatively, steel samples can be dissolved in the same way as samples of cast iron, but omitting the addition of sodium solution.

ALUMINIUM ALLOYS—

To 0·5 g of the sample in a polythene beaker add 10 ml of water and, in small portions, a total of 8 ml of hydrochloric acid. Cover the beaker with a clock-glass, and allow the reaction to subside. Add 15 ml of hydrogen peroxide (50 vol.) in small portions. Heat on an asbestos mat on a hot-plate until all reaction ceases. Cool and add 2 ml of hydrofluoric acid. Filter the solution into a polythene flask and dilute to 100 ml with water. (For alloys containing more than 2 per cent. of silicon, add 5 ml of sodium stock solution before finally making the solution up to volume.) Prepare a silicon calibration graph (0 to 250 p.p.m. of silicon) as for cast iron, but add 25 ml of aluminium stock solution in place of the iron. Addition of vanadium is rendered unnecessary by the presence of aluminium in both standard solutions and samples.

In every experiment the standard containing no added silicon was run as the reagent blank.

RESULTS

The results obtained for the determination of silicon in several standard samples are summarised in Table II. When analyses were performed in duplicate, the results are bracketed together in the table. The coefficient of variation at the 0·5 per cent. silicon level (in steel) was found to be 0·012 per cent., or 2·4 per cent. of the mean.

TABLE II

COMPARISON OF RESULTS FOR STANDARDISED MATERIALS

Sample No.	Type	Nominal composition	Silicon, per cent.	
			Standard analysis	Atomic absorption
N.B.S. 1013	Cement	CaO 64%; Fe_2O_3 3%; Al_2O_3 3%	SiO_2 24·1%	SiO_2 23·5% SiO_2 23·7%
N.B.S. 1015	Cement	CaO 61%; Al_2O_3 5%; MgO 4%, Fe_2O_3 3%	SiO_2 20·6%	SiO_2 20·0% SiO_2 19·9%
B.C.S. 206/2	Cast iron	P 1·37%; Mn 0·32%	3·42	3·43
B.C.S. 170/3	Cast iron	Mn 0·76%; P 0·71%; Cu 0·60%	2·47	2·53
Private sample	Cast iron	—	2·14	2·13 2·13
B.C.S. 241/1	High-speed steel	W 19·6%; Co 5·7%; Cr 5%	0·33	0·30
B.C.S. 251/1	Low alloy steel	Mn 1·5%; Mo 1·6%	0·41	0·39
B.C.S. 253/1	Low alloy steel	Ni 1%; Cr 1%	0·65	0·65 0·66
B.C.S. 336	Stainless steel	Cr 17·6%; Ni 9·5%; Mo 2·4%	0·51	0·51 0·53
B.C.S. 339	Chrome - vanadium steel	Cr 12·4%	0·36	0·36
B.C.S. 235	Stainless steel	Cr 18·6%; Ni 9·4%	0·82	0·83
B.C.S. 181/1	Aluminium alloy	Cu 4%; Mg 1·4%; Ni 2%	0·38	0·35 0·39
B.C.S. 216/1	Aluminium alloy	Cu 4%; Mg 0·74%; Mn 0·73%	0·74	0·72 0·72
B.C.S. 268	Aluminium alloy	Cu 1·34%; Mg 0·56%	2·43	2·46

DISCUSSION

Enhancement of silicon absorption by elements such as aluminium, calcium, sodium and vanadium can be explained in terms of suppression of the ionisation of silicon in the hot nitrous oxide flame (about 3000° C) by the added element. Evidence for ionisation in this flame exists for magnesium[8] and copper, both of which have ionisation energies just slightly lower than that of silicon. If this assumption is correct, it would appear that silicon is ionised to the extent of 10 to 15 per cent. No satisfactory explanation for the suppression of silicon absorption by phosphate is as yet forthcoming. The presence of phosphorus in steel and in cast iron has a negligible influence on the silicon response, presumably because of the complexing effect of iron itself. The magnitude of the effect of phosphorus on silicon in the absence of iron can be interpolated from Fig. 1, and an example of the determination of

silicon in cast iron in the presence of a comparatively high amount of phosphorus is included in Table II.

The majority of replicate results agree to within ±2 per cent. of the certificate value. When silicon is present as a minor constituent, as for instance in steel where it is usually present to an extent less than 1 per cent., a coefficient of variation of 2·4 per cent. of content was found; at higher silicon levels the precision improves significantly to give a coefficient of variation better than 1 per cent. The lowest concentration level at which silicon can be determined with acceptable accuracy depends mainly on sample dilution and instrumental conditions. As interfering effects can be largely overcome, the limits both of detection and determination are not influenced by the matrix elements. With a silicon sensitivity of 8 p.p.m. and a detection limit in aqueous solution of 3 p.p.m., concentrations of about 30 p.p.m. can be measured with a 10 per cent. coefficient of variation. This represents 0·03 per cent. of silicon if a 1-g sample is made up to 100 ml of solution. This is probably the practical limit of determination for silicon in steel or aluminium by the methods described, though clearly it can be improved by replication; lower concentrations can be determined with a greater proportional error.

The presence of silicon has hitherto caused difficulty in analytical methods, both because it has been thought to be difficult to bring completely into solution and also because of the interference effects it has caused. With the knowledge that silicon can be retained completely in solution, and not lost either by precipitation as silica or volatilisation as silicon tetrafluoride, a new approach can now be made to the complete analysis of many different materials by atomic-absorption spectrophotometry.

It should first be decided whether or not silicon is to be determined by atomic absorption, and if the silicon content is less than 20 per cent. it is reasonable to expect that it can. For silicon levels between 5 and 20 per cent. a small sample weight can be taken to ensure dissolution of silicon and to enable all or most major elements, including silicon, to be determined in this one solution. A second, more concentrated, solution, with silicon removed as silicon dioxide, may have to be prepared for the determination of minor elements. If silicon is present at a concentration of less than 5 per cent., larger amounts of sample (up to 1 g per 100 ml) should be used. Minor elements are determined in the same solution, and major elements after suitable dilutions have been made.

The presence of hydrofluoric acid necessitates the use of beakers and flasks constructed from material other than glass. Polythene beakers are quite satisfactory, provided strong heating is not required. Hard polythene measuring cylinders, which have been calibrated prior to use, enable volumes of 100 ml to be measured with an accuracy of better than 0·5 per cent.

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Determination of Sodium-22 in Rain Water by Using a Low Level β-Counter

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A method has been developed for the separation from rain water of sodium-22, sufficiently radiochemically pure for it to be measured by low level β-counting. A 50-litre sample of rain water is evaporated to about 1 litre and the concentrate passed through a cation-exchange column. The sodium is eluted with 0·7 N hydrochloric acid solution. The sodium fraction is then purified, precipitated with α-methoxyphenylacetic acid, converted into sodium chloride and counted in a low background anti-coincidence β-counter.

Schemes of analysis have been worked out and used for the determination of most of the radionuclides present in rain and tap waters. They include fission nuclides, natural activities resulting from the uranium and thorium series and manganese-54 which, although not a fission nuclide, has been present in fall-out since the Russian tests of 1961. Sodium-22 and sodium-24 are formed in small amounts in the upper atmosphere by cosmic-ray spallation of argon.[1] Sodium-22 is also formed by the fast-neutron reaction $^{23}Na(n,2n)^{22}Na$ in thermonuclear explosions. Sodium-22 was first detected in rain in 1957 (0·007 pCi per litre),[2] but a sharp increase was reported in 1963 following the first thermonuclear tests in the atmosphere.[3] During examinations of fall-out contamination in aircraft engine washings in this laboratory, in 1963, sodium-22 was suspected, although its presence was not later substantiated. Substantial amounts of sodium-22 had, however, been reported in the urine of eskimos eating caribou meat[4] and in milk.[5,6]

The half-life of sodium-22 is 2·6 years, and it decays by positron emission (90 per cent.) and electron capture (10 per cent.) to an excited state of neon-22. De-excitation to the ground state takes place by emission of a 1·28-MeV γ-photon.

γ-Spectrometry can be used to determine sodium-22, but the levels are much too low to be detected by an ordinary γ-spectrometer. Perkins and Nielson[5] have obtained results by using a 4096 channel multi-parameter memory spectrometer. Positron counting[3] is a much more specific way of determining sodium-22, but it was found too expensive to set up for the work that was to be undertaken in this laboratory.

Experimental

A 50-litre sample of rain water was collected on the roof of Cornwall House, Stamford Street, London, S.E.1, by using the sampling method of Wood, Metson and Richards.[7] The sample is reduced in volume by evaporation and the concentrate filtered to remove any insoluble matter.

The insoluble matter is treated with hydrofluoric and perchloric acids to remove silica and the residue treated with dilute hydrochloric acid, the solution being combined with the concentrate and made up to 1 litre with distilled water. A small aliquot is removed for the determination of sodium by flame photometry, and the remainder passed through a cation-exchange column, which absorbs all of the cations. The sodium and some of the potassium

and caesium are eluted from the column in the first fraction, with 0·7 N hydrochloric acid as eluant. The eluate is contaminated with ruthenium, which is removed by two ruthenium oxide scavenges, after which the filtrate is evaporated to dryness with perchloric acid. The perchlorate residue is extracted with anhydrous ethyl acetate, which dissolves sodium perchlorate in preference to caesium and potassium perchlorates.

The residue is filtered off and dry hydrogen chloride gas passed through the filtrate to precipitate sodium chloride. The sodium chloride is then dissolved in water and sodium precipitated with α-methoxyphenylacetic acid reagent, which separates the sodium from the remaining traces of potassium and caesium. The acid salt of the organic acid is converted into sodium chloride with butanol - dry hydrogen chloride gas mixture, filtered, mounted and counted in a low background anti-coincidence β-counter. The weight of sodium chloride gives the chemical recovery of the counting source, by using the sodium content of the rain water determined by flame photometry and making allowance for the sodium present in all of the reagents added.

Although the method takes about 7 days to complete, the actual practical work after the ion-exchange separation takes 1 day. Because of the time elapsed between the start and finish, any sodium-24 (half-life 15·4 hours) present initially would have decayed away before the source was counted.

METHOD

APPARATUS—

Low background (about 1 count per minute) anti-coincidence β-particle counters were used.

Ion-exchange column[8]—A column of length 20 cm and diameter 3 cm was prepared with Amberlite resin CE-120 (200 mesh) and converted into the hydrogen form with concentrated hydrochloric acid. The flow-rate used was 0·8 ml per minute.

De-mountable Perspex filter-stick, 16-mm internal diameter—Filter-papers, Whatman No. 42, 21-mm diameter, for use with filter-stick.

Infrared lamps.

Heating ring, 1200 watts.

Flask, 10-litre capacity.

Beakers and centrifuge tubes.

Platinum crucible.

Pipettes.

REAGENTS—

Use analytical-reagent grade reagents whenever possible and check them for the presence of radiochemical contamination.

Ruthenium carrier solution—Dissolve ruthenium trichloride in concentrated hydrochloric acid and dilute with water to give about 5 mg of ruthenium per ml of solution.

Hydrofluoric acid solution, 40 per cent., aqueous.

Hydrochloric acid, 10, 0·7 and 0·1 N.

Perchloric acid solution, 60 per cent. aqueous.

Bromine water—Saturate water with bromine.

Ethanol, 95 per cent. aqueous solution.

Butanol - hydrogen chloride—Saturate butanol with dry hydrogen chloride gas.

Ethyl acetate.

Tetramethylammonium hydroxide solution, 25 per cent. aqueous.

Dry hydrogen chloride gas—Prepare by dropping concentrated hydrochloric acid on to concentrated sulphuric acid.

α-Methoxyphenylacetic acid[9]—Dissolve 5·0 g of α-methoxyphenylacetic acid in 26·4 ml of absolute ethanol, add 4·1 ml of tetramethylammonium hydroxide solution and make up to 37·7 ml with distilled water. The reagent is 0·8 N with respect to the acid and 0·3 N with respect to the base.

The sodium salt precipitated is the acid salt [$C_6H_5(CH_3O)CHCOONa.C_6H_5(CH_3O)$-$CHCOOH$] containing 6·49 per cent. of sodium.

"Gelva solution"—A 5 per cent. w/v methanolic solution of poly(vinyl acetate).

PROCEDURE—

Step 1—Evaporate the 50-litre sample of rain water to about 900 ml on a heating ring. Filter the concentrate through a filter-paper (Whatman No. 30).

Step 2—Ignite the filter-paper containing the insoluble material in a platinum crucible, allow to cool, and then treat the residue with hydrofluoric and perchloric acid solutions to remove the silica; evaporate to dryness.

Step 3—Treat the residue with 10 ml of 10 N hydrochloric acid and 10 ml of water (remove and discard any remaining insoluble matter by filtration), and combine it with the main filtrate from step 1 and make up to 1 litre with water.

Step 4—Dilute 10 ml of this solution to 1 litre for the determination of sodium by flame photometry.

Step 5—Pass the remaining 990 ml through the ion-exchange column. Wash the column with 300 ml of 0·1 N hydrochloric acid. Elute the sodium from the column with 300 ml of 0·7 N hydrochloric acid, evaporate the eluate to about 20 ml and add 5 mg of ruthenium carrier, followed by 1 ml of bromine water.

Step 6—Add tetramethylammonium hydroxide solution (this is used because a strong base is required for complete precipitation) until the solution is alkaline, then add 2 ml of ethanol and boil to coagulate the ruthenium oxide precipitate. Remove the ruthenium oxide by filtration and repeat the ruthenium scavenge on the filtrate, and again filter off the ruthenium oxide.

Step 7—Acidify the filtrate from step 6 with 5 ml of perchloric acid solution and evaporate to dryness under an infrared lamp, and finally heat on a hot-plate to remove the last traces of perchloric acid. (All traces of perchloric acid must be removed because of risk of explosion with organic material.)

Step 8—Cool, add ethyl acetate to the residue and warm to dissolve the sodium perchlorate, filter and reject any insoluble matter.

Step 9—Pass dry hydrogen chloride gas into the filtrate to precipitate sodium chloride, centrifuge and discard the supernatant liquid.

Step 10—Dissolve the sodium chloride in water, add 10 ml of α-methoxyphenylacetic acid reagent and allow to cool in an ice-bath for 40 minutes, with occasional stirring. Transfer the tube to a water-bath at 20° C, stir for 5 minutes, centrifuge and discard the supernatant liquid.

Step 11—Add 5 ml of butanol - hydrogen chloride mixture to the precipitate to convert the sodium salt of α-methoxyphenylacetic acid into sodium chloride, centrifuge and discard the supernatant liquid. Wash the sodium chloride with 5 ml of butanol, centrifuge and discard the washing.

Step 12—Transfer the precipitate from step 11 with butanol to the filter-stick containing two tared Whatman No. 42 (21-mm diameter) filter-papers and wash once with 2 ml of butanol.

Step 13—Separate the filter-papers, dry in an oven at 80° C, re-weigh, mount on a 1-inch aluminium planchet by using Gelva solution and count in a low background β-counter. Check for the absence of caesium and potassium by counting the source covered with a 51 mg per cm^2 aluminium absorber. Average transmission for the counter used was 15 per cent. for sodium-22, 26 per cent. for caesium-137 and 50 per cent. for potassium-40.

Step 14—Calculate the chemical recovery from the weight of sodium chloride on the filter, the sodium content of the rain water and the sodium content of the reagents used after the ion-exchange separation.

STANDARDISATION OF THE β-COUNTER—

Use a standard sodium-22 solution (obtainable from the Radiochemical Centre, Amersham) to prepare a series of sources varying in thickness from 6 to 60 mg per cm^2. Count each source on a low level β-counter, both normally and covered with a 51 mg per cm^2 aluminium absorber, and plot an efficiency against source weight graph.

LIMITS OF DETECTION—

By using a counter with a background of 1·2 counts per minute and a counting time of 1440 minutes, the minimum detectable activity is 0·4 pCi of sodium-22, based on three standard deviations of the background count. This gives 0·008 pCi per litre for a 50-litre sample.

Results

Table I shows the amounts of total sodium found in rain water and the reagents used.

Table I

Content of inactive sodium found in rain and reagents

Sample	Sodium, p.p.m.
Rain water (1)	3·8
Rain water (2)	2·8
Rain water (3)	0·9
Tetramethylammonium hydroxide solution	800
α-Methoxyphenylacetic acid solution	100
Ruthenium carrier solution	0·6

Table II shows the radiochemical recovery of sodium-22 added to different types of water. The activity added was sufficient to make it possible to neglect in the calculation any sodium-22 present in the sample originally.

Table II

Radiochemical recovery of sodium-22

Type of water	Sodium-22 added, pCi	Sodium-22 found, pCi	Radiochemical recovery, per cent.
Distilled	928	890	96
Rain	4640	4509	97
Soft	4640	4500	97
Hard	4640	4310	93
Intermediate	4640	4456	96

Table III shows the decontamination factors for the principal radionuclides, with a half-life greater than 30 days, likely to be present in rain water. Manganese-54 was not included in these experiments because it is neither positron active nor β-active and hence would not be counted by a Geiger counter.

Results given in Table III were obtained by adding active isotopes to various samples of rain water and then taking the latter through the above procedure. The amount of contamination caused by each isotope was measured in the final sodium chloride source.

With ruthenium, the chemistry of which is complex, the active species was added in the form of ruthenium trichloride, which may not match the chemical species in fall-out. The recommended procedure, however, affords adequate decontamination from ruthenium species so far encountered in rain water.

Table III

Decontamination factors

Nuclide	Activity added, disintegrations per minute	Activity found, disintegrations per minute	Decontamination factor
Ruthenium-106/rhodium-106	8×10^6	50	$1{\cdot}6 \times 10^5$
Caesium-137	$2{\cdot}5 \times 10^4$	2	$1{\cdot}3 \times 10^4$
Strontium-90/yttrium-90	2×10^5	1	2×10^5
Cerium-144/praseodymium-144	$1{\cdot}25 \times 10^5$	3	$4{\cdot}2 \times 10^4$
Promethium-147	$4{\cdot}5 \times 10^5$	10	$4{\cdot}5 \times 10^4$
Zirconium-95	$8{\cdot}0 \times 10^4$	1	$8{\cdot}0 \times 10^4$
Antimony-125/tellurium-125*m*	$1{\cdot}25 \times 10^6$	15	$8{\cdot}5 \times 10^4$
*RaD.E.F.	$2{\cdot}22 \times 10^4$	0·8	$2{\cdot}7 \times 10^4$
Potassium-40	1416	0·6	$2{\cdot}3 \times 10^3$

* RaD.E.F. is an equilibrium mixture of lead-210 (RaD), bismuth-210 (RaE) and polonium-210 (RaF).

TABLE IV

RESULTS OBTAINED FROM RAIN-WATER SAMPLES

Date of collection of rain water	Activity of sodium-22, pCi per litre	Activity of caesium-137, pCi per litre
December, 1964, to February, 1965 ..	0·18 ± 0·03*	12·3
May to June, 1965	0·13 ± 0·02	18·8
October to November, 1965	0·04 ± 0·01†	2·7

* 90 per cent. confidence limits on counting.
† Very high rainfall.

Table IV shows the results obtained from rain-water samples, the results for caesium-137 being included for comparison.

These values are in close agreement with those obtained in June, 1964, by Perkins,[10] who used a multi-parameter γ-spectrometer. They are about half the peak levels observed by Rodel[3] in 1963.

Lal and Peters[11] calculated the production rate for sodium-22 produced by cosmic rays at latitude 50° N. If it is assumed that all of this is carried down uniformly in rain, the calculated activity in London rain water is 0·01 pCi per litre, which is considerably less than the observed values.

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An Investigation of the Degraded Neutron Flux in a 14-MeV Neutron-activation Cell

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Measurements have been made of the fluxes of thermal and indium resonance neutrons near the target of a 14-MeV neutron generator in a small irradiation cell. These measurements can be used to assess the importance of interfering reactions in fast-neutron activation analysis. For a fast-neutron output of 10^9 neutrons per second the measured sub-cadmium flux was $3{\cdot}8 \times 10^4$ neutrons per cm^2 per second, and the flux at the 1·4-eV indium resonance was $1{\cdot}3 \times 10^3$ neutrons per cm^2 per second per eV.

Fast-neutron activation analysis is often performed with the source in the middle of a relatively small cell. In these conditions there may be, at the sample position, a considerable flux of neutrons scattered from the walls with degraded energies, and it is useful to know the extent of this contamination to assess the importance of any interfering reactions. The most important neutrons are likely to be those with energies in the thermal and resonance regions. The fluxes of neutrons with energies below the cadmium cut-off and in the indium resonance region in a typical assembly have been measured in this Department. The results depend on the exact arrangement of moderating and absorbing material, but they will serve as a guide for workers with similar assemblies who wish to evaluate the necessity to take precautions against interfering reactions.

Experimental procedure

Neutrons (14 MeV) were produced by the D–T reaction at the centre of a cell about $1{\cdot}5 \times 1{\cdot}5 \times 2$ m. The walls consisted of concrete and earth on two sides and about 1 m of concrete on the others. The neutron output was measured by the associated particle technique, and the results are normalised to an output of 10^9 neutrons per second. The thermal-neutron flux was measured by irradiating two indium foils, one with a cadmium cover, in symmetrical positions 5 cm from the target. The resonance flux was measured by subsequently irradiating two indium foils, both in cadmium covers, but one also covered by 0·4 mm of indium, which served to shield that foil from neutrons with energies equal to the indium resonance. All of the indium foils were 1·27 cm in diameter and 0·28 mm thick.

The activities of the foils were measured with an end-window Geiger counter. This counter was calibrated by using an indium foil irradiated in a thermal-neutron facility. The activity of this foil was measured on the Geiger counter, and on a scintillation counter whose efficiency for indium-116*m* γ-rays was obtained from the calculations of Vegars, Marsden and Heath.[1]

Results

Thermal neutrons—

The activities of the bare and the cadmium-covered foils were followed for 1 hour after the end of the irradiation. Both decay curves showed the presence of a mixture of activities, but the difference fell with a half-life that agrees with the expected 54 minutes of indium-116*m*.

The saturation activity caused by neutrons below the cadmium cut-off was estimated to be $3{\cdot}03 \times 10^3$ disintegrations per second for a fast-neutron output of $4{\cdot}88 \times 10^8$ neutrons per second. A small correction needs to be applied for the depression of thermal-neutron flux within the foil; this was estimated from data compiled at the Argonne laboratory.[2] Assuming an activation cross-section of 155 barns, the calculated value of the thermal-neutron flux was $3{\cdot}8 \times 10^4$ neutrons per cm^2 per second for a fast-neutron output of 10^9 neutrons per second.

RESONANCE NEUTRONS—

The decay curves of the two foils again indicated a mixture of activities, but the difference between the counts fell with the expected half-life of indium-116*m*. The saturation disintegration rate caused by the resonance neutrons at the end of bombardment was estimated to be $0{\cdot}745 \times 10^3$ disintegrations per second for a fast-neutron output of $6{\cdot}7 \times 10^8$ neutrons per second. A correction for self-shielding must again be applied, and a correction is also necessary to allow for the difference in distribution within the foil of the activity produced by the resonance neutrons and that by thermal neutrons in the calibration foil. This has been estimated by assuming an exponential absorption of β-particles within the foil with a linear absorption coefficient, calculated by the method of Gleason, Taylor and Tabern,[3] of $1{\cdot}25 \times 10^2$ cm^{-1}.

To calculate the neutron flux, it is necessary to make assumptions about the shape of the neutron spectrum and the variation of the activation cross-section. If we assume that the activation is mainly caused by the large resonance at 1·4 eV, and the spectrum is constant over the width of this resonance, we can integrate on the assumption that the cross-section varies in accordance with the Breit - Wigner expression. If the peak cross-section is taken to be 30,000 barns and the width of the resonance 0·07 eV, then the observed activation corresponds to a neutron flux at the 1·4-eV indium resonance of $1{\cdot}3 \times 10^3$ neutrons per cm^2 per second per eV for a fast-neutron output of 10^9 neutrons per second.

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The Micro Determination of Calcium in Mammalian Hard Tissues

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A spectrophotometric method is described for the determination of microgram amounts of calcium. The procedure is extremely rapid and simple, enabling amounts of calcium in the range 2 to 3·5 μg per ml to be determined with an accuracy of about ±1 per cent. (standard deviation). The method, based on the ability of calcein (fluorescein-3,3′-bismethyliminodiacetic acid) to complex with calcium ion, measures the reduction in light absorption of a calcein solution at 506 nm on the addition of calcium.

THE analysis of many small enamel particles, 20 to 50 μg in weight, required a method of calcium determination that was both rapid and simple to carry out. An accuracy of about ±1 per cent. (standard deviation) was necessary in order to examine small variations in calcium concentration and calcium-to-phosphorus ratios. Existing techniques were either insufficiently sensitive or too laborious for this purpose.

Calcein (fluorescein-3,3′-bismethyliminodiacetic acid), a dye that forms a fluorescent complex with calcium selectively in the presence of magnesium, has proved valuable in previous investigations of mineralised biological tissues. The calcium - calcein complex was used as an indicator in the complexometric titration of calcium with EDTA.[1,2] To increase the sensitivity of this method, the authors attempted to determine calcium by measuring the amount of fluorescence with a spectrophotofluorimeter. Over a narrow range of concentrations of about 0·5 to 2 μg of calcium per ml, this technique proved moderately successful. Two difficulties were encountered, however, the first being the relationship between calcium concentration and fluorescence, which was not linear (Fig. 1), and the second, the instability of the photofluorimetric instruments available, which prevented accurate reproducibility.

