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Summaries of Papers in this Issue

The Determination of Trace Amounts of Aluminium and Other Elements in Iron and Steel by Atomic-absorption Spectrometry with Carbon Furnace Atomisation

A simple and rapid method is described for the determination of 0·0002–0·01 per cent. of acid-soluble aluminium and 0·0005–0·005 per cent. of acid-insoluble aluminium in iron and steel by using atomic-absorption spectrometry with carbon furnace atomisation. Samples are dissolved in nitric acid and analysed directly without pre-concentration. An initial investigation into the possibility of determining zinc, magnesium, manganese, vanadium, nickel and bismuth at the parts per million level in steel by a similar technique is also described and interferences are discussed.

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Analyst, 1975, **100**, 217–228.

A Kinetic Theory of Atomisation for Non-flame Atomic-absorption Spectrometry with a Graphite Furnace Part II. Analytical Applications of Kinetic Information for Copper

Kinetic information for the atomisation of copper in a heated graphite furnace has been used to derive analytical parameters that are encountered in atomic-absorption determinations. Equations have been derived to describe quantitatively pre-atomisation heating losses, the advantages to be gained from operating under stopped gas flow conditions and the relative merits of signal peak height and signal integration measurements.

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Analyst, 1975, **100**, 229–233.

Atomic-absorption Spectrophotometric Determination of Lead in Beverages and Fruit Juices and of Lead Extracted by Their Action on Glazed Ceramic Surfaces

A rapid procedure for the determination of lead in beverages and fruit juices by directly aspirating them into an atomic-absorption spectrophotometer is described. Concentrations of lead down to 0·1 p.p.m. can easily be determined. The standard deviation in the range 0·5–2·0 p.p.m. of lead is about 0·04 p.p.m. The accuracy of the method is good; although its sensitivity is slightly lower than that with methods in which condensation procedures are used, the proposed method is simpler and more rapid. Interferences caused by the different matrices of the samples are negligible in most instances investigated.

The method has been applied to samples of beverages and fruit juices that had been kept in glazed ceramic cups for 0·5 h in an attempt to compare the amount of lead extracted by these products with that extracted by 4 per cent. acetic acid solution, the reagent used for testing ceramic ware for extractable lead.

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Analyst, 1975, **100**, 234–237.

Colorimetric Determination of Vitamin D in Some Oily Pharmaceutical Preparations

A colorimetric method for the determination of vitamin D, based upon the use of anisaldehyde - sulphuric acid as a colour reagent, has been developed. The method enables 0·1–0·5 mg of calciferol to be determined with a mean percentage recovery of 100·2 ± 1·44 per cent., and it avoids the difficulties met with in the antimony(III) chloride colour reaction.

Both methods, as applied to oily injections of vitamin D, are compared, and a statistical analysis of the results reveals that the proposed method is the more precise, and has an accuracy equal to that of the antimony(III) chloride method.

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Analyst, 1975, **100**, 238–242.

A Thin-layer Chromatographic Test for the Identification of Some Drugs: Its Application to Steroids, Tetracyclines, Penicillins and Cephalosporins

The identity test described is based on the thin-layer chromatographic separation of the complex mixtures of substances formed by thermal degradation of small amounts of many organic substances applied as spots to thin-layer plates. Subsequent development of the plate, and the application of spray reagents when necessary, yields characteristic patterns that are suitable for comparison under 254- and 366-nm ultraviolet light with authentic specimens that have been similarly treated. The test has been found to give good discrimination within the groups of steroids, and the tetracycline, penicillin and cephalosporin antibiotics described in the European and British Pharmacopoeias. For the cephalosporin group, separation of the thermal degradation products by paper electrophoresis is an alternative that gives slightly better results.

The technique is flexible in that there is a wide choice of thin-layer chromatographic support media, heating conditions, developing solvents and spray reagents, which facilitates its application to different classes of compounds.

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Analyst, 1975, **100**, 243–248.

Fungicide Residues
Part IV. Determination of Residues of Carboxin in Grain by Gas Chromatography

A simple method for determining residues of carboxin in grain is presented. The carboxin is extracted from the sample with acetone in a Soxhlet extraction apparatus and, after concentration of the extract, is determined by gas - liquid chromatography using a nitrogen-selective detector. The presence of carboxin is confirmed by the use of a sulphur flame-photometric