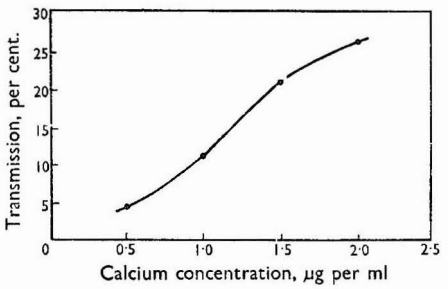

Fig. 1. Relationship between calcium concentration and fluorescence

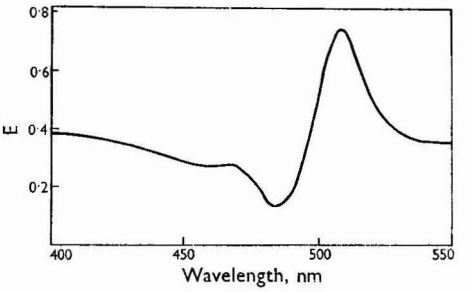

Fig. 2. Absorption spectrum of calcein solution read against a solution containing calcein and calcium

When calcium is added to an aqueous solution of calcein, the resulting fluorescence is accompanied by an apparent decrease in the brown colour of the solution. This prompted

an investigation of the absorption profiles of calcein and calcein - calcium. The profile results from a combination of fluorescence and light absorption. By reading the calcein blank against the calcium - calcein test solution, a maximum E value is obtained at 506 nm and a minimum at 480 nm (Fig. 2). If the cells are reversed the peaks are inverted, 480 nm becoming the maximum and 506 nm the minimum. It has proved possible to measure calcium concentration accurately by reading at 506 nm.

The calcein - calcium complex and the calcein solutions are not stable and the optical densities begin to decrease significantly about half an hour after making up the dye. If the dye solution is placed in an ice-bath, however, satisfactory results can be obtained for at least 30 minutes. This limitation is not very restrictive, as the procedure is so simple that several internal standards, together with twelve to sixteen test samples, can be analysed in 30 minutes. In the range 2 to 3·5 μg per ml, the determination has an accuracy of ± 1 per cent. (standard deviation). Outside this range, there is some deviation from linearity, as can be seen in Fig. 3.

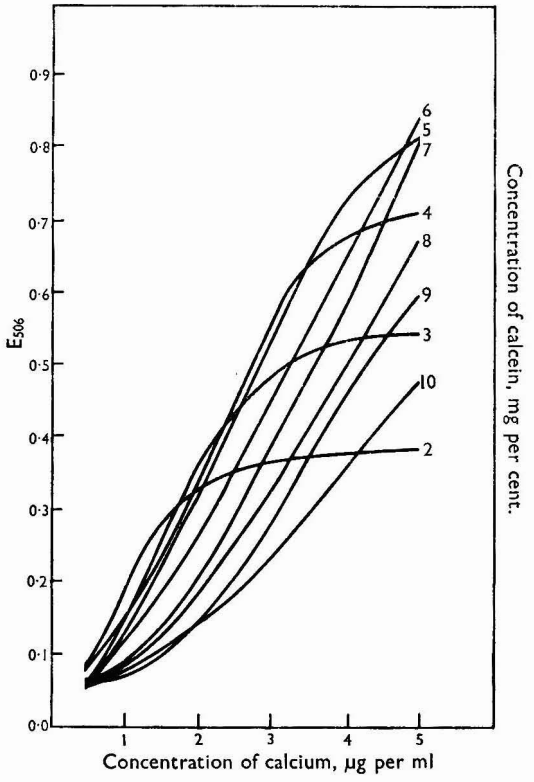

Fig. 3. Effect of increasing concentrations of calcein on calibration curves. The figure at the end of each curve refers to the concentration of calcein, mg per cent.

Experimental

Wavelength of maximum absorption—

Fig. 2 shows the absorption profile of a solution of calcium-free calcein, read against a calcium - calcein solution. The maximum at 506 nm and the minimum at 480 nm can be reversed by reading the solution of calcium - calcein against that of the calcium-free solution of dye.

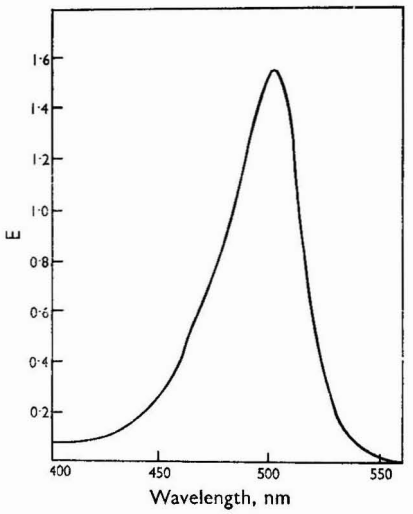

Fig. 4. Absorption spectrum of a solution containing calcein read against distilled water

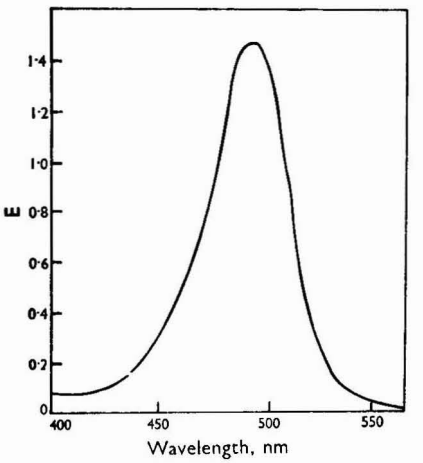

Fig. 5. Absorption spectrum of a solution containing calcein and calcium read against distilled water

The 506 nm maximum reading does not seem to correspond precisely with $\lambda_{max.}$ of calcein (Fig. 4), $\lambda_{max.}$ of calcium - calcein (Fig. 5) or to the excitation maximum of the calcium - calcein complex (Fig. 6). The readings obtained at this wavelength appear to result mainly

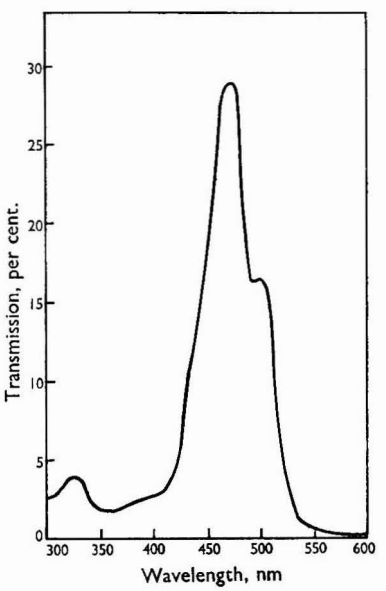

Fig. 6. Excitation spectrum of a solution containing calcium and calcein

from the fluorescence emitted by the calcium - calcein complex. This is suggested by the effects that increasing dye concentrations have on the readings, which can be explained by quenching, and, as the excitation maximum is so far from 506 nm, this suggests that there is also some reduction in absorption. There is perhaps also some reduction in the absorption

of the dye caused by the addition of calcium. Fig. 7 shows the fluorescence-emission spectrum of a calcium - calcein solution, and gives some indication of the extent of fluorescence at the wavelength of 506 nm.

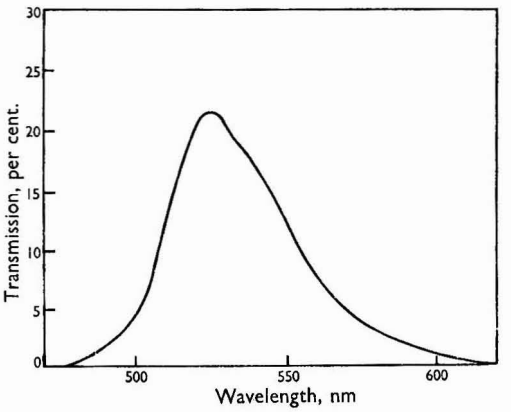

Fig. 7. Fluorescence-emission spectrum of a solution containing calcein and calcium

EFFECT OF DYE CONCENTRATION—

Fig. 3 shows how increasing concentrations of dye affect the calibration graphs. Above a certain concentration the readings fall, which could be caused by quenching of fluorescence by free calcein. This is strongly supported by the fact that increasing concentrations of dye affect fluorescence in a similar manner. The optimum concentration of the dye seemed to be about 5 mg per cent. In the present work a Koch-Light preparation was used.

EFFECT OF PERCHLORIC ACID CONCENTRATION—

Large variations in pH affect the optical density of the calcium - calcein complex and attempts were made to keep the concentration of perchloric acid fairly constant.

The particles of enamel, bone or dentine were weighed and dissolved in 2 M analytical-reagent grade perchloric acid. For a sample of enamel, x μg of the tissue was dissolved in x μl of acid. The dissolved enamel was diluted to $0{\cdot}12\,x$ ml with de-ionised water. This procedure arose from the fact that enamel contains about 36 per cent. of calcium, so that the dilution produced a solution containing an optimum concentration of calcium, *i.e.*, about 3 μg of calcium per ml. The amount of acid present was 8·33 μl of 2 M perchloric acid. For other tissues, *e.g.*, bone or dentine, a similar procedure was followed, the aim being to prepare solutions keeping the calcium and acid concentrations as close as possible at these levels.

The calibration solutions (*e.g.*, 2·0, 2·5, 3·0 and 3·5 μg of calcium per ml) also contained similar amounts of acid. As, however, these were made up by progressively diluting a stock solution, which contained both calcium and acid, the more dilute calcium calibration solutions contained also slightly less acid. Only the 3·0 μg per ml calcium calibration solution contained exactly 8·33 μl of 2 M perchloric acid per ml. The effect of this small variation in acid concentration on the determined values for calcium was below ± 1 per cent. The determination can tolerate a two-fold increase in the concentration of acid, *i.e.*, $2 \times 8{\cdot}33$ μl of 2 M perchloric acid per ml, with no significant reduction in accuracy. Higher concentrations of acid than this tend to produce lower readings.

EFFECT OF MAGNESIUM AND PHOSPHATE CONCENTRATIONS—

Magnesium can have an effect on the results. The readings of a 3·0 μg per ml calcium solution rose as the molar ratio of magnesium to calcium increased from 0 to 0·1 (Fig. 8). Subsequently, the increase took the form of a series of plateaux. When phosphate is present

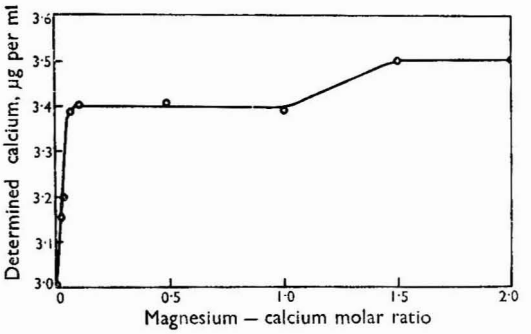

Fig. 8. Effect of increasing concentrations of magnesium on a solution containing 3·0 µg of calcium per ml

the effect of magnesium is reduced (Fig. 9). At a magnesium-to-phosphorus ratio of 0·08, the apparent increase in calcium concentration is about 1·3 per cent., at a magnesium-to-phosphorus ratio of 0·15, the increase in the apparent calcium concentration was 2·0 per cent., where a plateau was reached extending to a magnesium-to-phosphorus ratio of 1·5. Here a further increase in the determined calcium content occurred to about 4 per cent., where a

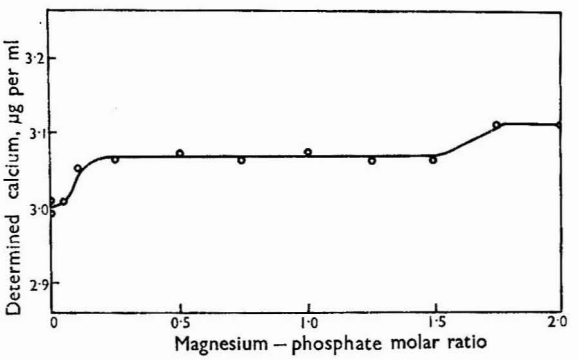

Fig. 9. Effect of increasing magnesium - phosphorus molar ratios in a solution containing 3·0 µg of calcium per ml

second plateau was reached. In the present context, *i.e.*, for bone, dentine or dental enamel (expected magnesium-to-phosphorus ratio about 0·04),[3] there is thus no significant interference by magnesium. The effect of greater concentrations of magnesium could be blanketed by adding magnesium to bring the magnesium-to-calcium molar ratio to between 0·15 and 1·0. This level corresponds to the plateaux shown in Figs. 8 and 9. Equimolar amounts of phosphate do not affect the determination of calcium.

Method

Reagents—

Calcein solution—Dissolve 5 mg of calcein in 25 ml of ice-cold 3 M potassium hydroxide. Make up to 100 ml with de-ionised water. Place this solution in an ice-bath. All determinations should be completed within 30 minutes of making up this dye solution.

Calibration solutions—

Calcium carbonate, 12·5 mg, dried for 1 hour at 105° C is dissolved in 13·9 ml of 2 M perchloric acid and diluted to 1 litre with de-ionised water. Aliquots of this solution, 10, 12·5, 15 and 17·5 ml, are diluted to 25 ml to give solutions containing 2·0, 2·5, 3·0 and 3·5 µg of calcium per ml, respectively.

PROCEDURE—

Make up the calcein solution immediately before the determination. Pipette 1 ml of water and 1 ml of calcein solution into a 1-cm path-length glass cuvette; mix thoroughly with a polythene rod. Retain this water - calcein blank throughout the procedure. In the second cuvette mix 1 ml of sample and 1 ml of calcein solution. Read the water - calcein blank against this test solution at 506 nm.

RESULTS

Fig. 10 shows a typical calibration graph.

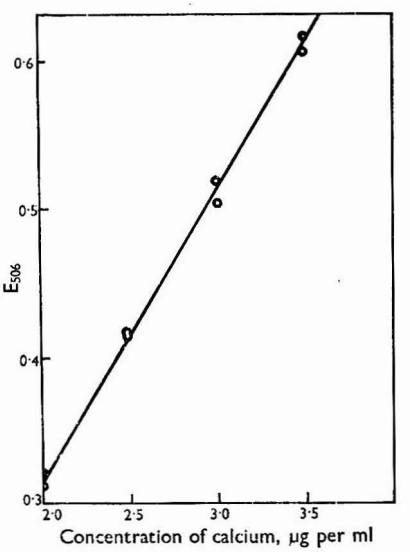

Fig. 10. Typical calibration curve for the range 2·0 to 3·5 µg of calcium per ml

Table I compares the results obtained by analysing solutions of bone, dentine and enamel by complexometric titration,[2] with those obtained by diluting the solutions 100 times and determining calcium by the technique described above.

TABLE I

COMPARISON OF TITRATION WITH SPECTROPHOTOMETRIC MICRO DETERMINATION

	Expressed as µg of calcium per ml of solution	
Material analysed	Titration results, duplicate determinations	Spectrophotometric results, triplicate determinations
Enamel	2·84	2·87
Dentine	3·28	3·33
Bone	2·99	2·96

DISCUSSION

The method described provides an extremely simple and rapid technique for the determination of micro amounts of calcium. The basis of the method, *i.e.*, the reduction in the apparent absorption of calcium - calcein solutions at 506 nm is empirical, as the peak does not seem to correspond to any single one of the spectra shown in Figs. 4, 5, 6 or 7. It is not clear to what extent it is caused by fluorescence and to what extent by absorption, but the accuracy and repeatability of the method, together with its great simplicity, justify the empirical approach. Some restriction is imposed by the instability of the dye, but the ease with which the determinations are performed permits the analysis of a fairly large number of samples in the 30 minutes available.

The calcium concentration of the solutions analysed should lie between 2·0 and 3·5 μg of calcium per ml. For mammalian hard tissues this presents no difficulty, as the approximate percentage of calcium is known.

The results presented in Table I agree with those obtained by an established complexometric procedure.

Interference by magnesium has been described. Its effect is appreciable in the absence of phosphate, but can be reduced by adding phosphate, or adjusting the magnesium concentration to within the range corresponding to the plateau shown in Fig. 8. For mammalian hard tissues the effect of magnesium is much reduced by the indigenous phosphate. The mechanism of this reduction is not clearly understood, but is probably caused by phosphate reducing the tendency of magnesium ions to aggregate with calcein molecules in solution.

The sensitivity of the procedure can be increased considerably by using micro cells.

We thank the Medical Research Council for a grant in support of this work, and Mr. G. Naylor for technical assistance.

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Determination of Hydrofluoric Acid in Inhibited Red Fuming Nitric Acid

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A simple method has been devised for directly measuring fluoride-ion concentrations in inhibited red fuming nitric acid, which is commonly used in propellents. The technique involves the use of a newly developed fluoride-sensitive electrode, and the concentrations are determined by measuring the potential developed between this electrode and a reference electrode. A special procedure, involving a buffer solution, was devised for weighing the inhibited nitric acid before neutralisation, dilution and measurement. The results are more accurate than those obtainable by the commonly used, and more complex, indirect methods. For the particular instance of inhibited red fuming nitric acid, the technique also proved superior to another proposed direct method in that it is effective with samples as small as 1 g.

RED fuming nitric acid is a common and effective oxidant for liquid-propellent missiles, but it is a very corrosive acid that will attack most metal containers. The corrosive action can be inhibited to a large extent by the addition of hydrofluoric acid in concentrations of about 0·5 M. For both assay and storage monitoring purposes, there is a need for a reliable technique of determining fluoride-ion concentrations in inhibited red fuming nitric acid.

Until recently the determination of fluorides has been difficult; the only analytical techniques available were based entirely on indirect methods, such as the effect of fluoride on redox potential of metal-ion complexes and on bleaching techniques such as that used by Johnson and Jones,[1] which involved the removal of iron from ferron by fluoride. However, the recent development of the Orion fluoride-ion electrode[R] makes it possible to use direct measurement techniques.[2] With this electrode, the direct measurement of fluoride-ion concentration is as simple as pH measurement.

One such technique, which provides a simple, sensitive and accurate means of determining the hydrofluoric acid in inhibited nitric acid, is described below. Another direct method, likewise based on the Orion electrode, was also considered and is discussed briefly; however, this second technique was found unsuitable for this analysis.

EXPERIMENTAL

GENERAL TECHNIQUE—

This method basically consists in weighing a solution of the inhibited nitric acid, neutralising the acid and measuring the potential developed between the fluoride-sensitive electrode and a convenient reference electrode, in this instance a saturated calomel electrode.

MATERIALS—

A standard fluoride stock solution was prepared from a weighed amount of analytical-reagent grade sodium fluoride. Aliquots of the stock solution were used to prepare known concentrations over the desired concentration range in a buffer solution of 0·1 M acetic acid and 0·1 M sodium acetate.

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PROCEDURE—

The procedure described below was designed specifically for the hydrofluoric acid concentration range usually used in inhibited nitric acid, *i.e.*, 0·4 to 0·8 per cent. w/w.

About 1 g of the inhibited nitric acid was weighed and diluted to 100 ml with 0·1 M acetic acid - 0·1 M sodium acetate buffer. A 5-ml volume of this solution was removed and diluted to 100 ml with the buffer solution. The amount of acid taken was determined accurately by weighing a screw-capped plastic vial containing 25 to 30 ml of buffer solution, and then adding about 1 ml of the sample with the aid of a disposable pipette and a 1-ml rubber suction cup. After the sample had been drawn into the pipette, the tip was quickly inserted into the weighed buffer solution and the acid forced out. In quick succession the vial was closed and re-weighed, and the solution diluted to 100 ml in a calibrated flask to provide the dilutions for measurement. In the preparation of the solutions, it is important that the pH be kept within the range of 4 to 6. The pH is slightly low, but a well poised buffer free from interfering anions is provided.

The final solution was then transferred to a beaker of suitable size, and the potential (in millivolts) measured with the Orion fluoride-ion electrode *versus* a saturated calomel reference electrode. As the resistance of the electrode is high, a high-impedance potentiometer is required. A Beckman expanded-scale pH meter was used for this particular experiment. At 25° C, $E = \epsilon + 0{\cdot}05915$ pF. The potentiometer was calibrated before running each group of samples. A standard fluoride solution was used to adjust the electrode potential to a predetermined value, which was obtained in this instance from a calibration graph prepared by plotting the log of the fluoride-ion concentration *versus* the potential in millivolts (Fig. 1); the reproducibility of these specific-ion electrodes is fairly well established as being about that of a good glass electrode for pH.

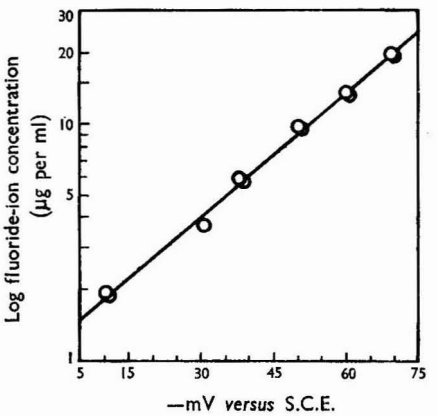

Fig. 1. Calibration graph with Orion fluoride electrode *versus* S.C.E. in 0·1 M acetic acid buffer. Measurements were made with a Beckman expanded-scale pH meter

RESULTS AND DISCUSSION

This method is rapid, simple and direct, and quite accurate, as indicated by the results given in Table I for known samples in similar ionic conditions. These samples were prepared by adding known amounts of sodium fluoride to fuming nitric acid to simulate the characteristics of inhibited red fuming nitric acid, so that the buffer solution would carry the same load as with an inhibited nitric acid sample. If the latter has a lower concentration of hydrofluoric acid than the prepared samples studied, it may be necessary to use a larger sample and to provide a greater buffer capacity for the additional acid. Instead of the 0·1 M acetic acid and sodium acetate, a 1·0 M system could be used.

In Table II, the results are compared with those obtained by a colorimetric procedure developed by Merwin[3] and Steiger,[4] which is based on the bleaching action of fluorine on the yellow colour produced by oxidising a solution of titanium sulphate with hydrogen peroxide. This method is at present required by U.S. Army Military Specification.

TABLE I

DETERMINATION OF HYDROFLUORIC ACID IN KNOWN SAMPLES PREPARED FROM SODIUM FLUORIDE AND FUMING NITRIC ACID

Sample No.	Hydrofluoric acid added, per cent.	Hydrofluoric acid found, per cent.
1	0·40	0·39
2	0·53	0·52
3	0·64	0·65
4	0·79	0·79
5	0·88	0·90
6	1·19	1·18

TABLE II

COMPARISON OF METHODS FOR MEASURING HYDROFLUORIC ACID IN INHIBITED RED FUMING NITRIC ACID

Sample No.	Merwin - Steiger method	Fluoride electrode
1	0·62	0·63
2	0·67	0·61
3	0·60	0·65
4	0·58	0·66
5	0·69	0·59
6	0·66	0·59
7	0·65	0·65
8	0·64	0·67

The unfavourable results from the Merwin - Steiger method were to be expected, as it requires about a 6-g sample to give sufficient fluoride ion to determine its concentration. The nature of inhibited nitric acid portends difficulty in any method that requires the neutralisation of more than a 1-g sample. In addition, the technique is complex and time consuming. The standard graph used in the Merwin - Steiger method must be checked frequently to be certain of the results obtained. This necessitates preparing several known samples and a blank for each set of test runs. Analysis of the sample and verification of the standard graph requires several hours.

Another direct method for determining fluoride-ion concentration investigated was a titration technique suggested by Lingane,[5] in which the Orion specific-ion electrode was likewise used. Thorium, calcium, aluminium and zirconium were tried as titrants for the fluoride ion, but this method was not amenable to the analysis of inhibited nitric acid. Thorium gave a nitration curve, but was of little value at the fluoride concentration that would permit the use of a 1-g sample. A solution of 0·1 M thorium was prepared and standardised with standard sodium fluoride. With such a high concentration of thorium, it was necessary to neutralise 6 to 7 g of sample to obtain a satisfactory curve. The problem of neutralisation was further complicated by the need to keep the volume of solution small so that the fluoride-ion concentration would not be too low. The other titrants did not give curves at all under the required conditions.

We thank Mr. Albert W. Saddler of Technical Micronics Control Inc. for supplying the results obtained by the Merwin - Steiger procedure, which are presented in Table II.

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Determination of the Total Silicon Content of Water

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A method is described for the determination of total silicon content of water based on evaporation, fusion and subsequent absorptiometric measurement of solutions of reduced β-molybdosilicic acid. The standard deviation for this procedure, by using samples of between 20 and 60 ml, was found to be 0·1 μg of silicon as silica.

To meet the specification requirements of new high-pressure boilers a method was required for the determination of lower levels of total silicon in water than had been previously necessary. The following method describes an adaptation of Morrison and Wilson's method,[1] in which the random errors at low concentration, contributed by atmospheric pollution of the evaporation and fusion stages, are reduced.

The method has been tested to determine the standard deviation of analytical results by carrying out silicon determinations on specially purified water.

EXPERIMENTAL

LABORATORY—

The method was carried out in a laboratory, shown in Fig. 1, that complied with the following requirements: maximum protection against solid particulate contamination at bench level; the minimum restriction on movement of personnel and on working facilities; and maintenance of the filtration and air control equipment causing the minimum interruption of work.

To achieve the above conditions, the maximum possible filtration efficiency of the air must be effected immediately before it passes over the work-bench area. The air, which is first passed through a large surface area 5-μm pre-filter followed by four absolute filters, enters the laboratory through eleven ceiling-mounted absolute filters directly above the work-bench area. Each filter operates at about 60 per cent. of its air-carrying capacity, and has an initial input velocity of 150 feet per minute. The pre-conditioned air is then guided down the working area of the bench by a 4-foot deep, clear Perspex screen installed over the leading edge of the entire bench area. The screen affords protection from operator contamination and from contamination generated within the floor area. The whole plant works on a once-through air system.

The optical density measurements were made in 10-cm cuvettes at 805 nm on a Unicam SP500 spectrophotometer, with de-ionised water in the reference cuvette. It is advisable to check the wavelength, as this may vary slightly from one instrument to another.

APPARATUS—

Heating block—This is a cast aluminium block (9 × 9 × 4 inches) fitted with four 250-watt heaters and thermostat, drilled and turned to take four 30-ml platinum crucibles and a thermometer. To ensure a good heat transfer between the block and the crucibles, a few millilitres of glycerol are introduced into each compartment, the temperature of the block being maintained at between 105° and 110° C.

Platinum crucibles—Platinum crucibles of 30-ml capacity are used. A series of fusion tests is carried out to determine whether any particular crucible gives results consistently different from the others. When not in use the crucibles should be stored in electronic-grade hydrofluoric acid.

Polythene bottles—Narrow-necked, 4-oz polythene bottles with caps are used in the colorimetric stage. Each bottle is cleaned initially with 0·75 ml of 5 N hydrochloric acid and 2 ml of 10 per cent. w/v ammonium molybdate in 100 ml of water, heated in a boiling water bath for 1 hour and then emptied. This procedure is repeated without the heating. The solutions are then treated as described in the colorimetric determination under "Procedure." Bottles that give high optical densities must not be used.

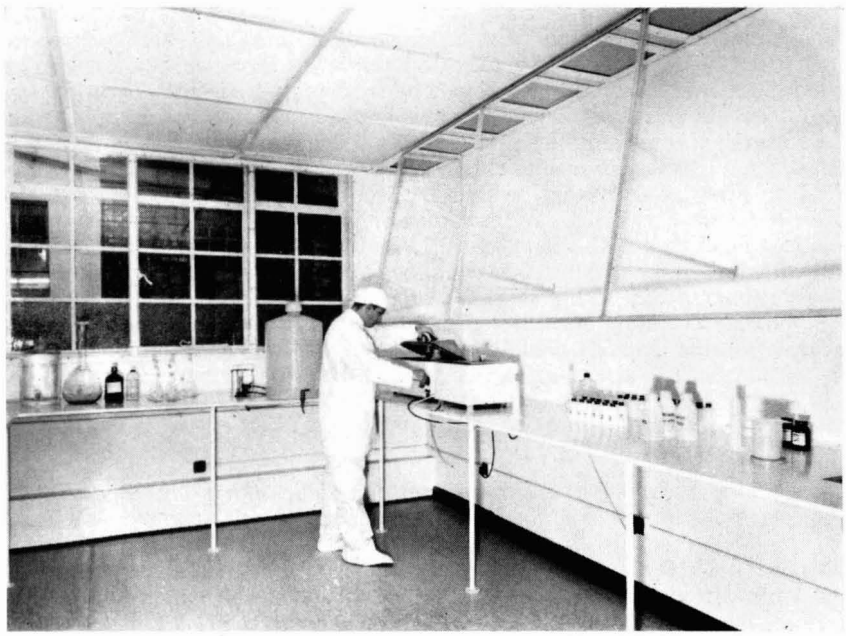

Fig. 1. The lay-out of the laboratory

[To face page 732

REAGENTS—

All reagents are of analytical-reagent grade unless otherwise stated. Polythene beakers, measuring cylinders and funnels should be used for making up reagents, which are then stored in polythene bottles.

Treated water—Mixed-bed de-mineralised water further treated with De-Acidite FF regenerated with 20 lb of sodium hydroxide per cu. foot. This water, which contains less than 0·001 p.p.m. of reactive silicon dioxide, is hereafter referred to as water.

Sodium carbonate—A 20 per cent. w/v aqueous solution of micro analytical-reagent grade sodium carbonate was made up and tested as follows.

Take 20 g from each batch, make up to 100 ml with water and filter through a Whatman No. 42 filter-paper into a polythene bottle. Carry out fusion blanks in duplicate on 1 ml of this solution from each batch and select further batches containing less than 5 μg of silicon dioxide per 1 ml of solution.

Ammonium molybdate—Make up a 10 per cent. w/v solution of ammonium molybdate tetrahydrate in water. This reagent is stable for at least 1 month.

Hydrochloric acid—Prepare 5 N hydrochloric acid by dilution of concentrated hydrochloric acid with water and standardisation against N sodium carbonate solution. It is found that 5 N hydrochloric acid, purchased as a standard solution, gives high reagent blanks.

Oxalic acid—Use a 10 per cent. w/v solution of oxalic acid dihydrate.

Reducing agent solution—Make up the first solution by dissolving 0·5 g of 1-amino-2-naphthol-4-sulphonic acid and 1·0 g of sodium sulphite, $Na_2SO_3.7H_2O$, in 50 ml of water, and filtering this solution through a Whatman No. 41 filter-paper into a polythene bottle. Make up a second solution by dissolving 27·4 g of sodium metabisulphite (anhydrous) in 150 ml of water, filtering and mixing with the first solution. This should be freshly made every 3 days.

Standard solution of silica—Fuse 1 g of pure silicon dioxide (Johnson Matthey Specpure grade) with 5 g of micro analytical-reagent grade sodium carbonate until a clear melt is obtained. Cool, dissolve the melt in water and make up to 1 litre. This solution contains 1000 p.p.m. of silica.

ANALYTICAL PROCEDURE

CALIBRATION—

Take 10 ml of the 1000 p.p.m. silica solution and dilute to 500 ml to obtain a 20 p.p.m. silica solution. Take 20 ml of this solution and dilute to 1 litre to obtain a solution containing 400 μg per litre of silica.

For calibration samples, pipette the required volumes of standard silica into clean 4-oz polythene bottles and make each up to 100 ml with water. To each bottle add 1 ml of the 20 per cent. w/v sodium carbonate solution, then proceed as described under "Colorimetric stage."

Prepare two calibration graphs, one from 20 μg to 1 μg and the other from 5 μg to 0·1 μg. The second graph can be obtained by further dilution of the 400 μg per litre silica solution. Both graphs are linear.

FUSION TESTS—

Test each crucible by carrying out at least two fusions in it. Transfer by pipette 1 ml of the 20 per cent. w/v sodium carbonate solution into each crucible and evaporate to dryness in the heating block. Fuse at bright red heat for 5 minutes with a Meker burner by using a nickel - chrome support and handling the crucible only with platinum-tipped tongs. Allow the melt to cool and dissolve it in water, the top of the heating block being used as a hot-plate. Make up to 100 ml and proceed as in "Colorimetric stage." If any crucible gives a high result, fuse in it 1 g of anhydrous sodium carbonate and swirl the melt so that it comes in contact with the whole of the inside of the crucible, dissolve this melt and discard. Repeat the fusion tests with 1 ml of the 20 per cent. w/v sodium carbonate solution.

REAGENT BLANK—

Into one of the prepared 4-oz polythene bottles place 100 ml of the water and 1 ml of the 20 per cent. w/v sodium carbonate solution (bottle A). Into a second bottle, B, place 5 ml of water and 1 ml of the 20 per cent. w/v sodium carbonate solution. Following the procedure described in the "Colorimetric stage" add the same volumes of the reagents to the contents of both bottles, except that after the addition of oxalic acid to bottle B add a further 95 ml

of water, then add the 1 ml of reducing agent. When the solutions are ready, measure their optical densities at 805 nm against de-ionised water. The bottle A gives the optical density of the reagent *plus* water blank; bottle B gives reagent blank. The difference in optical density between bottles A and B is equivalent to 95 per cent. of the reactive silicon content of the water as silica, used throughout the method.

EVAPORATION—

Two determinations can be completed in duplicate in 1 day with four crucibles, by carrying out 40 and 60-ml evaporations on each sample.

By using the platinum-tipped tongs remove the four crucibles from the hydrofluoric acid, rinse well with water and, to ensure complete removal of the hydrofluoric acid, heat in the Meker burner to bright red heat, then place in position in the heating block. Transfer by pipette into each crucible 1 ml of 20 per cent. w/v sodium carbonate solution. Allow the contents of the two crucibles selected for the 40-ml evaporations to evaporate to dryness and subject them to the "Fusion test" procedure. To the two remaining crucibles add the first 10-ml aliquot of the 60-ml evaporation samples.

Return the first two crucibles to the heating block, add a further 1 ml of the 20 per cent. w/v sodium carbonate solution and the first 10-ml aliquot of the 40-ml evaporation samples.

Introduce subsequent 10-ml aliquots of both 40 and 60-ml evaporation samples into their respective crucibles when the volume has been reduced to about 2 to 3 ml. When the evaporations are completed, fuse, dissolve the melt, make up to 100 ml and transfer the solutions to 4-oz polythene bottles. Carry out "Fusion test" procedure on the crucibles with 1 ml of the 20 per cent. w/v sodium carbonate solution.

COLORIMETRIC STAGE—

To each of the bottles containing samples add 1·5 ml of 5 N hydrochloric acid. Swirl the contents of the bottles and introduce into each 2 ml of the 10 per cent. w/v ammonium molybdate solution. Close bottles and set aside for 10 minutes. Add 2 ml of the 10 per cent. w/v oxalic acid solution to each, swirl, and add 1 ml of the reducing agent. Set aside for at least 15 minutes, after which the colour is stable for several hours. Measure the optical densities in a 10-cm cuvette at 805 nm against de-ionised water.

REPORTING—

The optical density measurements for the samples less the optical density of the fusion blanks gives the concentration of total silicon as silica in the samples.

The optical density of the fusion blanks less the optical density of the bottle A in the "Reagent blank" gives the concentration of non-reactive silicon as silica in the fusion blanks.

DEFINITIONS—

Reactive silicon content of water—Defined as those forms of silicon, mainly monomeric and dimeric silicic acids, that react with ammonium molybdate in 10 minutes under the conditions of the "Procedure" given above.

With the same calibration graph as that used for total silicon content take 100 ml of original sample, add 1 ml of the 20 per cent. w/v sodium carbonate solution and proceed as in "Colorimetric stage."

Total silicon content of the water—Defined as the silicon concentration determined after the evaporation and fusion procedure.

Non-reactive silicon—Defined as the difference between total and reactive silicon.

EFFECTS OF OTHER SUBSTANCES—

It was found that the increasing concentrations of neutral salts present in the samples caused a decrease in absorption.

The effect of 0·2 per cent. w/v neutralised sodium carbonate was found to decrease the optical density figures by about 10 per cent. for concentrations of silica between 0 to 20 μg per 100 ml.

	Silica			
Solutions, 100 ml	5 μg	10 μg	15 μg	20 μg
Sodium silicate solutions	0·167	0·334	0·503	0·672
Sodium silicate solutions *plus* 0·2 g of sodium carbonate ..	0·150	0·303	0·456	0·609

The effects of variation in concentration of reagents and interferences from other substances present were not investigated thoroughly. The method of Morrison and Wilson, although not identical, indicates that similar interferences would occur.[2,3]

Precision

The results are shown in Table I. The figures in the right-hand columns for each batch are the absorbance *minus* the corresponding blank. In each batch the first blank to be tested was assigned to the first observation on each solution tested, the second blank to the second observation on each solution.

Each of the following batches was carried out on consecutive days over a period of 1 week on one homogeneous batch of water.

Table I
Results

	Solution	1st Batch		2nd Batch		3rd Batch		4th Batch		5th Batch	
1	Fusion blanks..	0·042		0·042		0·038		0·044		0·040	
		0·037		0·042		0·034		0·041		0·043	
		0·0395	Mean	0·042	Mean	0·036	Mean	0·043	Mean	0·0415	Mean
2	20-ml evaporation	0·048	0·006	0·052	0·010	0·039	0·001	0·045	0·001	0·045	0·005
		0·048	0·011	0·038	0·004	0·045	0·011	0·042	0·001	0·048	0·005
		Mean	0·0085	Mean	0·007	Mean	0·006	Mean	0·001	Mean	0·005
3	40-ml evaporation	0·050	0·008	0·050	0·008	0·043	0·005	0·053	0·009	0·050	0·010
		0·040	0·003	0·044	0·002	0·049	0·015	0·050	0·009	0·054	0·011
		Mean	0·0055	Mean	0·005	Mean	0·010	Mean	0·009	Mean	0·0105
4	60-m levaporation	0·053	0·011	0·051	0·009	0·042	0·004	0·053	0·009	0·053	0·013
		0·047	0·010	0·053	0·011	0·041	0·007	0·044	0·003	0·048	0·005
		Mean	0·0105	Mean	0·010	Mean	0·0055	Mean	0·006	Mean	0·009

Calculations of within batch (Mo) and between (M_1) mean squares—

Solution	1	2	3	4
Coding X	1000 (X−0·034)	1000 (X−0·001)	1000 (X−0·002)	1000 (X−0·003)
ΣX^2	483	347	494	370
$(\Sigma B)^2/mn$	397	203	360	270
$\Sigma B^2/n$	454	267	413	313
m	5	5	5	5
n	2	2	2	2
M_1	14·3	16·0	13·3	10·8
Mo	6·0	16·0	16·2	11·4

Calculations for standard deviations—

Solution	2	3	4
$Sw^2 = Mo$	16	16·2	11·4
$Sb^2 = (M_1 - Mo)/n$	NS	NS	NS
$St^2 = M_1 + (n-1)Mo/n$	16	14·8	11·1

(NS = non-significant)

Decoding—

Data	Standard deviation	Solution 1	Solution 2	Solution 3	Solution 4
Coded units	Sw	2·5	4·0	4·0	3·4
	Sb	—	NS	NS	NS
	St	—	4·0	3·9	3·3
Original units	Sw	0·0025	0·0040	0·0040	0·0034
	Sb	—	NS	NS	NS
	St	—	0·0040	0·0039	0·0033

where Sw is the within-batch standard deviation, Sb the between-batch standard deviation and St the total standard deviation of single observation.

Conversion into silicon as silica—

Solution	St in optical density units	St in μg of SiO_2 per litre
2 20-ml evaporation	0·004	5
3 40-ml evaporation	0·004	3
4 60-ml evaporation	0·003	1·3

Discussion

This method has been developed to determine the standard deviation of analytical results, with a practical sample size, in order to meet high pressure boiler specifications which require a maximum of 20 μg per litre of total silicon as silica in feed water.

All statistical tests given in this paper are based on the 95 per cent. confidence limit.

The limit of detection for each of the evaporations is defined as $4{\cdot}652\sigma$ where σ is expressed in μg of silica per litre.

For 20-ml evaporations the standard deviation is 5 μg, and the limit of detection is $4{\cdot}652 \times 5 = 23{\cdot}3$ μg.

For 40-ml evaporations the standard deviation is 3 μg, and the limit of detection is $4{\cdot}652 \times 3 = 14{\cdot}0$ μg.

For 60-ml evaporations the standard deviation is 1·3 μg, and the limit of detection is $4{\cdot}652 \times 1{\cdot}3 = 6{\cdot}0$ μg.

When it is only necessary to detect 20 μg per litre or more of silicon as silica it would appear from the above figures that, under the conditions given in this paper, it would be sufficient to use 40-ml evaporations. However, should smaller concentrations of silica need to be detected, then 60-ml evaporations should be used.

It can be assumed that as in each evaporation the standard deviation was 0·1 μg, it should be possible to achieve lower limits of detection by using larger volumes of samples.

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Screening for Alkaloids in Toxicology by Using Thin-layer Chromatography: A Rapid System Simulating Paper Chromatography

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Although numerous thin-layer chromatographic systems for the separation of alkaloids have been reported, none of them compares in abundance of recorded R_F values with the citrate paper system of Curry and Powell. In order to overcome the slowness (which can be a serious disadvantage with this paper system in diagnosis of acute poisoning) without recourse to a new thin-layer chromatographic system and consequent sacrifice of results, a system has been developed that gives R_F values which, for practical purposes, are substantially the same as those in the paper system, for the limited range of drugs examined and for their extracts from horse urine. It is unlikely that the correlation is due to chance, and it is considered, therefore, that the more rapid thin-layer chromatographic system can be substituted for the paper system.

Although originally "alkaloid" was the name given to basic organic nitrogen compounds of plant origin, the definition has gained wider scope, largely because of the development by the pharmaceutical industry of synthetic drugs related chemically and pharmacologically to these "classic" alkaloids, and to the development of other basic nitrogenous drugs with no evolutionary origins at all in classic alkaloid chemistry. An analytical chemist confronted with the need to identify a compound in this class must now search a large and complex group. Chromatographic methods present the most useful approach initially, and the paper-chromatographic system described by Curry and Powell,[1] in which a citrate buffered paper and butanol - citric acid solvent are used, has many advantages over the other paper systems discussed by Clarke,[2] who has recorded R_F values on over 450 different alkaloidal compounds by using a slightly modified form of the system.

Because of the extensive results on R_F values now available (see also Jackson and Moss[3]) this is undoubtedly the best system for the screening of biological samples for the presence of this type of compound.

We have used a system of this kind for several years for the screening of race-horse urine and saliva samples for the presence of "dope," and for screening human body fluids when acute poisoning has occurred.

However, when a rapid result is required, as often happens in human poisoning, the time required for the paper system is a serious disadvantage (about 8 to 10 hours are required for a useful separation).

To shorten development time of the chromatogram, without recourse to a new system, which would have meant sacrificing a large amount of data, we have investigated the use of a thin-layer system, with cellulose powder,[4] analogous to the paper system of Curry and Powell.[1]

The thin-layer chromatographic system was evaluated by comparing R_F values with those obtained with the paper system when a limited number of drugs was used. A larger number of drugs have been compared on these systems by Sunshine, Fike and Laudesman[5] but without a statistical analysis. Pure drugs, and extracts from horse urine to which pure

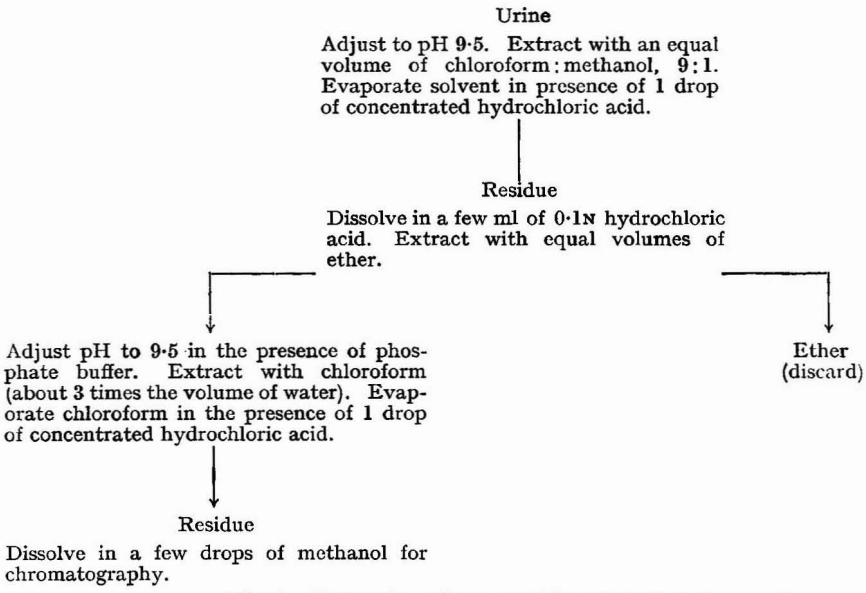

Fig. 1. Extraction scheme used for alkaloids in horse urine

drugs had been added, were spotted in methanol solution on the papers and thin-layer plates. Fig. 1 summarises the extraction procedure used. R_F values and standard deviations are listed in Table I. Most of these are derived from six separate chromatographic developments for each drug or urine extract investigated. The urine extracts used were from six separate extractions for each of the six drugs, each urine sample used being different (*i.e.*, 36 separate urine samples were used). It was hoped that this would allow for variations in R_F values caused by normal variations in composition of urine, and thus to simulate practical working conditions as far as possible. Although we always ran one thin-layer chromatogram and one paper system in adjacent tanks, with solvent that had been prepared in bulk for the day's experiment, we did not obtain any better agreement between thin-layer chromatographic and paper R_F values than by random comparison of R_F values obtained on different days.

Development time for the thin-layer chromatographic system was 1½ to 2 hours, and for the paper, 8 to 10 hours, with a 15-cm rise of solvent for each.

Table I

R_F values and standard deviations of drugs run on paper and thin-layer chromatograms

Clarke's figures are inserted for comparison

	R_F (paper)					R_F (thin-layer chromatogram)			
Drug	Urine extract	Standard deviation	Pure drug	Standard deviation	Clarke	Pure drug	Standard deviation	Urine extract	Standard deviation
Strychnine	27	3·1	23	5·0	24	26	4·3	28	4·3
Amphetamine	49	2·5	45	3·0	45	49	2·9	49	2·1
Nikethamide	85	1·5	88	2·6	83	85	2·7	82	1·9
Procaine	29	2·7	28	3·1	27	31	3·5	27	4·5
Methylamphetamine ..	54	5·2	53	4·8	52	55	4·8	53	6·4
Ephedrine	48	1·2	45	3·4	42	49	3·2	49	6·2
Atropine	—	—	33	4·1	33	40	3·4	—	—
Cocaine	—	—	39	3·1	42	42	4·1	—	—
Xylocaine	—	—	65	3·7	59	67	4·2	—	—
Methylphenidate ..	—	—	61	4·3	59	64	5·3	—	—
Phenothiazine	—	—	96	1·0	—	98	1·7	—	—
Methapyrilene ..	—	—	36	3·4	32	35	2·4	—	—

Experimental

Thin-layer chromatographic plates—A 50-g sample of CC41 thin-layer chromatographic cellulose powder (Chromedia-Whatman) was agitated with 100 ml of 5 per cent. w/v sodium dihydrogen citrate. Method of agitation is critical, and the object should be to ensure maximum agitation without aeration, otherwise crazing of adsorbent occurs during drying. We achieved this by using a laboratory stirrer with paddle blades. Plates (5 × 20 cm) were spread 0·25 mm thick with a Shandon "Unoplan" spreader, and left in the air for 15 minutes, followed by drying at 80° C for 1 hour in an oven with an internal circulating fan.

Plates were developed in tanks that had been equilibrated previously for at least 1 hour by the use, on the inside walls, of blotting-paper impregnated with solvent.

Buffered paper—Whatman No. 1 paper was dipped in a 5 per cent. w/v solution of sodium dihydrogen citrate, blotted, and dried in air at room temperature, essentially as described by Clarke.[1] No equilibration was used before development.

Solvent—For paper and thin-layer chromatograms, 9 parts of butanol were shaken with 1 part of 5 per cent. citric acid solution v/v and the solvent used the same day. Preparation of this solvent differs considerably from Clarke's method, but R_F values do not appear to be affected appreciably thereby. The advantage over Clarke's method is that this preparation is homogeneous. Clarke has, however, reported the preparation of a similar homogeneous solvent elsewhere[6] but does not correlate the results obtained by using this solvent with those reported elsewhere.[2]

Location—All spots were located by spraying with a mixture of 1 g of chloroplatinic acid, 18 g of potassium iodide and 600 ml of water.

We wish to thank Mr. John Giltrow for technical advice in preparing the films, and H. Reeve Angel and Co. Ltd. for a gift of CC41 cellulose powder. This work was undertaken as part of a research programme under the Scheme for the Suppression of Doping, financed by the Horserace Betting Levy Board, to whom we are grateful.

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Extraction Procedures in Chemical Toxicology

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Investigations into the separation by distillation, solvent extraction and the use of ion-exchange resins of drugs from biological materials, with particular reference to urine, are described and discussed.

The number of drugs that can be encountered in chemical toxicology may be described as countless.

Excellent techniques involving the use of ultraviolet spectrophotometry, thin-layer chromatography and spectrofluorimetry are available for the detection and determination of many drugs, and there is an extensive and useful literature on these subjects. Before any of these techniques can be applied, it is essential to effect separation of the substances, suspected or unknown, from the biological material under investigation. This material can include blood, urine, gastric contents, tissues (liver and brain) and foodstuffs, all varying greatly in chemical composition. Because of differences in chemical composition, especially the presence or absence of protein and fat, each type of material presents an individual problem. Urine, because it is a solution and usually contains neither fat nor protein, presents the simplest problem.

During the last 3 years, a chemical toxicology unit has been working in this Department, and its work is closely associated with that of the Poisoning Treatment Centre at the Royal Infirmary, Edinburgh. The latter deals with the diagnosis and treatment of about 1000 suspected cases of drug overdosage each year.[1] On rarer occasions, other substances, *e.g.*, cleaning fluids and weed killers may be involved. Few of the cases have proved fatal. The laboratory receives blood (withdrawn from patients on admission), gastric aspirate and lavage, and urine (collected for at least 8 hours after admission) for chemical analysis. Such tissues as liver and brain are generally not available.

Patients admitted to hospital with symptoms of so-called drug overdosage constitute a complex problem. In some cases poisoning is more or less obvious, whereas in others it is a question of deciding whether the symptoms are actually caused by drug overdosage or by organic disease. Drug overdosage may be caused by more than one drug. Frequently the evidence obtained from patient sources is inadequate or even misleading. In such cases, which are becoming more frequent, a system of screening is essential. Of the materials provided, urine has proved the most useful in this respect.

An examination of the literature has shown the inadequacy of the data relating to the separation of drugs from biological material, and the present paper is concerned with investigations into some aspects of this problem. The biological material that has received the greatest attention in these investigations is urine.

Most of the drugs examined possess high extinction ultraviolet spectra, and use has been made of this property, in conjunction with a recording ultraviolet spectrophotometer (Unicam SP800), to assess recoveries by the distillation and solvent-extraction procedures. In assessments with ultraviolet spectrophotometry, recordings were made against appropriate blanks.

Separation by distillation

Goldbaum and Domanski[2] have shown that many basic drugs can be recovered by distillation in the presence of sodium hydrogen carbonate. The technique is a modification of the usual steam-distillation procedure, but has the advantage that final volumes are much lower. The author[3] has applied this procedure to the examination of urine for the presence of some volatile basic drugs (phenothiazines) that could be detected by the use of ultraviolet spectrophotometry. The following is a description of the procedure as applied to urine.

Twenty-five millilitres of urine are diluted to 50 ml with water and 1 g of sodium hydrogen carbonate added. The mixture is distilled in an all-glass distillation apparatus, 30 ml of distillate being collected; 3 ml of 10 N hydrochloric acid are added and the ultraviolet spectrum is recorded over the range 200 to 350 nm (distillate A).

The distillate, which is now acidic, is diluted to 50 ml with water and the complete procedure repeated (distillate B).

The results obtained are shown in Table I.

TABLE I

DISTILLATION PROCEDURE

Basic substances—

1. Recoverable in distillate A but *not* in distillate B.

Recovery 50 to 80 per cent., depending on the nature of the substance, with the exception of mepyramine (recovery 25 per cent.) and ephedrine (recovery 15 per cent.). As compared with the original substances, the ultraviolet spectra are unchanged as the result of distillation.

Amphetamine	Propoxyphene	Methamphetamine
Tigloidine	β-Phenylethylamine	Phenmetrazine
Tranylcypromine	Benzphetamine	*N*-Methylephedrine
Chlorcyclizine	Chlorphedianol	Ephedrine
Pipradol	Meperidine	Chlorphentermine
Cyclizine	Methadone	Benzhexol
Amitryptyline	nor-Tryptyline	Pyridine
Nicotine	Imipramine	Phenindamine
Triplolidine	Phenyltoloxamine	Brompheniramine
Dextromethorphan	Chlorpheniramine	Methoxyphenamine
Diethylpropion	Chlorprothixene	Pheniramine
Trifluoperazine	Promethazine	Pipamazine
Trifluopromazine	Promazine	Methdilazine
Chlorpromazine	Trimeprazine	Methotrimeprazine
Methapyriline	Mepyramine	
Tripelennamine		

Triazine substances used as herbicides, Desmetryne, Atrazine, Simazine, Prometone and Prometryne.

2. The following substances are recovered in both distillates A and B. As compared with the original substances, the ultraviolet spectra are unchanged as the result of distillation.

Diphenhydramine	Diphenylpyraline	Orphenadrine

Hydrazine derivatives—

The following substances are recovered in distillate A but, as compared with the original substances, there are marked changes in the ultraviolet spectra. The "new" substances can be recovered in distillate B, but there are no further changes in the ultraviolet spectra.

Phenelzine	Isocarboxazid

Neutral and acidic substances—

The following are recovered in distillates A and B. The ultraviolet spectra are unchanged by the distillation procedures.

Phenacetin	Coumarin	Paramethadione
Troxidone	Methsuximide	
Monohydroxyphenols (phenol, *o*- and *p*-cresol, thymol, guaiacol and chloroxylenol)		
Benzoic acid	Salicylic acid	

Neutral substances—

The following substances are recovered in distillate A, there being no change in the ultraviolet spectra. They are recovered in distillate B, but there is a considerable change in the ultraviolet spectra.

Ethchlorvynol	Ethinamate	Methylpentynol
Methylpentynol carbamate		

Many basic drugs can be recovered by distillation. None, with the exceptions of diphenhydramine, orphenadrine and diphenylpyraline, is recoverable in distillate B. Some neutral drugs and, in particular, some acidic substances, *e.g.*, salicylic acid, benzoic acid and the monohydroxyphenols, are recovered in distillate B.

As evidenced by ultraviolet spectra, volatile substances were recovered unchanged, with the exception of the few recorded.

No attempt has been made to achieve full recovery. Recoveries over the range 0·5 to 2·0 mg were found to have a linear relationship, and those from urine and water were identical.

RECOVERY OF VOLATILE DRUGS FROM DISTILLATES—

With the exception of diphenhydramine, orphenadrine and diphenylpyraline, the basic drugs can be recovered by the addition of a slight excess of hydrochloric acid to the distillate and evaporation to dryness, preferably in an all-glass vacuum still.

Following the addition of sodium hydroxide to the distillate or a concentrate, the free bases can be extracted with diethyl ether, which can then be removed by evaporation at room temperature. There is no loss of base, except with methamphetamine, amphetamine, nicotine and β-phenylethylamine, when the losses are total if 1-mg amounts are involved.

Separation by extraction with organic solvents

Procedure with chloroform—

Chloroform, which is used universally as a solvent, was used in examinations carried out under the following conditions.

To 10 ml of aqueous solution containing 1 mg of the drug under investigation were added for acidic reaction, 1 ml of 10 N hydrochloric acid; for neutral reaction, 1 ml of 10 N hydrochloric acid, followed by solid sodium hydrogen carbonate until evolution of carbon dioxide gas ceased (pH about 7); and for alkaline reaction, 1 ml of 10 N sodium hydroxide.

Forty millilitres of chloroform were added and the mixture shaken vigorously for 2 minutes in an 8 × 1-inch glass-stoppered tube, the aqueous phase then being removed and rejected. Anhydrous sodium sulphate was added to effect dehydration. The mixture was filtered and 30 ml of the filtrate evaporated to dryness. Residues were dissolved in 10 ml of N hydrochloric acid or N sodium hydroxide and the ultraviolet spectra recorded against an either N sodium hydroxide or N hydrochloric (or sulphuric) acid. The aqueous phases were appropriate blank over the range 200 to 350 nm.

In a further series of experiments, 30 ml of chloroform extract were shaken with 10 ml of separated and the ultraviolet spectra recorded against an appropriate blank over the range 200 to 350 nm.

The procedures have been applied to 10-ml volumes of urine, and the results obtained are shown in Table II.

Table II

Influence of reaction (acid, neutral and alkaline) on extraction with chloroform

A. Extraction with chloroform
+ Substance extracted
− Substance not extracted

	Acid	Neutral	Alkaline
Sulphonamides—			
Sulphaguanidine	−	−	−
Phthalylsulphathiazole	−	−	−
Succinylsulphathiazole	−	−	−
Sulphacetamide	−	−	−
Sulphamethizole	−	−	−
Sulphafurazole	−	−	−
Sulphaphenazole	−	−	−
Sulphasomizole	+	+	−
Sulphamethoxydiazine	+	+	−
Sulthiamine	+	+	−
Sulphasomidine	−	+	−
Sulphamethoxypyridazine	−	+	−
Sulphamezathine	−	+	−
Sulphanilamide	−	+	−
Sulphapyridine	−	+	−
Sulphamerazine	−	+	−
Sulphathiazole	−	+	−
Sulphadimethoxine	−	+	−
Sulphadiazine	−	+	−
Hydrazine derivatives—			
Nialamide	−	+	−
Isocarboxazide	+	+	−
Iproniazid	−	+	−
Coumarins—			
Dicoumarin	+	+	−
Warfarin	+	+	−
4-Hydroxycoumarin	+	−	−
7-Hydroxycoumarin	+	−	−

TABLE II (*continued*)

	Acid	Neutral	Alkaline
Indanedione—			
Phenindione	+	+	−
Xanthine derivatives—			
Caffeine	+	+	+
Theobromine	+	+	−
Theophylline	+	+	−
Monohydroxyphenols—			
Phenol	+	+	−
o- and *p*-Cresol	+	+	−
Thymol	+	+	−
Chloroxylenol	+	+	−
Guaiacol	+	+	−
Miscellaneous—			
Barbiturates	+	+	−
Acetyl-*p*-aminophenol	+	+	−
Phenylbutazone	+	+	−
Oxyphenbutazone	+	+	−
Phenytoin	+	+	−
Morphine	−	+	−
Chlordiazepoxide	−	+	+
Chlordiazepoxide lactam	+	+	−
Nitrazepam	−	+	−
Oxazepam	+	+	−
p-Aminophenol	−	+	−
Phenylacetylurea	+	+	−
Bibenzonium (bromide)	+	+	−
Captodiamine	−	+	−
Vanillic acid diethylamide	+	+	+
Glutethimide	+	+	+
Methaqualone	+	+	+
2-Amino-5-chlorobenzophenone*	+	+	+
2-Amino-5-nitrobenzophenone†	+	+	+

* Produced from chlordiazepoxide, its lactam and oxazepam when these substances are heated with 5 N hydrochloric acid in a boiling water bath for 1 hour.

† Produced from nitrazepam when this substance is heated with 5 N hydrochloric acid in a boiling water bath for 1 hour.

B. Extraction of chloroform solutions with aqueous N hydrochloric (or sulphuric) acid or N sodium hydroxide

\+ Substance extracted
− Substance not extracted

	N Hydrochloric acid	N Sodium hydroxide
Barbiturates	−	+
Phenytoin	−	+
Warfarin	−	+
7-Hydroxycoumarin	+	+
4-Hydroxycoumarin	+	+
Sulphapyridine	+	+
Sulphadiazine	+	+
Sulphamethoxydiazine	+	+
Sulthiamine	+ (Trace)	+
Dicoumarin	−	+
Phenylbutazone	−	+
Oxyphenbutazone	−	+
Acetyl-*p*-aminophenol	+	+
Morphine	+	+
Theobromine	+	+
Theophylline	+	+
Caffeine	−‡	−
Methaqualone	−	−
Glutethimide	−	−
Iproniazid	+	+
Phenylacetylurea	−	−
Oxazepam	+	+
Bibenzonium	−	−
p-Aminophenol	+	+ (Turns brown)

‡ Extracted with 5 N hydrochloric acid.

Reference has not been made to well defined acidic substances, *e.g.*, salicylic acid, as their properties are well defined. A substance such as salicylic acid is extractable with chloroform from aqueous solutions with an acidic, but not a neutral, reaction. Salicylic acid can be recovered from chloroform by extraction with aqueous solutions of sodium hydroxide.

Reference has also not been made to well defined bases, *e.g.*, quinine, for the same reasons. Quinine can be extracted with chloroform from aqueous solutions with either neutral or alkaline reactions and from chloroform with aqueous solutions of mineral acids.

It has been recorded[4] that sulphonamides may interfere in the ultraviolet detection and determination of barbiturates; this can occur only with sulphonamides that are soluble in chloroform.

Theobromine and theophylline can interfere in the ultraviolet determination of barbiturates, and both are constituents of numerous pharmaceutical preparations. Caffeine presents unusual extraction properties, as it can be extracted by chloroform from aqueous solutions with an acidic, neutral or alkaline reaction. Extraction of caffeine from chloroform solution cannot be effected with aqueous N sodium hydroxide or N hydrochloric (or sulphuric) acid, but it can with aqueous 5 N hydrochloric acid.

The anomalous behaviour of bibenzonium (bromide) merits attention; its extraction as a halide complex probably occurs with acidic and neutral solutions.

Chlordiazepoxide, the lactam, oxazepam, and nitrazepam[5] are converted into chloro- or nitroaminobenzophenones when heated with 5 N hydrochloric acid for 1 hour in a boiling water bath. Such amines are extracted with chloroform, and heptane, not only from neutral and alkaline aqueous solutions, but also from those with acidic reactions; it is possible that halide complexes are extracted from acidic aqueous solutions.

Extraction from a neutral solution will include two groups of substances, *viz.*, weak acids and bases. Some distinction can be made by extraction of the chloroform solution with either aqueous solution of alkali or mineral acid. This is not an entirely clear-cut procedure because ampholytes, *e.g.*, acetyl-*p*-aminophenol, are extracted from chloroform solution under both conditions.

It has been found[3] that some of the phenothiazine sulphoxides, *e.g.*, chlorpromazine sulphoxide, could be recovered by chloroform extraction from aqueous solutions with a neutral or alkaline reaction, by using the technique described above, whereas others, *e.g.*, perphenazine, could not. To recover the latter group, it was found necessary to use the following modified procedure.

To 10 ml of an aqueous solution are added 1 ml of 10 N sodium hydroxide and 40 ml of chloroform. The mixture is shaken vigorously for 2 minutes. Without separation of the phases, dehydration is effected by the addition of anhydrous sodium sulphate. The mixture is filtered and 30 ml of the filtrate are evaporated to dryness.

Many of the substances listed in Table II can be extracted from chloroform solution with aqueous solutions of sodium hydroxide or mineral acid, or both. Methaqualone and glutethimide are exceptions in that they can only be recovered by the removal of the solvent by evaporation.

Bases can be recovered from solution in chloroform by extraction with an aqueous solution of a mineral acid. For this purpose one has a choice of acids, but in some instances correct selection is important. Sulphuric acid must be used in preference to hydrochloric acid if, as with quinine and quinidine, use is to be made of fluorimetry. Sulphuric acid must be used for the extraction of some phenothiazine drugs from chloroform, as in some instances hydrochloric acid is inadequate because of the formation of chloroform-soluble halide complexes.[3,6]

For extraction purposes, 4 parts of chloroform to 1 part of urine have been used. Under these conditions, emulsion formation is extremely rare. Only one extraction with solvent was carried out and although complete recovery is not attained, recoveries were found to be linearly related over a range of 0·25 to 2·0 mg, hence quantitative results could be obtained. It would appear from the literature that this concept is being generally accepted.

The use of a dehydrating agent, such as anhydrous sodium sulphate, ensures clean, almost water-free extracts, the compositions of which are more readily reproducible. It would appear that this concept also is being more readily accepted.

Reference should be made to a factor that could influence the effect of the washing of chloroform extracts. Substances that can be recovered by extraction of chloroform solutions with aqueous solutions of alkali or mineral acid may be divided into the following groups.

(i) Those extracted by aqueous solutions of alkali but not by those of mineral acid, *e.g.*, acids such as the barbiturates.

(ii) Those extracted by aqueous solutions of mineral acid but not by those of alkali, *e.g.*, bases such as quinine.

(iii) Those extracted by aqueous solutions of both alkali and mineral acid. Ampholytes, *e.g.*, the sulphonamides, behave in this manner. In addition, other substances, such as theophylline and 7- and 4-hydroxycoumarin, behave in this manner but cannot be described as ampholytes.

The "washing" of organic solvent extracts has been general practice, but this has been avoided by the author as such a procedure could result in losses of "unknown" constituents.

The separations are preferably carried out in glass-stoppered measuring cylinders or tubes that can be centrifuged, and a teat-pipette is useful for the separation of top layers.

These extraction procedures have been applied to normal urine, and the ultraviolet spectra obtained have been recorded.[3]

USE OF OTHER SOLVENTS—

Many other solvents have been used for extraction purposes.

Diethyl ether is a good general solvent, as it has a low boiling-point and hence can be readily removed by evaporation. Unfortunately, it is highly inflammable, contains anti-oxidants and is not particularly selective. When used as an extractant for urine, many normal constituents are extracted.

Ethyl acetate is another good general solvent but is not particularly selective in its action. Because of their insolubility in other organic solvents, it is used for the extraction of chlorothiazide and hydrochlorothiazide from urine.[7,8] No details have been published concerning the limitations imposed by the pH of the aqueous solution on the extraction of these substances by ethyl acetate.

Benzene is a selective solvent but, unfortunately, it possesses an intense ultraviolet spectrum, so that traces can cause complications if ultraviolet spectrophotometry is used.

Heptane is a selective solvent, and has been successfully used in determinations involving phenothiazine drugs, imipramine etc.; it is particularly useful in spectrofluorimetry.

Morphine can be extracted with organic solvents from neutral aqueous solutions or aqueous solutions containing ammonia; chloroform is not an efficient solvent for this purpose.

TABLE III

SOLVENTS, OTHER THAN CHLOROFORM, USED FOR THE EXTRACTION OF DRUGS FROM AQUEOUS SOLUTIONS

Solvent	Substance
Benzene	Quinine, Quinidine*[9] Caffeine[10]
Heptane	Phenothiazine drugs[3,11,12,13] Imipramine[6,11] Phenylbutazone[14] Desipramine† Trimipramine
The following are not extractable by heptane: sulphonamides, barbiturates and oxyphenbutazone (a drug and a metabolite of phenylbutazone)	
Ethyl acetate	Ethchlorvynol[15] Chlorothiazide[7]‡ Hydrochlorothiazide[8]‡

* Selectively separated from metabolites.
† A drug and also a metabolite of imipramine.
‡ Not soluble in chloroform.

Chloroform - isopropyl alcohol (3 + 1) or butanol - benzene (1 + 1) are more efficient but are not particularly selective and, as a result, many constituents of normal urine are also extracted. Results are summarised in Tables III and IV.

TABLE IV

INFLUENCE OF pH ON THE EXTRACTABILITY OF SOME DIURETIC DRUGS (ALL INSOLUBLE IN CHLOROFORM) WITH ETHYL ACETATE FROM AQUEOUS SOLUTIONS

Substance	Acid	Neutral	Alkaline
Chlorothiazide	+	+	−
Hydrochlorothiazide	+	+	−
Bendrafluazide	+	+	−
Hydroflumethiazide	+	+	−

SEPARATION WITH ION-EXCHANGE RESINS

Ion-exchange resins have not received much application in chemical toxicology, possibly because the procedures involved are slow in comparison with other extraction procedures.

The author[16 to 22] has examined the use of ion-exchange resins, not only in connection with problems associated with chemical toxicology, but also with problems associated with clinical biochemistry. To achieve some degree of uniformity, these studies have been confined mainly to the use of one resin, Dowex 50W × 12 (200 to 400 mesh). It has been

TABLE V

BEHAVIOUR OF SOME BASIC AND OTHER SUBSTANCES WHEN PASSED THROUGH A COLUMN CONTAINING DOWEX 50W × 12

A

Present in the 0·1 N hydrochloric acid eluate and, therefore, not retained	Barbiturates, phenytoin, phenylbutazone, oxyphenbutazone and diazotisable amines obtained by the action of hot solution of alkali or mineral acid on chlorothiazide and hydrochlorothiazide
Retained by the resin and eluted with 0·5 N hydrochloric acid	Ammonium ion and many amino-acids
Retained by the resin and eluted with N hydrochloric acid	Many amino-acids, sulphonamides, adrenalin, theobromine, theophylline, *p*-aminophenol and isoprenaline
Retained by the resin and eluted with 2·5 N hydrochloric acid	Cystine, histidine, caffeine, isoniazid, morphine and the diazotisable amine obtained from oxyphenbutazone by the action of hot mineral acids
Retained by the resin and eluted with 5 N hydrochloric acid	Nicotine, codeine, brucine, strychnine, amphetamine, paraquat and diquat
Retained by the resin and eluted with 8 N hydrochloric acid	Quinine, paludrine, mepacrine, the aminobenzophenones, produced from chlordiazepoxide, oxazepam and nitrazepam by the action of hot mineral acid,[5] and the diazotisable amine produced from phenylbutazone by the action of hot mineral acid[23]

B

Substance	Applied in hydrochloric acid	Eluted with
Methyldopa[21]	0·1 N	N Hydrochloric acid
Meperidinic acid[17]	0·1 N	6 N Ammonia solution
Bretylium, Hexamethonium, Guanethidin[22]	N	5 N Hydrochloric acid

These substances are recovered from eluates by evaporation to dryness in an all-glass vacuum still.

Morphine[17,18]	N	(*a*) 2·5 N Hydrochloric acid (*b*) 6 N Ammonia solution
Codeine, Brucine, Strychnine[17,18]	N	(*a*) 5 N Hydrochloric acid (*b*) 4 N Ammonia solution - ethanol (20 + 80)

These substances are recovered from eluates by solvent extraction.

found that with some drugs, there is no alternative to the use of cation-exchange resins for extraction purposes. The following procedure was used.

The column characteristics were: resin (cation exchange), Dowex 50W × 12 (H^+form), 200 to 400 mesh, height 70 mm and diameter 10 mm.

Substances (about 1 mg) in 100 ml of 0·1 N hydrochloric acid were applied to the column and the column was washed with 100 ml of 0·1 N hydrochloric acid. Elution was then carried out with 100-ml volumes of 0·5, 1, 2·5, 5 and 8 N hydrochloric acid in that order.

The procedure has been used extensively in urine analysis. Urine can be applied to the column either as 10 ml of urine *plus* 1 ml of 10 N hydrochloric acid and diluted to 100 ml with water or as 100 ml of urine *plus* 10 ml of 10 N hydrochloric acid. The particular technique used depended on the nature of the substances to be recovered. Some results are summarised in Table V. The procedures used for the recovery of certain drugs are also referred to in Table V. In several instances, *e.g.*, methyldopa, bretylium, meperidinic acid, guanethidine and hexamethonium, because of their insolubility in organic solvents, this is the only procedure available to achieve concentration.

Eluates obtained from cation-exchange resins are not entirely suitable for direct examination by ultraviolet spectrophotometry because of absorbance caused by background material derived from the resin material. With isoprenaline, it has been found satisfactory, provided that an adequate blank has been carried out. During the earlier work on this subject, the author used colorimetric methods to obtain evaluation of the technique, especially as many of the substances examined, *e.g.*, many amino-acids, did not show absorbance within the ultraviolet range.

Discussion

There were at least two objectives concerned in the present investigation, and the attainment of these cannot be described as complete.

Because of the lack of results given in the literature, information was required concerning separation techniques, which are a necessary preliminary to any chemical toxicological procedure. It was hoped to formulate a scheme of approach that could be applied to the examination of the new drugs that are constantly appearing. For routine purposes, only the distillation and solvent-extraction procedures are used. Chloroform is the solvent of the first choice, as low blank values are obtained when applied to normal urine. Ion-exchange resins are used only as a last expedient.

The second objective was to obtain sufficient information that could be applied to the rapid screening of urine for the presence of drugs, a procedure that is becoming more and more essential in hospital practice. The following screening procedures have been developed and are now used, followed by ultraviolet spectrophotometry over the range 200 to 350 nm.

(1) Distillation of urine in the presence of sodium hydrogen carbonate.

(2) Extraction with chloroform of urine with a neutral reaction, then (*a*) evaporation of the chloroform extract to dryness and dissolution of the residue in N hydrochloric acid; (*b*) extraction of the chloroform solution with N sodium hydroxide; and (*c*) extraction of the chloroform solution with N sulphuric acid.

(3) Extraction with chloroform of urine with an alkaline reaction, then (*a*) evaporation of the chloroform extract to dryness and dissolution of the residue in N hydrochloric acid; and (*b*) extraction of the chloroform solution with N hydrochloric or sulphuric acids, followed by 5 N hydrochloric acid.

The subsequent procedures can be completed within 1 hour.

Extraction with chloroform of urine with an acidic reaction is not normally carried out as many normal ultraviolet-absorbing substances are also extracted and their presence can confuse interpretations.

It should be emphasised that although the coverage for screening purposes is large, it is not complete.

The analyst should be presented with the specimens of his choice in order that a satisfactory assessment can be made. This is not always the practice as clinicians tend to place much emphasis on blood levels and, as a result, blood may be the only material supplied for analysis. Some clinicians are loath to obtain urine from comatose patients. It should be noted, however, that in certain cases, *e.g.*, amphetamine intake and paraquat poisoning,[24]

blood levels are usually so low that detection and determination, respectively, are not really possible. Because of the much higher concentrations encountered in urine, the latter is the most suitable material for chemical analysis, at least under these circumstances.

The conjugation and metabolism of drugs are important factors to be considered. Several important books and reviews on these subjects have been written,[25,26,27] but our knowledge of them is still incomplete. With some drugs, such factors must be taken into consideration if satisfactory results are to be obtained. Morphine is excreted almost entirely in conjugated form, thus necessitating the use of hot, acid hydrolysis before applying any analytical procedure. Following the ingestion of paracetamol or phenacetin, the recovery of products as *p*-aminophenol, as the result of hot, acid hydrolysis, appears to produce the most satisfactory results.[28] The barbiturates pose another interesting problem. Following the ingestion of certain barbiturates, *e.g.*, amylobarbitone and pentobarbitone, unchanged barbiturates may not be detectable in the urine. By using special solvent techniques (not described in this paper) it is possible to detect and determine large amounts of metabolites, particularly of the hydroxylated type.[29,30,31,32]

Ultraviolet spectrophotometry, with a recording instrument, has been used extensively in the present investigations. Reference must be made to the work of Bradford and Brackett,[33] who have described an extensive scheme involving the use of ultraviolet spectrophotometry and designed mainly for the examination of materials such as suspected pills, capsules and narcotics. Their investigations were not concerned with urine examination.

The author expresses his thanks to the many pharmaceutical firms who supplied samples of drugs without cost.

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Recommended Methods for the Evaluation of Drugs

PREPARED BY THE JOINT COMMITTEE OF THE PHARMACEUTICAL SOCIETY AND THE SOCIETY FOR ANALYTICAL CHEMISTRY ON METHODS FOR THE EVALUATION OF DRUGS

The Chemical Assay of Cascara Dry Extract, Cascara Tablets and Cascara Bark

THE Panel, which was set up to recommend standard methods of assay of drugs and their preparations containing anthraquinones, has published Reports on the "Chemical Assay of Senna Fruit and Senna Leaf"[1] and on the "Chemical Assay of Aloes."[2] The work has now been extended to cascara bark and to certain of its preparations. The constitution of the Panel was: Professor J. M. Rowson (Chairman), Dr. C. Daglish, Professor J. W. Fairbairn, Miss B. Gartside (resigned October, 1967), Miss H. M. Perry and Mr. H. A. Ryan, with Mr. P. W. Shallis as Secretary.

REPORT

Cascara, the dried bark of *Rhamnus purshiana* DC., is an anthraquinone purgative which is administered as a dry extract usually in the form of sugar-coated tablets; a liquid extract and an elixir are also used.

The most important constituents of the bark are (*a*) four primary glycosides, *cascarosides A* and *B* (glucosides of barbaloin), *cascarosides C* and *D* (glucosides of chrysaloin); (*b*) *aloins*, barbaloin, chrysaloin, which are *C*-glycosides; smaller amounts of (*c*) *O-glycosides* and of (*d*) free *anthraquinones* are also present. The cascarosides are almost tasteless, but the aloins are extremely bitter.

Detailed published information on the relative biological activities of the cascarosides and of the aloins is lacking; such preliminary information as was available to the Panel indicated that both groups of compounds possessed similar activities but that they differed in the speed of their action.

The work of Fairbairn and Simic[3] has shown that in the preparation of cascara dry extract from the bark a significant loss of total glycosides occurred and that the proportion of cascarosides to aloins was lower in the extract than in the bark. Because of the thermolability of the cascarosides, their different speed of action and their tastelessness, the Panel agreed with the recommendation of Fairbairn and Simic[3] that a chemical method of evaluating cascara should estimate the cascarosides separately from the aloins. Such a pair of figures can give some measure of the care exercised in the pharmaceutical manufacture of extracts of cascara and can also be used to detect adulteration of such preparations by the addition of aloes.

Several methods have been published for the chemical assay of cascara, and that recommended by Fairbairn and Simic[3] was selected by the Panel as the basis for their work. The dry extract was examined first, because of its frequent use in the form of tablets. The method was then applied to the assay of dried cascara bark.

CHEMICAL ASSAY

The method recommended by the Panel for the assay of cascara preparations is set out in the Appendixes. The use of 70 per cent. ethanol as an extracting solvent in stage (*i*) was found to be satisfactory. In stage (*ii*) the free anthraquinones are removed by extraction with carbon tetrachloride; relatively large volumes of this solvent are used to obviate the formation of troublesome emulsions. The presence of 1 per cent. of sodium chloride in the aqueous phase was ineffective for this purpose. The cascarosides are then separated from the aloins by partition in the system ethyl acetate - water. Fairbairn and Simic[3] have shown that this partition is 96 to 99 per cent. effective, but the use of recently water-saturated ethyl acetate is essential. They have also shown that the *O*-glycosides only represent some 10 per

cent. of total anthracene glycosides in the extract and that these are fairly evenly distributed between the two phases when partitioned in stage (*ii*). The Panel concluded that the separate estimation of this small amount of *O*-glycosides would be an unnecessary elaboration of the method.

TABLE I

RESULTS OF COLLABORATIVE ASSAYS OF SEVEN SAMPLES OF CASCARA DRY EXTRACT

By the method described in Appendix I

Sample	Laboratory	Aloins as anhydrous barbaloin, per cent.	Cascarosides as cascaroside A, per cent.	Total glycosides as cascaroside A, per cent.
CDE1	A	4·32 4·84	6·10 6·86	13·32 14·95
	B	3·93 3·93	6·76 6·77	13·33 13·34
	C	4·21 —	6·34 6·97	13·38 —
	D	4·00 4·26	6·18 6·27	12·87 13·39
	E	4·01 4·04	6·80 6·53	13·92 13·28
	Mean	4·17	6·56	13·53
	s.d.	0·29	0·31	0·59
	s.d. as % of mean	7·0	4·7	4·4
CDE2	A	8·53 8·53	7·73 7·38	22·07 21·70
	B	8·30 8·03	7·87 7·96	21·84 21·47
	C	8·12 6·71	6·72 7·90	20·36 19·23
	D	7·60 7·70	7·10 6·80	19·90 19·90
	E	7·88 8·04	7·20 6·57	20·45 20·08
	Mean	7·94	7·32	20·70
	s.d.	0·53	0·52	0·99
	s.d. as % of mean	6·7	7·1	4·8
CDE3	A	8·07 8·03	6·98 6·88	20·53 20·36
	B	7·15 7·38	7·11 6·81	19·14 19·28
	C	6·96 7·46	6·82 7·60	18·53 20·15
	D	7·60 7·40	6·60 6·70	19·40 19·23
	E	7·30 7·35	6·68 6·47	18·94 18·83
	Mean	7·47	6·87	19·44
	s.d.	0·35	0·32	0·68
	s.d. as % of mean	4·7	4·7	3·5
CDE4	A	5·68 5·48	4·70 4·76	14·25 13·96
	B	5·23 5·23	4·45 4·28	13·24 13·08
	C	5·71 4·29	4·72 4·79	14·31 12·00
	D	5·00 5·20	4·30 4·50	12·71 13·21
	E	5·31 4·70	4·27 4·29	13·19 12·19
	Mean	5·18	4·51	13·21
	s.d.	0·43	0·22	0·79
	s.d. as % of mean	8·3	4·9	6·0
CDE5	A	3·62 3·54	6·89 7·08	13·01 13·06
	B	3·43 3·49	7·65 7·36	13·56 13·28
	C	3·35 3·65	6·61 6·99	12·27 13·16
	D	3·60 3·50	7·20 6·90	13·21 12·87
	E	3·39 3·78	6·52 6·87	12·26 13·26
	Mean	3·54	7·00	12·99
	s.d.	0·13	0·32	0·43
	s.d. as % of mean	3·7	4·6	3·3
CDE6	A	2·48 2·49	6·15 6·34	10·35 10·57
	B	2·46 2·45	6·85 6·66	11·04 10·82
	C	2·83 2·77	6·03 5·90	10·82 10·87
	D	2·40 2·50	6·50 6·30	10·53 10·53
	E	2·51 2·59	6·17 6·16	10·43 10·55
	Mean	2·55	6·31	10·65
	s.d.	0·14	0·29	0·22
	s.d. as % of mean	5·5	4·6	2·1
CDE7	A	3·38 3·44	7·01 6·82	12·71 12·62
	B	3·41 3·42	7·75 7·58	13·53 13·38
	C	3·36 3·07	6·73 6·05	12·42 11·24
	D	3·60 3·50	7·20 6·90	13·21 12·87
	E	3·29 3·02	6·94 6·62	12·51 11·74
	Mean	3·35	6·96	12·62
	s.d.	0·18	0·48	0·71
	s.d. as % of mean	5·4	6·9	5·6

The aloins present in the ethyl acetate fraction are freed from solvent and are converted into the free anthraquinones by the drastic condition of stage (*iii*), in which iron(III) chloride and hydrochloric acid are used. The liberated anthraquinones are then extracted with carbon tetrachloride and are estimated spectrophotometrically in N sodium hydroxide solution. Oxidation and estimation procedures of stage (*iii*) are the same as those of our Report on the Chemical Assay of Aloes.[2]

The cascarosides present in the aqueous phase of stage (*ii*) are estimated in stage (*iv*) by the same procedure as that specified for aloins. The $E^{1\%}_{1cm}$ value for cascaroside A is derived from the work of Fairbairn and Simic.[3]

Results for total glycosides are obtained by summation of the values for cascarosides and for aloins, re-calculated as equivalent cascarosides. Investigations by members of the Panel have shown that results for total glycosides by this process of summation are the same as those obtained by a separate, direct estimation of total glycosides and omitting the ethyl acetate - water partition in the second part of stage (*ii*). Such a direct estimation of total glycosides would, however, be an acceptable procedure.

CASCARA DRY EXTRACT

Members of the Panel have applied the method of Appendix I to the assay of seven different samples of cascara dry extract available on the English market. All spectrophotometers were initially checked by using a common standard sample of ammonium cobalt sulphate solution. The results of these assays are given in Table I, from which it will be noted that, in general, satisfactory concordance of results was obtained within and between laboratories, but that greater variation existed in the results for aloins than in those for cascarosides or for total glycosides. The extinction ratio value *R* for aloin assays was within the range 1·8 to 2·1; for cascaroside assays it was between 1·9 and 2·3.

CASCARA TABLETS

Cascara tablets are a convenient and widely used dosage form of cascara dry extract; they are generally sugar-coated. This preparation may be assayed by the method already described. To allow for individual tablet variation, a number of tablets, equivalent to about 2·5 g of dry extract, should be taken; they are disintegrated by soaking and by trituration in water before the addition of ethanol to about 70 per cent. content as an extracting solvent. An aliquot is then assayed as set out in Appendix II. Results are expressed in terms of milligrams per tablet.

TABLE II

RESULTS OF COLLABORATIVE ASSAYS OF TWO SAMPLES OF CASCARA TABLETS

By the method described in Appendix II

Sample	Laboratory	Aloins as anhydrous barbaloin, mg per tablet	Cascarosides as cascaroside A, mg per tablet	Total glycosides as cascaroside A, mg per tablet
CT1	A	4·43 4·78	8·14 8·31	15·63 16·39
	B	4·56 4·60	9·15 8·94	16·85 16·55
	C	5·18 5·10	9·38 9·05	18·12 17·67
	D	4·73 4·93	9·16 9·13	17·07 17·37
	E	5·16 5·12	8·08 8·15	16·79 16·79
	Mean	4·86	8·75	16·92
	s.d.	0·28	0·51	0·69
	s.d. as % of mean	4·1	5·8	4·1
CT2	A	4·45 4·83	9·98 10·05	17·51 18·22
	B	4·86 4·63	10·90 11·03	19·12 18·89
	C	4·44 4·67	11·10 10·10	18·56 17·89
	D	4·28 4·15	10·57 10·31	17·72 17·26
	E	4·59 4·91	9·41 9·32	17·17 17·62
	Mean	4·58	10·28	17·99
	s.d.	0·25	0·63	0·68
	s.d. as % of mean	5·5	6·1	3·8

Two commercial samples of sugar-coated cascara tablets were assayed by members of the Panel using the method of Appendix II. The figures obtained are recorded in Table II and these show satisfactory concordance of results within and between laboratories.

Cascara bark

The Panel has shown that the method of Appendix I can be applied, with minor modifications, to the assay of samples of cascara bark. The details are given in Appendix III. Maceration of the powdered bark in 70 per cent. ethanol overnight gave satisfactory extraction.

Table III

Results of collaborative assays of five samples of cascara bark

By the method described in Appendix III

Sample	Laboratory	Aloins as anhydrous barbaloin, per cent.	Cascarosides as cascaroside A, per cent.	Total glycosides as cascaroside A, per cent.
CB1	A	2·02 2·06	4·45 4·79	7·86 8·26
	B	2·60 2·50	4·70 4·68	9·10 8·90
	D	2·23 2·24	4·77 4·73	8·51 8·48
	E	2·31 2·12	4·27 4·60	8·18 8·19
	Mean	2·26	4·62	8·44
	s.d.	0·20	0·18	0·41
	s.d. as % of mean	8·8	3·9	4·9
CB2	A	1·10 1·20	6·25 6·15	8·14 8·21
	B	1·27 1·37	6·31 6·12	8·49 8·48
	D	1·24 1·21	6·15 6·13	8·23 8·16
	E	1·39 1·50	5·72 5·66	8·09 8·23
	Mean	1·29	6·06	8·25
	s.d.	0·13	0·24	0·15
	s.d. as % of mean	10·0	4·0	1·8
CB3	A	1·56 1·63	5·31 5·57	7·98 8·34
	B	1·91 1·95	5·45 5·25	8·69 8·56
	D	1·73 1·82	5·25 5·24	8·14 8·28
	E	1·93 1·99	4·82 4·77	8·09 8·14
	Mean	1·82	5·21	8·28
	s.d.	0·16	0·28	0·24
	s.d. as % of mean	8·8	5·4	2·9
CB4	A	1·58 1·55	7·42 7·65	10·13 10·32
	B	1·83 1·84	7·37 7·29	10·50 10·43
	D	1·74 1·75	7·10 7·37	10·02 10·30
	E	1·79 1·81	6·32 6·45	9·31 9·48
	Mean	1·74	7·12	10·06
	s.d.	0·11	0·48	0·44
	s.d. as % of mean	6·3	6·7	4·4
CB5	A	1·52 1·55	5·94 6·01	8·54 8·66
	B	1·79 1·86	5·78 5·63	8·83 8·79
	D	1·72 1·72	5·94 6·02	8·83 8·90
	E	1·83 1·64	4·97 5·15	8·04 7·89
	Mean	1·70	5·68	8·56
	s.d.	0·13	0·41	0·39
	s.d. as % of mean	7·6	7·2	4·6

Five commercial samples of cascara bark were assayed by the method of Appendix III and the results obtained by members of the Panel are set out in Table III. These show good concordance within and between laboratories, although variation does exist between results of assays for aloins. The figures for the ratio value R for cascaroside assays were between 1·9 and 2·2; for aloin assays they were 1·7 to 1·9 and these lower values may suggest slight contamination in the aloin assay.

Investigation of liquid preparations of cascara

Cascara liquid extract is a concentrated aqueous extract of the bark, to which ethanol is added to give a final content of 21 to 24 per cent. v/v. The Panel has devoted considerable effort to its assay by application of the method of Appendix I. A sample of about 3 g, accurately weighed, of liquid extract was diluted to 100 ml with 70 per cent. ethanol and a 10-ml aliquot of the dilution was assayed by the method of Appendix I. Three commercial

samples of cascara liquid extract were examined and within each laboratory good concordance of results was obtained. But for each constituent in each of the three samples, involving 45 duplicate assays, there was very wide deviation between results from the different laboratories. Typical results are those for sample CLE2 in Table IV.

TABLE IV

RESULTS OF COLLABORATIVE ASSAYS OF SAMPLES OF CASCARA LIQUID EXTRACT

CLE2 assayed by the method of Appendix I; CLE4 assayed within the period of 1 week

Sample	Laboratory	Aloins as anhydrous barbaloin, per cent. w/w	Cascarosides as cascaroside A, per cent. w/w	Total glycosides as cascaroside A, per cent. w/w
CLE2	A	1·09 1·07	1·12 1·10	2·96 2·89
	B	1·56 1·55	1·26 1·32	3·88 3·93
	C	1·06 1·06	1·28 1·15	3·04 2·93
	D	1·59 1·58	1·41 1·32	4·06 3·96
	E	0·98 0·95	1·13 1·07	2·78 2·68
	Mean	1·14	1·22	3·31
	s.d.	0·48	0·12	0·57
	s.d. as % of mean	42·1	9·8	17·2
CLE4	A	1·54 1·50	1·15 1·15	3·75 3·66
	B	1·63 1·58	1·29 1·37	4·03 4·03
	C	1·33 1·57	1·11 1·06	3·33 3·68
	D	1·79 1·71	1·30 1·34	4·30 4·20
	E	1·33 1·31	1·12 1·08	3·34 3·28
	Mean	1·53	1·20	3·76
	s.d.	0·16	0·12	0·37
	s.d. as % of mean	10·5	10·0	9·8

A detailed study of sample CLE1 in one laboratory over a 3-month period showed a fall in aloin content from 0·98 to 0·35 per cent., but the cascarosides remained constant in amount. An examination of the two other samples CLE2 and CLE3 in the same laboratory detected no similar degradative changes over a period of 3 to 4 months. Examination of the three samples in the four other laboratories after this period of 3 to 4 months failed to produce any evidence of disappearance of aloins in CLE1; and all results within each of these four laboratories were in general agreement at the beginning and end of the period of time, but disagreement between laboratories was still marked.

Attempts were next made to eliminate possible errors due to ageing of the preparation, also to the occurrence of precipitation in the liquid extract itself and in the initial dilution with 70 per cent. ethanol. A freshly filtered sample CLE4 was sent out from a central stock to each laboratory, where it was assayed under closely detailed conditions and within the period of 1 week. The results obtained are given in Table IV, from which it will be noted that deviations of about 10 per cent. were obtained in the assays of each of the compounds. The storage of four separate portions of this sample for a 3-month period under different conditions in one laboratory resulted in one portion showing a fall in aloins content to 0·48 per cent. with only a slight loss of cascarosides. No significant changes occurred in the three other portions.

Preliminary work on cascara elixir also gave divergent results, and the Panel has concluded that the method of Appendix I cannot be applied with any confidence to liquid preparations of cascara. It stresses that concordant results on such a preparation within a laboratory are unlikely to agree with those obtained in a second laboratory.

CONCLUSION

The Panel recommends the methods of Appendixes I, II and III for the chemical assay of cascara dry extract, cascara tablets and cascara bark. It cannot recommend any process for the assay of liquid preparations of cascara.

The Panel does not have within its terms of reference the establishing of standards for the materials assayed, but it recommends that cascara and its preparations be assessed on the content of total glycosides and on the proportion of cascarosides present therein.

It also draws attention to the greater variability of both figures for cascara dry extract than for the bark itself. Thus the total glycoside content of five samples of cascara bark were within the range 8·2 to 10·1 per cent. (Table III), and the amount of cascarosides varied between 55 and 73 per cent. of the total glycosides present, whereas for the seven samples of cascara dry extract the range for total glycoside content was 10·65 to 20·7 per cent. (Table I), and the amount of cascarosides varied between 34 and 59 per cent. of the total glycosides present.

Appendix I

RECOMMENDED METHOD FOR THE CHEMICAL ASSAY OF CASCARA DRY EXTRACT

REAGENTS—

Carbon tetrachloride.
Iron(III) chloride solution, 60 *per cent. w/v*—Analytical-reagent grade.
Hydrochloric acid, 36 *per cent. w/w.*
Methanol.
Sodium hydroxide, N.
Distilled water.
Ethanol, 70 *per cent. v/v aqueous.*
Ethyl acetate.

PROCEDURE—

(*i*) Weigh accurately about 0·5 g of powdered extract and place it in a 100-ml calibrated flask with 80 ml of 70 per cent. ethanol. Shake the mixture occasionally, allow to stand overnight, make the volume up to 100 ml with 70 per cent. ethanol, shake well and filter through a Whatman No. 4 filter-paper.

(*ii*) Transfer 10 ml of this solution into a separating funnel, add 10 ml of water and extract with two or three 40-ml portions of carbon tetrachloride. Wash the combined carbon tetrachloride extracts with one 10-ml portion of water, reject the carbon tetrachloride layer and return the washings to the aqueous layer.

Extract the combined aqueous layers with five 60-ml portions of freshly prepared water-saturated ethyl acetate and reserve both layers for further work. (Prepare the water-saturated ethyl acetate by shaking 300 ml of ethyl acetate with 30 ml of water for 3 minutes and then allowing the layers to separate.)

(*iii*) *Aloins.* Transfer the combined ethyl acetate extracts to a suitable flask, distil off the solvent and evaporate just to dryness. Dissolve the residue in 0·3 to 0·5 ml of methanol, rinse out with water into a 50-ml calibrated flask and make up to 50 ml with water.

Place 20 ml of this solution in a round-bottomed 100-ml flask containing 2 ml of 60 per cent. iron(III) chloride solution and 12 ml of hydrochloric acid. Attach a water-cooled, double-surface condenser to the flask, place the flask in a bath of continuously boiling water (so that the water level is above that of the liquid level in the flask) and heat for 4 hours.

Cool the solution, transfer it into a separating funnel, and rinse out the round-bottomed flask successively with 3 to 4 ml of water, 3 to 4 ml of N sodium hydroxide and 3 to 4 ml of water, adding these rinsings to the contents of the separating funnel.

Extract the contents of the separating funnel with three 20-ml portions of carbon tetrachloride. Wash the combined carbon tetrachloride layers with two 10-ml portions of water. Reject the washings. Extract the carbon tetrachloride layer with one 15-ml portion and with successive 5-ml portions of N sodium hydroxide until the final sodium hydroxide extract is colourless. Combine the alkaline extracts, heat them in a shallow dish on a bath of boiling water for 5 minutes, with constant stirring to remove carbon tetrachloride, cool and adjust to 50 ml with N sodium hydroxide. Determine the extinction of this solution in a 1-cm cell at 440 nm and at the maximum, at 500 nm, against N sodium hydroxide in a similar cell. The readings should be taken within 1 hour of beginning the alkaline extraction with one 15-ml portion of N sodium hydroxide (step (*iii*), line 13) and particular care should be taken to carry out these stages of the estimation in subdued light.

Calculate the percentage of aloins present as anhydrous barbaloin on the assumption that the $E^{1\%}_{1\,cm}$ value at 500 nm of the red solution obtained from anhydrous barbaloin is 209. Also calculate the ratio value $E_{500\,nm}/E_{440\,nm}$. If the ratio value is less than 1·7, reject the result. Re-calculate the percentage of aloins present as cascaroside A on the assumption that the $E^{1\%}_{1\,cm}$ value at 500 nm of the red solution obtained from cascaroside A is 125.

(*iv*) *Cascarosides.* Transfer the aqueous layer, reserved in stage (*ii*), line 6 above, to a 50-ml calibrated flask and make up to 50 ml with water.

With 20 ml of this solution, carry out the iron(III) chloride oxidation, N sodium hydroxide extraction and colorimetric determination as described above for *Aloins*, stage (*iii*), lines 4 to 21 (beginning at "Place 20 ml of this solution . . ." to ". . . subdued light.").

Calculate the percentage of cascarosides present as cascaroside A on the assumption that the $E^{1\%}_{1\,cm}$ value at 500 nm of the red solution obtained from cascaroside A is 125. Also calculate the ratio value $E_{500\,nm}/E_{440\,nm}$. If the ratio value is less than 1·8, reject the result.

(*v*) The percentage of total glycosides present as cascaroside A is obtained by adding together the values for aloins in stage (*iii*) re-calculated as cascaroside A and for cascarosides in stage (*iv*).

Appendix II

RECOMMENDED METHOD FOR THE CHEMICAL ASSAY OF CASCARA TABLETS

PROCEDURE—

(*i*) Place a number of tablets, equivalent to about 2·5 g of dry extract, in a glass mortar, add 5 to 8 ml of water, and allow to soak for 15 minutes. Then triturate to a smooth paste, transfer to a 500-ml calibrated flask with the remainder of a total of 150 ml of water, and make up to 500 ml with absolute ethanol. Filter if necessary.

(*ii*) to (*v*). Take 10 ml of this solution and proceed as in Appendix I (*ii*) to (*v*). Calculate results as the weight in milligrams present in each tablet of aloins, cascarosides and total glycosides as cascaroside A.

Appendix III

RECOMMENDED METHOD FOR THE CHEMICAL ASSAY OF CASCARA BARK

Proceed as in Appendix I (*i*) to (*v*) but use the following modifications:

Stage	(*i*) line 1	Weigh accurately about 1 g of powdered cascara bark . . . .
Stage	(*ii*) line 5	Extract the combined aqueous layers with three 60-ml portions of freshly prepared water-saturated ethyl acetate . . . .
Stage	(*iii*) lines 17 and 18	Determine the extinction of this solution in either a 1-cm or 2-cm cell at . . . .

REFERENCES

1. Report of the Joint Committee of the Pharmaceutical Society and Society for Analytical Chemistry, *Analyst*, 1965, **90**, 582.
2. Report of the Joint Committee of the Pharmaceutical Society and Society for Analytical Chemistry, *Ibid.*, 1967, **92**, 593.
3. Fairbairn, J. W., and Simic, S., *J. Pharm. Pharmac.*, 1964, **16**, 450.

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Recommended Methods of Analysis of Pesticide Residues in Foodstuffs

REPORT BY THE JOINT DIMETHOATE RESIDUES PANEL

SET UP JOINTLY BY THE SCIENTIFIC SUB-COMMITTEE ON POISONOUS SUBSTANCES USED IN AGRICULTURE AND FOOD STORAGE, THE ANALYTICAL METHODS COMMITTEE OF THE SOCIETY FOR ANALYTICAL CHEMISTRY AND THE ASSOCIATION OF BRITISH MANUFACTURERS OF AGRICULTURAL CHEMICALS

The Determination of Dimethoate Residues in Fruits and Vegetables

THE Panel was set up by the Scientific Sub-Committee of the Advisory Committee on Pesticides and other Toxic Chemicals, the Association of British Manufacturers of Agricultural Chemicals and the Analytical Methods Committee of the Society for Analytical Chemistry to establish by collaborative study an accurate and reproducible method for the determination of dimethoate residues in crops. German, Italian and United States' workers also joined the Panel. The Panel first met at the end of 1964 and undertook a series of collaborative studies, at first with Chilwell and Beecham's method[1] and then with Frehse's method (H. Frehse, private communication). This report describes the findings of the Panel and recommends Frehse's method for determining dimethoate residues in apples, pears, cauliflowers, peas, cabbages, blackcurrants, olives and oranges. A semi-quantitative identification procedure is also described.

Members of the Panel are listed in Appendix III.

LITERATURE SURVEYED BY THE PANEL—

At its first meeting the Panel carefully considered the methods available for the determination of dimethoate residues in fruits and vegetables and also an assessment, subsequently published by Smart,[2] of three of the more important methods. He found that Laws and Webley's general method[3] was satisfactory for residues of dimethoate in sprouts, lettuces and apples, but not in peas. Chilwell and Beecham's method was also satisfactory at the 1 or 2 p.p.m. level of added dimethoate in cabbages, lettuces, apples and peas. Giang and Schechter's method,[4] however, gave variable results. Fukel'man[5] also investigated Chilwell and Beecham's method and proposed some modification. de Pietri-Tonelli[6] has reviewed the methods available for dimethoate residues analysis. Since this review, several further relevant papers have been published and were considered by the Panel. George, Walker, Murphy and Giang[7] described a method involving reaction with 1-chloro-2,4-dinitrobenzene in methanolic sodium hydroxide, and used it for determinations on a range of plants and vegetables. Engst and Kubel[8] used thin-layer chromatography for the quantitative determination of dimethoate residues, and Mitsui and Suzuki[9] also published a method for the thin-layer chromatographic separation and colorimetric analysis of dimethoate residues. Abbott, Bunting and Thomson[10] used multi-band chromatoplates for the determination of residues of dimethoate in a variety of crops. Smart and Hill[11] described paper and thin-layer chromatographic separations of some polar organophosphorus insecticides extracted and cleaned-up by Frehse's method.

Ashworth[12] has described a gas - liquid chromatographic method for determining dimethoate residues in flue-cured tobacco.

P=S dimethoate is the active ingredient of formulated dimethoate and is gradually metabolised *in vivo* to the more toxic P=O dimethoate. Thus, as residues of dimethoate on a crop decrease with time, the toxicity of the residue per microgram of insecticide may increase, although the weathered residue rarely contains more than 10 to 20 per cent. of the total dimethoate residue in the form of the oxygen analogue. Data, both published and unpublished, on the toxicity and residues of P=O dimethoate were scrutinised closely by the Panel. The Panel considered that a strong case could not be made out for a method to

determine P=O dimethoate separately from P=S dimethoate, and hence the recommended method determines both P=S and P=O dimethoates together. Evidence for the presence or absence of P=S and P=O dimethoate individually by a semi-quantitative identification procedure is also described.

Chilwell and Beecham's method

After considering the available methods, the Panel selected Chilwell and Beecham's micro distillation procedure for initial collaborative study. The method had given good reproducible blank and recovery values in the hands of several workers and was robust, standing up well to Youden's test.[13] The dilute tin(II) chloride solution used for developing the molybdenum-blue colour was stabilised by addition of hydrazinium sulphate.

Table I

Recovery of added dimethoate from apples and cabbages by Chilwell and Beecham's method

	Collaborative study No. 1				Collaborative study No. 2	
	Net recovery from apples of		Net recovery from sprouts of		Net recovery from apples of	
Laboratory	0·5 p.p.m., p.p.m.	2·0 p.p.m., p.p.m.	0·5 p.p.m., p.p.m.	2·0 p.p.m., p.p.m.	0·5 p.p.m., p.p.m.	2·0 p.p.m., p.p.m.
1	0·24*	2·1†	—	—	0·7	1·8
	0·6*	1·5†	—	—	0·35	1·8
					0·35	1·8
					0·34	1·7
2	0·44	0·86	0·13	1·26	0·48	1·85
	0·36	1·02	0·30	0·74	0·65	2·05
	0·27	1·08	0·34	1·52	0·63	1·70
					0·54	1·73
					0·39	2·05
					0·56	1·94
3	0·37	1·54	0·45	1·09	0·46	1·42
	0·51	1·69	0·28	1·01	0·28	1·46
	0·43	1·42	0·21	0·83	0·23	1·38
4	0·36	1·42	0·41‡	1·40‡	0·58	1·69
	0·47	1·44	0·39‡	1·62‡	0·49	1·82
			0·39‡	1·65‡	0·42	1·85
5	0·50	—	—	—	0·45	1·83
					0·49	1·83
					0·48	1·81
6	0·17	1·08	0·29	0·96	0·51	1·62
	0·23	0·78	0·46	0·96	0·42	1·46
	0·57	1·00	0·65	1·34	0·34	1·54
	0·34			1·02		
				1·56		
7	—	—	—	—	0·80‡	1·2‡
					0·62‡	1·30‡
					0·30‡	1·54‡
					0·28‡	1·54‡
8	—	—	0·40	1·12	0·48	1·87
			0·42	1·10	0·47	1·83
				1·20	0·40	2·05
				1·22		
				1·30		
		1·0 *p.p.m. level*		1·0 *p.p.m. level*		
9	0·33	0·88	0·34	0·73	—	—
	0·35	0·74	0·40	0·81		
	0·37	0·72	0·38	0·76		

* Added, 0·45 p.p.m. † Added, 1·80 p.p.m. ‡ By using a modified method.

In the first collaborative study, members of the Panel recovered recrystallised P=S dimethoate from untreated apples and Brussels sprouts at the 0·5 and 2·0 p.p.m. levels.

When studying recovery in this and subsequent collaborative work the pesticide was added before maceration. The results obtained are given in Table I. Reagent and crop-blank values are given in Table II. Although the blank values were generally reasonable, the

TABLE II

BLANK VALUES FOR REAGENTS AND UNTREATED CROPS BY CHILWELL AND BEECHAM'S METHOD

	Collaborative study No. 1			Collaborative study No. 2	
Laboratory	Reagent blank, p.p.m.	Apple blank (*less* reagents), p.p.m.	Sprout blank (*less* reagents), p.p.m.	Reagent blank, p.p.m.	Apple blank (*less* reagents) p.p.m.
					0·29
					0·29
1	0·15	Nil	—	0·30	0·17
	0·14	—	—	0·30	0·27
	0·16	—	—	0·27	0·30
	0·13	—	—	—	0·30
					0·38
					0·47
2	0·14	0·05	0·08	0·14	Nil
		0·08	Nil	0·11	0·14
		0·09	0·07	0·11	0·06
3	0·04	0·10	0·03	0·18	0·22
	0·04	0·09	0·04	0·10	0·13
	0·05	0·08	0·06	0·10	0·05
4	—	<0·02	0·02*	0·04	0·15
			0·08*	0·01	0·09
			0·11*	0·04	0·14
5	0·04	0·04	—	0·03	0·04
	0·02	0·08	—	0·04	0·02
	0·02	0·06	—	0·04	0·03
6	0·20	Nil	Nil	0·50	0·36
	0·12	0·02	Nil	0·58	Nil
	0·12	0·20	0·06	0·41	Nil
	0·20	0·13	0·11	0·46	Nil
		0·07			
		0·15			
7	—	—	—	0·08*	0·12*
				0·08*	0·12*
				0·08*	0·04*
				0·08*	0·04*
8	—	—	—	0·05	0·32
				0·10	0·32
					0·33
					0·32
					0·33
					0·32
					0·53

* By using a modified method.

Panel considered that the recovery values were somewhat disappointing. Several laboratories reported difficulty in filtering the homogenised extract and, for the second collaborative study, an acetone extraction was used, followed by chloroform partition and micro distillation, as in Chilwell and Beecham's original method. It was noted that the reduction of the molybdophosphate complex should be conducted in strongly acidic solution, otherwise the blue colour does not develop properly. In the initial study, some laboratories suggested that the calibration graph was not linear at about 3 p.p.m., or above, and so more reducing agent was used to reduce the molybdophosphate complex subsequently and is advocated in the recommended method. The end of the cold finger should be ground to give greater surface area for condensation of the micro distillate.

The second collaborative study was carried out on apples, again at 0·5 and 2·0 p.p.m. levels, and the results obtained for recoveries are given in Table I, and those for blanks in Table II. The mean recovery of P=S dimethoate from apples at the 0·5 p.p.m. level was 0·46 p.p.m., with a standard deviation of $\pm$0·11 p.p.m., and at the 2·0 p.p.m. level 1·76

p.p.m., with a standard deviation of ±0·19 p.p.m. (neglecting the results from laboratory 7 in which a modified method was used). The Panel considered these recovery values satisfactory for a residue method, but noted the confusing variation in blank values not only between laboratories but sometimes within laboratories. High reagent blanks could in some instances be ascribed to phosphorus in the perchloric acid or to the hydrazine reagent. To avoid "spitting" during the perchloric acid digestion, some members used an asbestos mat on top of the electric hot-plate to counteract uneven heating effects, or micro Kjeldahl flasks with gas burners. One laboratory investigated the modified Chilwell and Beecham procedure on other crops; blank values of 0·05 to 0·10 p.p.m. were obtained with cabbages and 90 to 95 per cent. of added P=S dimethoate was recovered at the 2 p.p.m. level; high blank values with cauliflowers, blackcurrants and peas could be reduced to an acceptable level by treating the chloroform extract with Nuchar C190N before micro distillation; recoveries with the latter crops were 80 to 90 per cent. This laboratory reported 60 per cent. recovery of P=O methoate from apples at the 2 p.p.m. level when using the modified procedure. Another laboratory reported only 20 per cent. recovery of added P=O dimethoate from apples.

Frehse's method

At this stage Frehse introduced to the Panel his method involving the use of an aluminium oxide column clean-up. It consisted of extraction with acetone, evaporation of the acetone, filtration, extraction of the aqueous extract with chloroform, purification of the chloroform extract by chromatography on a Brockman Grade V aluminium oxide column, followed by evaporation of the eluate, wet ashing the residue and molybdenum-blue determination of phosphorus. The details are essentially those set out in Appendix I. The method had given good recoveries and low blank values with a range of crops in Frehse's laboratory. It was,

Table III

Blank values for reagents and untreated crops by Frehse's method

	Collaborative study No. 3			Collaborative study No. 4		
Laboratory	Reagent blank, p.p.m.	Apples blank (*less* reagents), p.p.m.	Cauliflowers blank (*less* reagents), p.p.m.	Reagent blank, p.p.m.	Peas blank (*less* reagents), p.p.m.	Cabbages blank (*less* reagents), p.p.m.
1	0·32	0·13	0·10	0·32	0·15	—
	0·31	Nil	0·07	0·30	0·08	—
2	0·14	Nil	0·03	—	—	—
	0·10	Nil	0·01			
	0·14	0·02	Nil			
	0·03	0·04	0·16	0·01	0·17	0·12
	0·02	0·06	0·10	0·01	0·17	0·06
	0·02	0·04	0·13	Nil	0·14	0·11
4	0·30	0·01	Nil	—	—	—
	0·19	Nil	Nil			
	0·22	Nil	0·04			
5	0·07	0·15	0·07*	0·07	Nil	0·02
	0·07	0·17	0·10*	0·07	0·05	0·05
	0·05	0·15		0·09	0·01	0·03
7	0·06	0·05	0·15	0·04	0·07	0·07
	0·06	0·05	0·15	0·06	0·07	0·07
	0·06			0·06	0·07	0·09
	0·08				0·09	
	0·08					
	0·09					
8	0·05	0·20	0·05	—	—	—
	0·03	0·24	Nil			
		0·21	Nil			
		0·23				
		0·42				
		0·43				
		0·14				

* By using Super-Cel filtration.

TABLE IV

RECOVERY OF KNOWN AMOUNTS OF ADDED DIMETHOATE FROM APPLES, CAULIFLOWERS, PEAS AND CABBAGES BY FREHSE'S METHOD

	Collaborative study No. 3				Collaborative study No. 4			
	Net recovery from apples of		Net recovery from cauliflowers of		Net recovery from peas of		Net recovery from cabbages of	
Laboratory	0·5 p.p.m., p.p.m.	2·0 p.p.m., p.p.m.	0·5 p.p.m., p.p.m.	2·0 p.p.m., p.p.m.	0·5 p.p.m., p.p.m.	2·0 p.p.m., p.p.m.	0·5 p.p.m., p.p.m.	2·0 p.p.m., p.p.m.
1	0·47	1·7	0·44	1·5	0·38	1·2		
	0·29	1·8	0·29	1·6	0·28	1·4		
2	0·38	1·47	0·43	1·77	—	—	—	—
	0·53	1·83	0·38	1·78				
	0·52	1·83	0·34	1·74				
3	0·39	1·57	0·50	1·48	0·41	1·28	0·36	1·45
	0·35	1·50	0·37	1·46	0·39	1·52	0·39	1·62
	0·42	1·63	0·32	1·60	0·36	1·55	0·36	1·56
4	0·37	1·83	0·40	1·81	—	—	—	—
	0·47	1·70	0·70	1·75				
	0·56	1·63	0·46	1·77				
5	0·47	2·08	0·46*	1·95*	0·51	1·88	0·48	1·95
	0·44	1·97	0·50*	1·97*	0·52	1·97	0·50	1·96
	0·52	2·00	0·48*	2·01*	0·53	2·03	0·47	1·99
7	0·46	1·92	0·44	1·48	0·47*	1·79	0·49†	0·9†
	0·48	1·94	0·44	1·52	0·49*	1·79	0·51†	0·91†
			0·44	1·54	0·51*	1·81	0·53†	0·95†
				1·54	0·51*			0·99†
8	0·50	1·91	0·37	1·93	0·43	2·0	—	—
	0·50	1·73	0·44	1·86	0·41	1·7		
	0·50	1·89	0·63	1·63	0·43	1·7		
Mean	0·46	1·83	0·45	1·73	0·43	1·69	0·44	1·76
Standard deviation ..	±0·06	±0·17	±0·10	±0·18	±0·06	±0·26	±0·06	±0·19

* By using Super-Cel filtration.
† 0·54 p.p.m. added at 0·5 p.p.m. level and 1·08 p.p.m. at 2 p.p.m. level.

therefore, submitted to collaborative study to compare it with Chilwell and Beecham's method. Recovery of added P=S dimethoate from apples and cauliflowers was first examined in the third collaborative study, and then from peas and cabbages in the fourth collaborative study. Blank values for these studies are given in Table III and recovery values in Table IV. High reagent blanks obtained in two laboratories were ascribed to impurities in the aluminium oxide and in the acetone used. It is important to cool the aqueous extract thoroughly before

TABLE V

RECOVERY OF ADDED DIMETHOATE FROM SUNDRY CROPS BY FREHSE'S METHOD

		Net recovery	
Crop	Total blank	0·5 p.p.m. level, p.p.m.	2·0 p.p.m. level, p.p.m.
Blackcurrants	0·14	0·53	—
	0·11	0·49	
Orange flesh	0·15	—	1·91
	0·14		1·93
Orange peel	0·09	—	1·95
	0·13		1·83
Pears	0·05	0·43	1·82
	0·05	0·43	1·84
	0·05	0·32	1·81
Olives	0·07	0·78	—
	0·10	0·61	
		0·53	

filtering, otherwise high blank values can occur. Results for the method with other crops were obtained in individual laboratories and are set out in Table V. With olives it was necessary to extract the aqueous solution with two 50-ml volumes of hexane before partition with chloroform, otherwise oils interfered seriously with the wet-oxidation stage. Although it took slightly longer to run single determinations with Frehse's method than with Chilwell and Beecham's method, several determinations could more easily be run together, and the Panel considered it to be more reliable than the latter.

The Panel tested the recovery of P=O dimethoate by Frehse's method for the fifth collaborative study. Recovery values, at 0·5 p.p.m. level, are set out in Table VI.

TABLE VI

RECOVERY OF A KNOWN AMOUNT OF P=O DIMETHOATE FROM APPLES BY FREHSE'S METHOD

Laboratory	Net recovery of 0·5 p.p.m. of P=O dimethoate, p.p.m.		
2	0·35	0·40	0·36
3	0·24	0·24	0·24
5	0·30	0·35	0·28
7	0·29	0·31	0·33
8	0·48	0·36	0·41
Mean	0·33		
Standard deviation ..	±0·07		

As a final test of Frehse's method the Panel recovered unknown amounts of P=S dimethoate from locally purchased apples. The amounts of dimethoate found by the collaborating laboratories are set out in Table VII.

TABLE VII

RECOVERY OF UNKNOWN AMOUNTS OF ADDED DIMETHOATE FROM APPLES BY FREHSE'S METHOD

Laboratory	Net recovery of 0·70 p.p.m., p.p.m.	Net recovery of 1·40 p.p.m., p.p.m.
1	0·62, 0·57	0·32, 0·93
3	0·66, 0·61	1·31, 1·36
4	0·61, 0·75	0·88, 0·94
5	0·73, 0·78	1·56, 1·53
7	0·67, 0·69	1·39, 1·41
8	0·94, 0·94	1·39, 1·39
10	0·80, 0·83	1·39, 1·58
11	0·58, 0·78	1·19, 1·13
Mean	0·72	1·29
Standard deviation ..	±0·12	±0·23

A slight modification of the original procedure for evaporating the acetone after extraction was checked and found to be satisfactory, and has been incorporated into the method.

The Panel recommends Frehse's method, set out in detail in Appendix I, for the quantitative determination of dimethoate residues in fruits and vegetables.

SEMI-QUANTITATIVE IDENTIFICATION OF DIMETHOATE

At its initial meeting the Panel considered that if the quantitative recommended method were not specific for dimethoate a semi-quantitative identification step should be recommended for use alongside it to establish any residues determined as dimethoate. A semi-quantitative identification of P=O dimethoate was also considered necessary to give an approximate assessment of the amount of P=O dimethoate in the residue, thus enabling a more accurate toxicological evaluation (P=O dimethoate may be ten times more toxic than P=S dimethoate). It was clearly advantageous if a semi-quantitative identification step for P=O and P=S dimethoates could be worked out following the extraction and clean-up used in the proposed quantitative method, rather than initiate a new extraction and clean-up procedure.

THIN-LAYER CHROMATOGRAPHY—

One laboratory, therefore, investigated various published and unpublished paper and thin-layer chromatographic techniques on the cleaned-up extract from Frehse's method. The problem appeared to be not only one of identifying dimethoate in a sample known to have been sprayed with the insecticide, but also that of identifying P=O and P=S dimethoates in a sample whose spray history was not known. To this end, nearly thirty other organophosphorus insecticides and metabolites were screened to see whether they were likely to interfere with the determinations. Identification of 0·1 p.p.m. insecticides, particularly of the P=O metabolite, was sought. In this laboratory, difficulty was encountered with Abbott's multi-band identification technique; the R_F values were appreciably higher than reported in the literature but clean-up of cabbage extracts was good. Other laboratories, however, had achieved satisfactory results. The silica gel - kieselguhr (1 + 1) system, used in the multi-band plates, provided a good separation in two dimensions, and gave acceptable identification to a first approximation. Alumina plates were not satisfactory as they gave poor separation in the solvents required to move the pesticide from the origin. Steller's method[14] gave a useful separation, but crop interferences were observed.

Modifications, involving both paper and thin-layer chromatography, of Bates'[15] formamide-impregnated systems were worked out and gave good identification of both P=O and P=S dimethoates in a wide range of crops. Some further clean-up was, however, necessary with some crops. Adequate sensitivity was obtained with the 4-(*p*-nitrobenzyl)pyridine reagent of Watts.[16] This work was subsequently published by Smart and Hill.[11]

The use of polyamide plates was also investigated and adequate separation achieved by using a two-dimensional technique, but cauliflowers and peas, even after further clean-up, did not give satisfactory chromatograms.

As the silica gel - kieselguhr (1 + 1) and formamide-impregnated, silica gel thin-layer chromatographic systems were the most successful, these were examined collaboratively by the Panel. Laboratories investigated the methods on several crops and found them generally satisfactory, except that in some instances overloading was contributing to some distortion of R_F values of the pesticides extracted from crop materials compared with standards run at the sides of the plates. Smaller aliquots (corresponding to 5 g of crop material) of cleaned-up extracts were, therefore, taken for the eighth collaborative study on the same thin-layer chromatographic systems, and the effect of brief drying of the plates at 110° C between runs to regularise R_F values was also investigated.

Distortion of R_F values and interferences were less with the smaller samples, and 0·1 p.p.m. (0·5 μg) of P=S and P=O dimethoates was detectable on silica gel - kieselguhr plates. The formamide-impregnated plates were somewhat less sensitive; 0·2 p.p.m. (1 μg) of the P=S and P=O dimethoates was generally identifiable, but crop constituents were nearly absent. Baking the silica gel - kieselguhr plates for 5 minutes at 100° C between developments was considered advantageous. The Panel therefore recommends the silica gel - kieselguhr semi-quantitative identification system as first action, but that if crop interferences confuse the identification the formamide-impregnated system should be used. Details of the identification procedures are set out in Appendix II.

If an aliquot of the chloroform extract is used for thin-layer chromatography before being cleaned-up on the alumina column, the identification is not so clear-cut and in some instances may not be possible.

Approximate R_F values to be expected with the recommended chromatographic systems are—

(*a*) With silica gel - kieselguhr plates: P=S dimethoate 0·6 in chloroform - acetone (9 + 1) and 0·8 in chloroform - acetone (2 + 1); and P=O dimethoate 0·2 in chloroform - acetone (9 + 1) and 0·4 in chloroform - acetone (2 + 1).

(*b*) With formamide-impregnated plates: P=S dimethoate 0·5 to 0·6 in 1,2-dichloroethane - benzene (2 + 1) and 0·7 in *cis*-1,2-dichloroethylene; and P=O dimethoate: 0·05 to 0·07 in 1,2-dichloroethane - benzene (2 + 1) and 0·1 to 0·15 in *cis*-1,2-dichloroethylene.

GAS - LIQUID CHROMATOGRAPHY—

The Panel also considered the application of gas - liquid chromatography to the determination of dimethoate. Several laboratories looked at the problem independently by using the Aerograph phosphorus detector.

One laboratory had investigated[17] Apiezon on Chromosorb G, allowing identification of a fraction of a nanogram of P=S dimethoate and about 10 ng of P=O dimethoate, but obtained better results with an X.E. 60 cyanosilicone-impregnated column. The latter showed a sensitivity of 0·2 to 0·3 ng of P=S dimethoate and 2 ng of P=O dimethoate, the peaks having retention times of 95 and 75, respectively, with reference to 100 for parathion. Butane-1,4-diol succinate also showed promise. Another laboratory had used the X.E. 60 cyanosilicone-impregnated column with extracts of fortified crops obtained by the recommended method. With no alumina column clean-up, peas had given a 0·04 p.p.m. blank, 76 per cent. recovery of P=S dimethoate and 107 per cent. of P=O dimethoate. With column clean-up, recoveries were 50 per cent. for P=S dimethoate and 80 per cent. for P=O dimethoate. A column temperature of 150° to 200° C was used for P=S dimethoate and 120° C for P=O dimethoate.

One laboratory reported that a successful column for P=S and P=O dimethoates was 2 per cent. diethylene glycol succinate on 80 to 100-mesh Gas Chrom Q. The retention times of P=S and P=O dimethoates, relative to parathion (100), were 135 and 108.

Another laboratory used an S.E.30 silicone-impregnated column for identifying P=S dimethoate in acetone extracts of cherries and plums. The response of the detector was linear in the range 1 to 10 ng. Apiezon L (0·5 per cent.) was more promising for detection of P=O dimethoate.

In the co-ordinating laboratory a 2 foot × $\frac{1}{8}$-inch stainless-steel column packed with 5 per cent. S.E.30 silicone gum supported on 80 to 100-mesh Chromosorb W and pre-treated with hexamethyldisilazane was used. To avoid decomposition of dimethoate it was necessary to treat the column by repeated injection of dimethoate initially, and then daily before use with 1 μg of dimethoate. The oven temperature was 215° C, when 0·1 ng of P=S dimethoate was detectable. Menazon, vamidothion, vamidothion sulphoxide, demeton-S-methyl sulphoxide and sulphone did not interfere. Untreated apples, peas, cauliflowers, blackcurrants and cabbages extracted and cleaned-up by the recommended method gave little or no response, and recoveries of 0·1 to 0·3 p.p.m. of P=S dimethoate were usually 70 to 120 per cent. Pears needed additional clean-up. P=O dimethoate could probably be determined at 150° to 160° C at 0·3 p.p.m. Formamide from impregnated thin-layer chromatographic plates interfered when spots on such plates were scraped and eluted. Spots from silica gel - kieselguhr plates needed to be extracted by using a Soxhlet apparatus.

The Panel was unable to recommend a gas - liquid chromatographic method at this stage.

Appendix I

RECOMMENDED METHOD FOR QUANTITATIVE DETERMINATION OF DIMETHOATE RESIDUES IN FRUITS AND VEGETABLES

APPARATUS—

Macerator.

Chromatographic column—A column, with internal diameter about 2 cm and length 30 cm, with tap and fitted with detachable reservoir, was used.

Absorption spectrophotometer and 4-cm cells.

REAGENTS—

All reagents should be of analytical-reagent grade when possible.

Acetone—Re-distillation may be necessary.

Chloroform.

Carbon tetrachloride.

Aluminium oxide—Neutral, Brockman Grade V (with 15 per cent. of water).

Perchloric acid solution, 72 per cent., aqueous, sp. gr. 1·70.

Nitric acid, sp. gr. 1·42.

Ammonium molybdate solution, 5 per cent., aqueous.

Sulphuric acid, 0·6 N.

Tin(II) chloride - hydrazinium sulphate solution—Dissolve 2·0 g of hydrazinium sulphate in 1 litre of 0·6 N sulphuric acid, cool to about 10° C and add 1·0 g of tin(II) chloride, from a recently opened bottle. This solution becomes clear after about 12 hours in a refrigerator and can be kept for 2 weeks.

Isobutyl alcohol.

Ethanol.

Potassium dihydrogen orthophosphate solution (for preparation of calibration graph)—Dissolve 43·9 mg of potassium dihydrogen orthophosphate in 100 ml of water and dilute 100-fold to give a solution containing 1 μg of phosphorus per ml.

PREPARATION OF CALIBRATION GRAPH—

By pipette introduce a suitable volume of standard potassium dihydrogen orthophosphate solution into a 100-ml separating funnel. Add 2 ml of perchloric acid and make up to 30 ml with water. Add 5 ml of 5 per cent. ammonium molybdate solution and 15 ml of isobutyl alcohol. Shake the mixture for 90 seconds, allow the layers to separate and discard the lower aqueous layer. Wash the isobutyl alcohol with 15 ml of 0·6 N sulphuric acid, shaking the mixture for 60 seconds. Allow to separate and discard the lower layer. Wash the isobutyl alcohol into a 50-ml calibrated flask with ethanol, add 0·5 ml of hydrazinium sulphate - tin(II) chloride solution and make up to 50 ml with ethanol, mixing thoroughly. Read the optical density at 735 nm in a 4-cm cell against water in the reference cell.

EXTRACTION—

Macerate a 250-g sample of crop with 500 ml of acetone for 1 minute and filter through a Buchner funnel. Again macerate the "cake" that is left with 250 ml of acetone for 1 minute and filter through the Buchner funnel. Combine the acetone extracts and make up to 1 litre with acetone. Take 200 ml (corresponding to 50 g of crop material) and evaporate off the acetone under reduced pressure at a temperature not higher than 60° C. Cool the flask thoroughly under the cold water tap. Filter the residue and wash the filter-paper with water. Transfer to a separating funnel, making the final volume up to 150 to 200 ml. Extract three times with 200 ml of chloroform. (Emulsions are rarely encountered if the volume of chloroform is equal to, or greater than, the volume of the aqueous extract.) Any semi-solid interface should be left with the aqueous phase at each extraction. Combine the chloroform extracts and filter through Whatman No. 1 paper. Evaporate just to dryness under reduced pressure at a temperature not higher than 60° C.

If the method cannot be completed the same day, solutions containing crop extracts should be stored at 0° C.

COLUMN CHROMATOGRAPHY—

Prepare a chromatographic column of 10 g of aluminium oxide in a liquid phase consisting of a mixture of chloroform and carbon tetrachloride (1 + 1). Dissolve the residue from the chloroform extraction in 10 ml of chloroform - carbon tetrachloride (1 + 1), introduce the solution into the column, and allow it to run at a rate of about one drop per second. When the level of the solvent has reached the top of the alumina, add a further 10 ml of chloroform - carbon tetrachloride (1 + 1), rinsing out the flask. When this has reached the top of the alumina, elute the column with 120 ml of the chloroform - carbon tetrachloride mixture. After about 50 ml of solvent have run through the column, its rate of flow can be increased to two drops per second. Evaporate the solvents and, by using chloroform, transfer the residue to the flask for wet oxidation.

WET ASHING—

Evaporate the chloroform, add 5 ml of distilled water, five drops of concentrated nitric acid, 2 ml of 72 per cent. perchloric acid and a small glass bead to the residue in the flask and heat to dense white fumes for several minutes. Cool, add a few millilitres of water, and also a few drops of nitric acid if the solution is coloured, and again heat to white fumes. Cool and transfer to a 100-ml separating funnel with water, making the volume up to 30 ml.

COLORIMETRIC DETERMINATION—

Add 5 ml of ammonium molybdate solution and 15 ml of isobutyl alcohol. Shake the mixture for 90 seconds, allow the layers to separate and discard the lower aqueous layer. Wash the isobutyl alcohol layer with 15 ml of 0·6 N sulphuric acid, shaking it for 60 seconds. Allow to separate and discard the lower layer. Wash the isobutyl alcohol layer into a 50-ml

calibrated flask with ethanol, add 0·5 ml of hydrazinium sulphate - tin(II) chloride solution and make the volume up to 50 ml with ethanol, mixing thoroughly. Read the optical density at 735 nm in a 4-cm cell against water in the reference cell.

Read the corresponding amount of phosphorus from the calibration graph and multiply it by 7·40 to express the results as dimethoate.

Appendix II

RECOMMENDED METHOD FOR SEMI-QUANTITATIVE IDENTIFICATION OF DIMETHOATE RESIDUES IN FRUIT AND VEGETABLES

REAGENTS—

Silica gel G (Merck, for chromatography).
Kieselguhr G (Merck, for chromatography).
Silica gel HF (Merck, for chromatography).
Formamide—General-purpose reagent.
1,2-Dichloroethane.
cis-*1,2-Dichloroethylene*—Obtained from Ralph N. Emanuel, 3–4 Leather Market, London, S.E.1.
4-(p-*Nitrobenzyl)pyridine*—Obtained from Ralph N. Emanuel. Prepare a 2 per cent. solution in acetone.
Tetraethylenepentamine, 10 per cent. in acetone.
Active carbon, Nuchar C190N.
Acetonitrile, redistilled.
Hexane, redistilled.
Magnesium oxide—Chromatographic grade.
Standard solutions of P=S and P=O dimethoates—These were prepared in chloroform and should be stored at 0° C in the dark.

THIN-LAYER PLATES—

(*a*) *Silica gel - kieselguhr plates*—Slurry 20 g of silica gel G, 20 g of kieselguhr G and 80 ml of water for $1\frac{3}{4}$ minutes and spread the mixture over five 20 × 20-cm plates by using a spreader set at 0·25 mm. After initial air-drying, dry the plates in the oven at 110° to 120° C for at least 1 hour and allow them to stand in a desiccator cabinet to cool.

(*b*) *Formamide-impregnated silica gel plates*—Slurry 30 g of silica gel HF with 100 ml of 20 per cent. formamide in ethanol for 1 minute, allow to stand for 1 minute to remove air bubbles and spread the mixture over four 20 × 20-cm plates by using a spreader set at 0·5 mm. With some batches of silica gel HF it is necessary to vary the amount of formamide solution to obtain a slurry of suitable consistency. After spreading, air-dry the plates for 5 to 10 minutes and then oven-dry them at 50° C for 10 minutes before storing in a desiccator cabinet.

(*a*) *Further clean-up for silica gel - kieselguhr plates*—Evaporate an aliquot, corresponding to 5 g of fruit or vegetable, of the eluate from the alumina column of the recommended quantitative method (Appendix I) nearly to dryness on a water-bath, with a gentle stream of air. Take up the residue in 25 ml of acetonitrile and extract four times with 10 ml of hexane, shaking the mixture for 1 minute on each occasion. Discard the hexane extracts. Add 0·2 g of Nuchar C190N and 0·4 g of magnesium oxide to the acetonitrile solution and shake it for 5 minutes. Filter through a Whatman 2V (No. 12) paper and concentrate the filtrate in a stream of air just to dryness on a water-bath. (A test-tube with a drawn-out lower end is suitable for this stage.) Take up in 20 to 25 μl of chloroform for chromatography. With apples, pears, cabbages and blackcurrants there is no need for acetonitrile - hexane partition, or magnesium oxide treatment, and the chloroform - carbon tetrachloride eluate can be shaken directly with 0·2 g of Nuchar C190N, filtered and concentrated for chromatography.

If the method cannot be completed the same day, solutions containing crop extracts should be stored at 0° C.

(*b*) *Further clean-up for formamide-impregnated plates*—The details of the procedure for further clean-up for formamide-impregnated plates are identical with those for silica gel - kieselguhr plates, except that magnesium oxide is omitted when the 0·2 g of Nuchar C190N is added to the acetonitrile solution, and the same provision applies with apples, pears, cabbages and blackcurrants.

CHROMATOGRAPHY (TWO DIMENSIONAL)—

(*a*) *With silica gel - kieselguhr plates*—With a suitable capillary, carefully apply the solution in successive 2 to 3-μl amounts at a spot 2·5 cm from the edges of two adjacent sides. Also apply P=S and P=O dimethoates (say 1 μg of each) 2 cm from the far ends of the two adjacent sides and 2·5 cm from the edges. Develop in chloroform - acetone (9 + 1), in a tank that has been allowed to equilibrate, until the solvent front is 3 cm from the top edge. Dry at 100° C for 5 minutes. Develop at right angles in chloroform - acetone (2 + 1), in a tank that has been allowed to equilibrate, until the solvent front is 3 cm from the far edge. Allow the plate to dry in the air. Spray with 15 to 20 ml of 4-(*p*-nitrobenzyl)pyridine solution in acetone. Heat for 10 minutes at 110° C in an oven. Spray with tetraethylenepentamine solution, whereupon the pesticides appear as blue spots on a white background. Compare the position and intensity of the sample spots with the standards.

(*b*) *With formamide-impregnated plates*—With a suitable capillary, carefully apply the solution in successive 2 to 3-μl amounts at a spot 2·5 cm from two adjacent sides. Also apply P=S and P=O dimethoates (say 1 μg of each) 2 cm from the far ends of the two adjacent sides and 2·5 cm from the edges. Develop in 1,2-dichloroethane - benzene (2 + 1), in a tank that has been allowed to equilibrate, until the solvent front is 3 cm from the top edge. Allow to dry in the air at room temperature. Develop at right angles, in *cis*-1,2-dichloroethylene, in a tank that has been allowed to equilibrate, until the solvent front is 3 cm from the far edge. Allow to dry in the air. Spray with 15 to 20 ml of 4-(*p*-nitrobenzyl)pyridine solution in acetone. Heat for 10 minutes at 110° C in the oven. Spray with tetraethylenepentamine solution, whereupon the pesticides appear as blue spots on white background. Compare the position and intensity of the sample spots with the standards.

Appendix III

MEMBERSHIP OF THE PANEL

The Panel consisted of D. C. Abbott (Chairman), the late E. D. Chilwell, W. B. Chapman, H. Crossley (until 23rd May, 1966), H. Frehse, N. Gandolfo, K. A. Lord, A. F. Machin, A. Müller, R. A. Savidge (from 4th October, 1966), N. A. Smart (Secretary), R. W. Storherr (from 16th September, 1966), R. P. Tew and B. Vasta (until 6th September, 1966).

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Book Reviews

STEROID HORMONE ANALYSIS. Edited by HANS CARSTENSEN. Volume 1. Pp. xiv + 493. London: Edward Arnold (Publishers) Ltd.; New York: Marcel Dekker Inc. 1967. Price £10 10s. 0d.

This is designated as the first of two volumes; there is, however, no indication of the subjects to be treated in the second. The seven chapters of Volume 1 cover a diversity of topics, with emphasis on practical procedures, but with great variation in the amount of detail included.

Chapter 1, by E. Bojesen and co-authors, deals with the determination of steroid hormones through the use of sulphur-35 sulphonylating reagents. The principles of these isotope derivative methods, and the meticulous procedures essential for their success, are clearly and fully described in an authoritative manner.

In chapters 2 and 3, E. Caspi *et al.* present short but effective introductions to the use of infrared and nuclear magnetic resonance spectrometry in steroid investigations. Tables of the principal correlations are included, as well as instructive examples of typical applications. Practical details are essentially limited to the preparation of potassium bromide microdiscs and pellets, and the use of microcells for absorption spectrometry of solutions. The importance of dilution in the characterisation of intramolecular hydrogen bonding is stressed (although not exemplified in the illustrations on pp. 87 to 89).

Chapter 4 deals with the chromatography of steroids on paper. The article (184 pages) is by far the longest in the book; unfortunately, in the reviewer's opinion it is also the least successful. Ideally, descriptions of experimental procedures should expose all of the essential particulars and illustrate optional details, without labouring the obvious. Admittedly, the craft of paper chromatography presents special difficulties in this respect. This chapter contains many useful practical directions and informed observations, but the author's prolix style is somewhat infelicitous for the reader. Moreover, several relevant topics seem to have escaped mention: examples are the observations of several authors concerning the mechanism of the Zimmermann reaction (p. 246); the effects of "isotope fractionation" during chromatography (p. 290); intramolecular hydrogen bonding in 21-hydroxy-20-oxosteroids in relation to the lowered polarity of the 21-hydroxyl group (pp. 216 and 236); and the decomposition of steroids as a possible source of loss, in their elution from paper (p. 311). The author's definition of chromatography, based on that proposed by Williams and Weil in 1952, omits the condition of mobility of one phase which, in the reviewer's experience, is generally regarded as an intrinsic requirement of the process, rather than a "convention" as suggested in this chapter.

Chapter 5, by K. B. Eik-Nes and co-authors, is a crisp and critical discussion of the biogenesis, secretion and metabolism of testosterone, and of published methods for its determination in urine and plasma. The basic principles and salient features of the methods are tabulated.

B. A. Knights, in chapter 6, presents in a concise style a very readable survey of gas chromatography in relation to the analysis and characterisation of steroids. Detailed procedures are not given, but are readily accessible via the well annotated tables, which provide a key to the most important analytical applications published up to 1965.

Steroid conjugates form the subject of the final chapter, by J. R. Pasqualini. Extraction, purification, synthesis, hydrolysis and isolation methods are all briefly discussed. As in the two preceding chapters, much useful information is condensed in tabular form.

Author and subject indexes complete the volume.

A few grammatical inaccuracies have escaped correction, and the following factual errors seem worthy of mention. In Table III, p. 150, the 3rd column total is incorrect. On p. 438 the constants ("K") refer to solvolysis rates (k), not equilibria, and their values should be $\times 10^{-5}$, not $\times 10^{5}$: symbols for hydrogen atoms are missing from Fig. 3 on the same page.

This well documented book is of value as a reference volume for research workers concerned with steroid hormone analysis. Its usefulness is, however, somewhat reduced by the excessive delay in publication, and is likely to be limited by the unduly high price. C. J. W. BROOKS

ENZYMES. By D. W. Moss, M.A., M.Sc., Ph.D. Pp. viii + 110. Contemporary Science Paperbacks 15. Edinburgh and London: Oliver & Boyd. 1968. Price 7s. 6d.

NATURAL HIGH POLYMERS. By C. T. GREENWOOD, Ph.D., D.Sc., F.R.I.C., F.R.S.E., and E. ANN MILNE, Ph.D. Pp. viii + 128. Contemporary Science Paperbacks 18. Edinburgh and London: Oliver & Boyd. 1968. Price 7s. 6d.

"There is no end to the buying of books, and to study books closely is a weariness to the flesh" is James Moffat's rendering of the well known passage in Ecclesiastes. To the truth of this every chemist can testify, as his science is an expanding universe of knowledge and its exposition, with which he can never hope to keep pace except perhaps in his own sub-galactic sub-unit. But every chemist worth his salt is seized by a desire to keep in touch, if only superficially, with what is happening outside the particular star that he is engaged in pursuing, and to make this easy for him is a boon and a praiseworthy venture. This is achieved in the two books under review, and if they are typical of the series, its success is assured. The intention claimed in the prefaces to these volumes of initiating the sixth-former and the undergraduate, or even just satisfying an enquiring mind is ably fulfilled; the exposition is clear and logical, the reading is easy and there is no weariness to the flesh.

Natural High Polymers deals with proteins, including their synthesis in nature (the "code" is clearly explained), polysaccharides, lignin and rubber, and inorganic polymers, such as the silicates. A most interesting section in Enzymes is that on their rôle in reactions in the body. Some overlapping in the treatment of the subjects of these two volumes is unavoidable in giving an over-all picture of the subjects, but they fortify each other.

Both volumes can be thoroughly recommended for giving an up-to-date general survey to the chemist in another field or to the scientist in another discipline. J. I. M. JONES

ION EXCHANGE IN MIXED AND NON-AQUEOUS MEDIA. By JOHANN KORKISCH. SOME NEUTRAL BIFUNCTIONAL ORGANOPHOSPHORUS COMPOUNDS AS SOLVENT EXTRACTANTS. By JEROME W. O'LAUGHLIN. ANION EXCHANGE IN NITRATE SOLUTIONS. By JOHN FARIS and ROBERT F. BUCHANAN. ANALYTICAL CHEMISTRY OF NEPTUNIUM. By G. A. BURNEY, E. K. DUKES and H. J. GROH. THE ANALYTICAL CHEMISTRY OF POLONIUM. By ROBERT C. LANGE. Progress in Nuclear Energy, Series IX: Analytical Chemistry, Volume 6. Pp. vi + 260. Oxford, London, Edinburgh, New York, Paris and Frankfurt: Pergamon Press. 1966. Price 90s.

The monographs on Analytical Chemistry in the series "Progress in Nuclear Energy" will be known to analytical workers for their coverage of subjects of non-nuclear as well as of nuclear interest. Volume 6 is concerned with aspects of two general topics, separation techniques and the analytical chemistry of selected elements, in this case neptunium and polonium. Specific titles of the chapters are Ion Exchange in Mixed and Non-aqueous Media by Johann Korkisch, Some Neutral Bifunctional Organophosphorus Compounds as Solvent Extractants by Jerome W. O'Laughlin, Anion Exchange in Nitrate Solutions by John Faris and Robert F. Buchanan, Analytical Chemistry of Neptunium by G. A. Burney, E. K. Dukes and H. J. Groh and The Analytical Chemistry of Polonium by Robert C. Lange.

The first and longest chapter in the book, which is by Korkisch, systematically considers the behaviour of many elements in mixed or non-aqueous media in the presence of cation or anion exchangers. A large amount of distribution data is included in graphs and tables, and the application of selected systems to actual separations is demonstrated by the inclusion of elution curves. O'Laughlin first briefly discusses methods of syntheses of neutral bifunctional organophosphorus compounds and then describes extraction behaviour of the elements with these compounds by reference to liquid - liquid extraction experiments and reversed-phase partition chromatography; a final section considers the theoretical implications of experimental results. Information on the extraction of more than seventy elements from nitric acid solution by a strongly basic anion exchange is given in the chapter by Faris and Buchanan, who also consider anion exchange in organic solvent - nitric acid mixtures.

The last two chapters on the analytical chemistry of neptunium and polonium cover similar ground for the two elements, summarising properties, methods of separation and techniques of quantitative determination.

The first three chapters on separations, which constitute some three quarters of the total length of the book, contain much information on elemental distributions and are likely to be a useful starting point for those workers interested in applying the systems considered to their own particular problems. The last two chapters are short but in the space available the authors concentrate on the analytical aspects of the chemistry of neptunium and polonium and, therefore, the "signal-to-noise" ratio for analysts is high. Nevertheless, there is always the danger that books covering a number of diverse and rather specialised subjects will appeal only in part to prospective readers and this book is more likely to appear on library shelves than in the collection of the private individual.

T. B. PIERCE

FLUORESCENCE. THEORY, INSTRUMENTATION AND PRACTICE. Edited by GEORGE G. GUILBAULT. Pp. xxviii + 697. London: Edward Arnold (Publishers) Ltd.; New York: Marcel Dekker Inc. 1967. Price 145s.

The text of this book constitutes an expansion of nineteen papers presented at a 2-day symposium of the Analytical Division of the A.C.S. held in Miami Beach in April, 1967. Not infrequently the proceedings of symposia published in this way are chiefly of interest to those who attended the meeting or who were prevented from attending by force of circumstances. Generally, such published proceedings have appeal only over a limited period of time.

This book, however, is refreshingly different, for it covers virtually all possible areas of luminescence phenomena, both molecular and atomic, and penetrates from applied aspects, *e.g.*, analytical applications of various luminescence phenomena, through to instrumentation for the study of fluorescence, etc., kinetics and other fundamental aspects of the subject area. There is no doubt that this is an important text and that it cannot safely be neglected by anyone who is interested in this fascinating corner of spectroscopy. Subjects dealt with include Structure and Environmental Factors in Fluorescence, Laser Excitation of Vibrational Fluorescence (I.R. Fluorescence), Theory of Luminescence Polarisation of Fluorescence, Fluorescence of Metal Chelate Compounds, Analytical Phosphorimetry, Atomic Fluorescence in Flame Media and Electroluminescence of Condensed Hydrocarbons.

Although all sections are equally praiseworthy in their approach to the various topics dealt with, from an analytical viewpoint there can be little doubt that the contribution from Winefordner and Mansfield on atomic-fluorescence spectroscopy and from Bard, Santhanam, Cruser and Faulkner on the other "new" technique of electroluminescence are of outstanding importance. These new analytical techniques bid fair to supplement or even replace some of the much use dinstrumental techniques currently in our armoury for inorganic and organic trace analysis. I cannot stress too strongly how significant these are. There is no doubt whatsoever that, even for these two sections alone, this book represents a milestone along the highway of analytical chemistry.

The book is expensive for Europeans, but it is a really worthwhile publication.

T. S. WEST

TITRATION IN NON-AQUEOUS MEDIA. By I. GYENES C.Sc. English translation edited by D. COHEN, M.A., Ph.D., A.R.I.C., and I. T. MILLAR, B.Sc., Ph.D., F.R.I.C. Pp. xiv + 461. London: Iliffe Books Ltd; Princeton and New Jersey: D. Van Nostrand Company, Inc. 1968. Price 75s.

Non-aqueous titration is an expanding technique that finds use both in research and in quality-control laboratories, especially in the pharmaceutical industry. In consequence, there is a need for a comprehensive reference work on the subject. This book attempts to fill the gap that has hitherto been present in the literature, with a discussion of the theoretical and practical aspects of non-aqueous titration.

The first half of the book deals with some of the theoretical aspects and includes a detailed discussion of the solvents that can be used. The theoretical section begins with the development of acid - base concepts, a subject that is too often taken for granted, and concludes with an account of the methods of end-point detection, *viz.*, potentiometry, visual indicators and photometric titration.

The latter half is devoted to descriptions, with experimental details, of methods of analysis for all of the main classes and most of the sub-classes of organic compounds. In addition, accounts of both redox and complexometric titrations are included. As much information as possible is given in these sections to enable the practising analyst to carry out the procedures described on the

macro-, semimicro- and micro-scale. Also, in many instances, worked calculations of the results are included.

Nearly 900 references are cited, although the author does not consider these to represent a complete coverage of the available literature. The aim of the book is to assist the chemist working in industrial laboratories on routine analysis, but it can also be recommended as a standard reference work on the general subject of non-aqueous titrations.

R. M. DAGNALL

CHEMICAL ANALYSIS FOR IRONFOUNDRIES. SELECTED METHODS. Recommended by the BCIRA Methods of Analysis Sub-Committee. By THE BRITISH CAST IRON RESEARCH ASSOCIATION. Pp. 215. London: George Allen & Unwin Ltd. 1967. Price 42s.

In 1959 the British Cast Iron Research Association published what was intended to be the first part of "Selected Methods of Analysis of Foundry Materials." This first issue was restricted to the analysis of pig and cast iron, and it was intended to follow it up with further parts covering alloy irons, ferro-alloys, slags, refractories, fuels and residual elements. The new book, published under a slightly modified title, represents a change in policy for, in addition to replacing the previous work (100 pages), it covers the analysis of all types of irons and also ferro-alloys and slags within the compass of a single volume of 215 pages. The outcome is a concise work that covers all of the essential day-by-day requirements of the ironfoundry chemist. The decision to exclude methods of analysis for refractories, fuels, trace and residual elements is based on the premise that they are rarely required in ironfoundry laboratories. If this point is accepted, perhaps with some reservations about the growing importance attached to trace elements and residuals, there is no doubt that it has helped to keep the size of the volume within the bounds of a handy laboratory bench book. By the same token, theoretical matter has been restricted to a brief outline of the chemical principle of each method.

The first section, dealing with pig and cast irons, includes a comprehensive treatment of the problem of sampling. Traditional techniques and up-to-date methods, such as the rotary sampling machine and evacuated glass-tube sampling, are all described in considerable detail.

The choice of methods of analysis is excellent. All are established methods of proved merit and considerable use has been made of British Standard and other authoritative methods. Alternative methods have been included for each element when this has been possible without reducing the quality standard the authors have set for themselves.

Methods for the analysis of ferro-alloys are mainly concerned with the principal element and one or two important minor elements. The methods given for the analysis of foundry slags are based on well known techniques, with the possible exception of the method for fluorine, for which the authors can claim some originality.

The appendices dealing with equipment, etc., laboratory techniques and hazards and the use of spectrophotometers complete a very useful volume. There is no doubt that this book will become widely used, not only in ironfoundry laboratories, but also in steelworks laboratories and, indeed, wherever the analysis of ferrous materials is practised. It is equally adaptable for the use of students and experienced chemists, but it is essentially a laboratory handbook for the practical analyst in the ironfoundry industry.

B. BAGSHAWE

DRITTES KOLLOQUIUM ÜBER METALLKUNDLICHE ANALYSE MIT BESONDERER BERÜCKSICHTIGUNG DER ELEKTRONENSTRAHL - MIKROANALYSE, WIEN, 25–27 OKTOBER, 1966. Pp. iv + 290. *Mikrochimica Acta*/Supplementum II. Vienna and New York: Springer-Verlag. 1967. Price DM59; $14.75: to subscribers to *Mikrochimica Acta* DM53.10; $13.40.

This volume, issued as a supplement to *Mikrochimica Acta*, contains the twenty-seven papers presented at the third annual conference held in Vienna in October 1966, on electron-beam microanalysis. With the exception of a prepared discussion on one paper, together with the author's reply, no discussions of the papers are given. Most of the authors are from universities and industrial research laboratories in Germany.

More than a quarter of the papers are concerned with applications, generally metallurgical, of the "microsonde," a form of X-ray emission spectroscopy in which simultaneous analysis and observations of an extremely small specimen area are possible. Papers are presented showing that by incorporating with this equipment such refinements as scanning devices, laser beams and the electron microscope, it is possible to obtain detailed pictures of, for example, inclusions in steels, the distribution of oxygen in copper - titanium alloys and of impurity particles in silicon

wafers. Examples are also reported of the use of an electron-beam microanalyser in the study of the calcium oxide - magnesium oxide system, the diffusion of silicon in molybdenum and in the examination of surface films on metals. One paper describes the production of quite extraordinary colour pictures of the structure of metal samples by superimposition of several oscillograph pictures, each taken with a different colour filter.

There are several valuable, if somewhat more sober, papers on correction factors and Kossel diagrams, as well as detailed descriptions of equipment, and the whole volume presents a good conspectus of recent developments in this interesting field. J. W. PRICE

GAS EFFLUENT ANALYSIS. Edited by WILLIAM LODDING. Pp. xii + 220. London: Edward Arnold (Publishers) Ltd.; New York: Marcel Dekker Inc. 1967. Price 95s.

The study of reactions in solids by measurement of a physical parameter while a sample is being heated is a well established technique. Many such reactions are accompanied by the evolution of gases, and the identification and measurement of these gases is the subject of this book.

This is the first volume of a series on Thermal Analysis, and contains seven chapters by different authors. A short review of history and theory is followed by a description of apparatus for gas evolution and detection. The second chapter contains a survey of thermal-conductivity detectors, their operation and calibration, an outline of gas chromatography and several examples of the application of these detectors to thermodynamic and kinetic studies. The application of mass spectrometry is described and illustrated by several examples of mass-spectrometric thermal analysis. Pyrolysis gas chromatography is illustrated mainly by its application to polymers. A short chapter describing some simple chemical absorption techniques is followed by a review of infrared methods, which includes an extensive study of the potential of a multiple-scan interference spectrometer. The final chapter reviews the detection of condensation nuclei evolved during thermal analysis.

Apparatus is well illustrated and numerous examples of the application of each technique are described. Most chapters contain an extensive bibliography and there is a comprehensive author index, but the subject index seems inadequate. Occasionally there is a tendency to give a short over-all description of a technique rather than concentrating on the peculiar requirements of that technique when applied to evolving gases. As a result, the description may be inadequate for those who are unfamiliar with the technique. Apart from this defect I found this book gave a useful review of an extensive subject.

The format and printing of the book are very clear and it appears to be free from typographical errors. G. M. S. DUFF

RECOMMENDED METHODS OF SAMPLING ALUMINIUM SCRAP. By the ORGANISATION OF EUROPEAN ALUMINIUM SMELTERS. Pp. vii + 43. London: Organisation of European Aluminium Smelters. 1968. (*Gratis* from OEA, 3 Albemarle Street, London, W.1.)

The segregation of miscellaneous scrap metal is a difficult problem, and any information that minimises this task, and the expense, is welcomed.

In this small publication, which is also available in French and German, the emphasis is not on "spot-testing," as might have been expected for the aluminium industry, but on the provision of a representative (melted) sample for subsequent chemical or spectrographic analysis.

The recommendations are approved by the Technical Committee of the Organisation of European Aluminium Smelters, and aim to give general guidance in the sampling of aluminium and aluminium alloy waste and scrap. W. T. ELWELL

SPECTROSCOPIC TRICKS. Edited by LEOPOLD MAY. Pp. xiv + 333. London: Adam Hilger Ltd. 1968. Price 84s.

This book consists of a collection of "Tricks" and Notes, which appeared in the journal *Applied Spectroscopy* over the period from 1959 to 1965. The tricks consist of new devices and modifications to mainly commercially available instruments that have been invented by practising spectroscopists. For example, in one item a method for introducing powders into flames is described, and several others are concerned with sample cells and disc holders, useful in infrared spectroscopy. These are reproduced from the journal without further comment. The book is divided into eight sections applicable to different techniques, by far the longest of which are those devoted to emission spectroscopy and infrared spectroscopy. There are smaller sections on mass spectroscopy, X-ray spectroscopy and ultraviolet - visible spectroscopy, etc. It is perhaps anomalous to find atomic absorption in the emission-spectroscopy section.

The original publication of these tricks has been of considerable value to spectroscopists. Whether this collation at a later date, when some will already be in general use, is of the same value is questionable. Additionally, even allowing for the current high prices of technical literature, the price of this book, which contains no original material, still seems exorbitant. To someone who does not regularly read *Applied Spectroscopy*, however, the book represents an interesting collection of ideas, some of which were familiar and some not. JOHN M. OTTAWAY

DIE ANALYTISCHE CHEMIE IN DER ERGEUGENDEN UND VERARBEITENDEN HÜTTENINDUSTRIE. Edited by VEREIN DEUTSCHER EISENHÜTTENLEUTE. Pp. iv + 391. Dusseldorf: Verlag Stahleisen MbH. 1968. Price DM59.

This is a collection of nineteen papers, mostly by different authors, on various aspects of metallurgical analysis, presented at a symposium in Essen at the beginning of 1965. It is intended to supplement the four-volume "Handbuch für das Eisenhütten-Laboratorium," dealing with questions—basic theory, historical matters, new techniques—which do not fit into the framework of the Handbook.

As usual in a work of this kind, the essays vary widely in value and authority. I shall not attempt to comment on all of them. The first, by Horst Wünsch, on the application of statistics to chemical analysis, is a model of its kind, and the second, by three authors, on sampling in the iron and steel industry, confirms one's impressions that this is a problem that is tackled much more systematically on the continent than here. In the contribution by Hans-Wolfgang Nürnberg on polarography, on the other hand, there is a clear indication that Germany lags behind, and a high proportion of the literature references are to British work. Alfred Stetter's 10 pages on X-ray analysis give no literature references at all, and it is hard to see in what way this supplements the excellent treatment in Volume II of the Handbook (119 references).

Several essays deal with gas analysis, including one on non-ferrous metals, and there is some overlapping here. Dr. Koch attempts to compress his encyclopaedic knowledge of metal-phase identification into 17 pages, and is not helped by the poor quality of the reproduction of some of the photographs.

A noticeable omission from the essay on trace analysis, and others that might be expected to include it, is any mention of atomic-absorption spectrophotometry. G. M. HOLMES

CHEMICAL ENVIRONMENT IN THE AQUATIC HABITAT (Proceedings of an I.B.P. Symposium held in Amsterdam and Nieuwersluis 10–16 October 1966). Edited by H. L. GOLTERMAN and R. S. CLYMO. Pp. xii + 322. Amsterdam: North-Holland Publishing Company. 1967. Price 70s.

An unusual book for an analytical chemist to be reviewing one might think at first glance—yes indeed, but one dealing with a topic that is rapidly becoming of increasing importance to a great number of analytical chemists. The reviewer is not qualified to comment upon the chemical oceanography and limnology of the subject, but these subjects are built upon the answer to "How much?" That is the work of analytical chemists. Indeed the opening paper cuts the analytical chemist down to size by regarding him as a high grade technician. However, by considering the problems that are given or discussed during this symposium, we can see the reason for this, as one of the tasks of the meeting was to produce a manual for the non-existent "routine" analyses that are needed to help the biologist solve his difficulties.

The papers are of four main types and are conventionally catalogued as the problem of organic substances, their chemical determination and biological fate; the availability of phosphorus, silica and iron in water; problems in the interaction of water and bottom sediments; and technical papers reviewing old and proposed new methods of analysis.

It may be a broad general statement but it seems that the analytical chemist with all the power of his arsenal is needed desperately in the first two categories: in the third possibly not so much, but in the fourth he is very much at home.

As the book is the proceedings of a conference, detailed criticism of individual papers would be out of place in this review. However, there are several important papers concerned with the determination of orthophosphate in water, total carbon analysis in water pollution control, automatic methods for the analysis of natural waters, etc.

This book is not one for the analytical chemist who wants a ready reference to solve his problems, but as a fascinating and stimulating book on what analytical chemists will probably be doing in the near future it will handsomely repay reading. G. NICKLESS

Summaries of Papers in this Issue

Determination of Trace Amounts of Cobalt in Alumina by Atomic-absorption Spectroscopy

After the dissolution of alumina by hydrochloric acid in a sealed tube at 270° C traces of cobalt are determined by measurement of atomic absorption at 240·7 nm. In one of the two procedures described cobalt, in the range 50 to 250 p.p.m. in alumina, is determined by aspiration directly into an air - acetylene or nitrous oxide - acetylene flame, and in the other procedure cobalt, in the range 10 to 100 p.p.m. in alumina, is determined by co-precipitation on hydrated manganese dioxide, followed by extraction into isobutyl methyl ketone as its 8-hydroxyquinoline complex. The extract is sprayed into an air - propane flame and absorbance measurements are made as before. No interferences were found from the other elements likely to be present in alumina.

The nitrous oxide - acetylene flame is less favourable than air - acetylene for the determination of cobalt because of the unfavourable effect of the strongly reducing cyanogen zone of the flame and because of considerable loss of atomic cobalt caused by ionisation.

B. FLEET, K. V. LIBERTY and T. S. WEST

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

Analyst, 1968, **93**, 701–708.

The Determination of Silicon by Atomic-absorption Spectrophotometry, with Particular Reference to Steel, Cast Iron, Aluminium Alloys and Cement

The determination of silicon by atomic-absorption spectrophotometry in silicon-containing and siliceous materials has been investigated. Means are outlined by which many types of sample can be completely dissolved without loss of silicon, thus avoiding the need for fusion. Full operating details are given, and the results, accuracy and sensitivity of the method are discussed.

W. J. PRICE and J. T. H. ROOS

Pye Unicam Ltd., York Street, Cambridge.

Analyst, 1968, **93**, 709–714.

Determination of Sodium-22 in Rain Water by Using a Low Level β-Counter

A method has been developed for the separation from rain water of sodium-22, sufficiently radiochemically pure for it to be measured by low level β-counting. A 50-litre sample of rain water is evaporated to about 1 litre and the concentrate passed through a cation-exchange column. The sodium is eluted with 0·7 N hydrochloric acid solution. The sodium fraction is then purified, precipitated with α-methoxyphenylacetic acid, converted into sodium chloride and counted in a low background anti-coincidence β-counter.

B. A. BURDEN

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

Analyst, 1968, **93**, 715–719.

An Investigation of the Degraded Neutron Flux in a 14-MeV Neutron-activation Cell

Measurements have been made of the fluxes of thermal and indium resonance neutrons near the target of a 14-MeV neutron generator in a small irradiation cell. These measurements can be used to assess the importance of interfering reactions in fast-neutron activation analysis. For a fast-neutron output of 10^9 neutrons per second the measured sub-cadmium flux was $3{\cdot}8 \times 10^4$ neutrons per cm^2 per second, and the flux at the 1·4-eV indium resonance was $1{\cdot}3 \times 10^3$ neutrons per cm^2 per second per eV.

P. E. FRANCOIS

Department of Physics, University of Aston in Birmingham, Gosta Green, Birmingham 4.

Analyst, 1968, **93**, 720–721.

The Micro Determination of Calcium in Mammalian Hard Tissues

A spectrophotometric method is described for the determination of microgram amounts of calcium. The procedure is extremely rapid and simple, enabling amounts of calcium in the range 2 to 3·5 μg per ml to be determined with an accuracy of about ± 1 per cent. (standard deviation). The method, based on the ability of calcein (fluorescein-3,3′-bismethyliminodiacetic acid) to complex with calcium ion, measures the reduction in light absorption of a calcein solution at 506 nm on the addition of calcium.

C. ROBINSON and J. A. WEATHERELL

Biological Research Unit, Dental School and Hospital, University of Leeds.

Analyst, 1968, **93**, 722–728.

Determination of Hydrofluoric Acid in Inhibited Red Fuming Nitric Acid

A simple method has been devised for directly measuring fluoride-ion concentrations in inhibited red fuming nitric acid, which is commonly used in propellents. The technique involves the use of a newly developed fluoride-sensitive electrode, and the concentrations are determined by measuring the potential developed between this electrode and a reference electrode. A special procedure, involving a buffer solution, was devised for weighing the inhibited nitric acid before neutralisation, dilution and measurement. The results are more accurate than those obtainable by the commonly used, and more complex, indirect methods. For the particular instance of inhibited red fuming nitric acid, the technique also proved superior to another proposed direct method in that it is effective with samples as small as 1 g.

E. F. CROOMES and R. C. McNUTT

Army Propulsion Laboratory and Center, Research and Development Directorate, U.S. Army Missile Command, Redstone Arsenal, Alabama 35809.

Analyst, 1968, **93**, 729–731.

Determination of the Total Silicon Content of Water

A method is described for the determination of total silicon content of water based on evaporation, fusion and subsequent absorptiometric measurement of solutions of reduced β-molybdosilicic acid. The standard deviation for this procedure, by using samples of between 20 and 60 ml, was found to be 0·1 μg of silicon as silica.

P. M. BAKER and B. R. FARRANT

Analytical Laboratory, The Permutit Co. Ltd., Chiswick, London, W.4.

Analyst, 1968, **93**, 732–736.

Screening for Alkaloids in Toxicology by Using Thin-layer Chromatography: A Rapid System Simulating Paper Chromatography

Although numerous thin-layer chromatographic systems for the separation of alkaloids have been reported, none of them compares in abundance of recorded R_F values with the citrate paper system of Curry and Powell. In order to overcome the slowness (which can be a serious disadvantage with this paper system in diagnosis of acute poisoning) without recourse to a new thin-layer chromatographic system and consequent sacrifice of results, a system has been developed that gives R_F values which, for practical purposes, are substantially the same as those in the paper system, for the limited range of drugs examined and for their extracts from horse urine. It is unlikely that the correlation is due to chance, and it is considered, therefore, that the more rapid thin-layer chromatographic system can be substituted for the paper system.

P. E. HAYWOOD and M. S. MOSS

Forensic Laboratory, Equine Research Station, Newmarket, Suffolk.

Analyst, 1968, **93**, 737–739.

Extraction Procedures in Chemical Toxicology

Investigations into the separation by distillation, solvent extraction and the use of ion-exchange resins of drugs from biological materials, with particular reference to urine, are described and discussed.

S. L. TOMPSETT

Department of Clinical Chemistry, University of Edinburgh, Royal Infirmary, Edinburgh 3.

Analyst, 1968, **93**, 740–748.

The Chemical Assay of Cascara Dry Extract, Cascara Tablets and Cascara Bark

Report prepared by the Joint Committee of the Pharmaceutical Society and the Society for Analytical Chemistry on Methods for the Evaluation of Drugs.

Joint Committee of the PHARMACEUTICAL SOCIETY and the SOCIETY FOR ANALYTICAL CHEMISTRY

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

Analyst, 1968, **93**, 749–755.

The Determination of Dimethoate Residues in Fruits and Vegetables

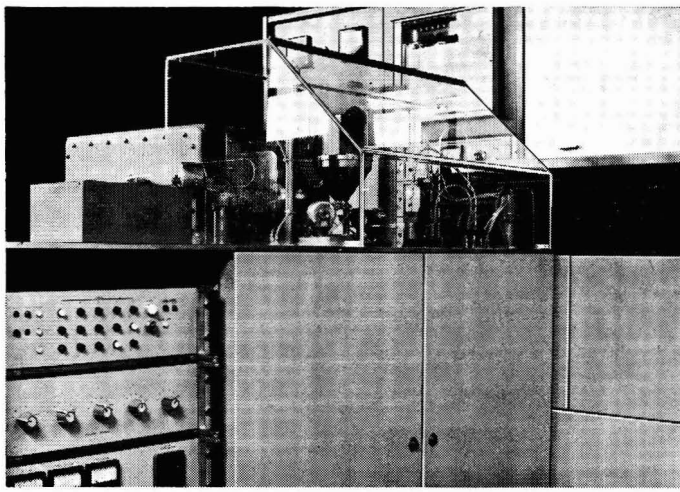
Report prepared by the Joint Dimethoate Residues Panel.

Panel set up jointly by the Scientific Sub-Committee on Poisonous Substances Used in Agriculture and Food Storage, the ANALYTICAL METHODS COMMITTEE of the SOCIETY FOR ANALYTICAL CHEMISTRY and the ASSOCIATION OF BRITISH MANUFACTURERS OF AGRICULTURAL CHEMICALS

N. A. SMART, *Secretary of the Panel*:

Ministry of Agriculture, Fisheries and Food, Plant Pathology Laboratory, Hatching Green, Harpenden, Herts.

Analyst, 1968, **93**, 756–766.



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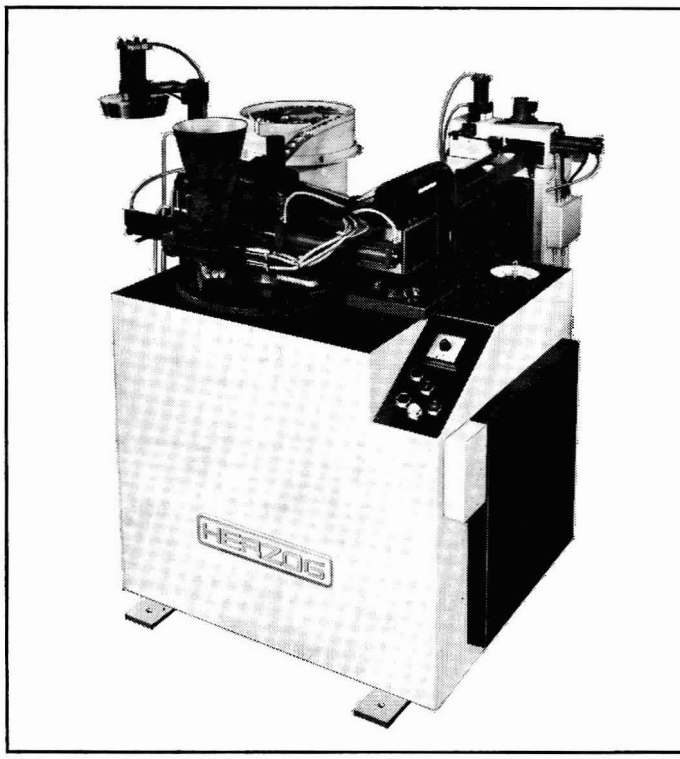

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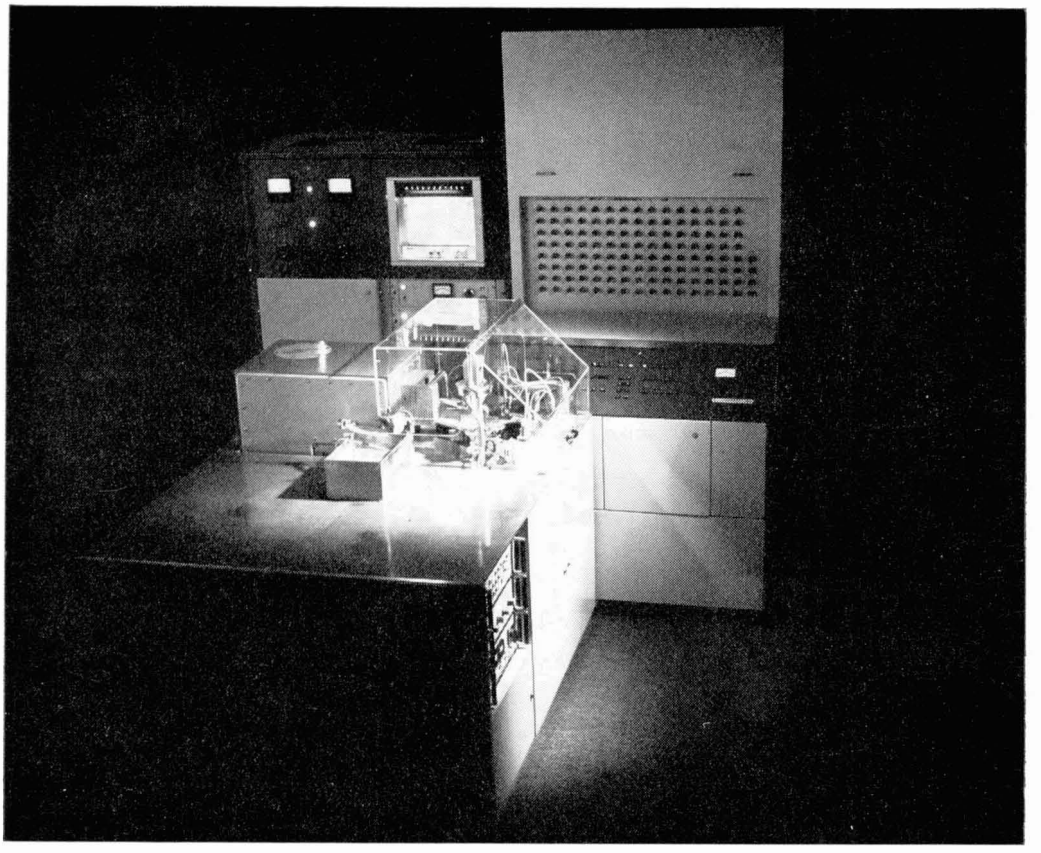

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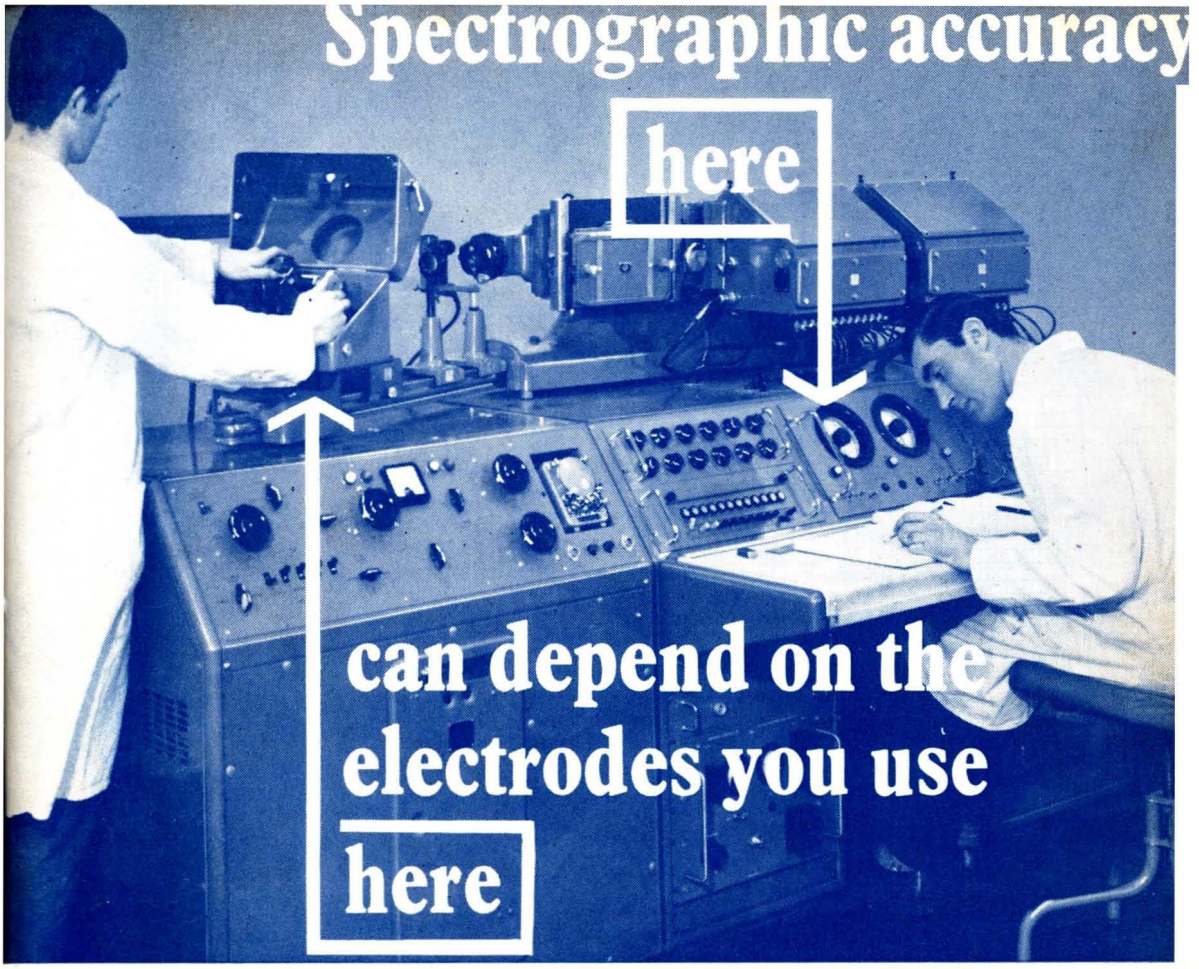

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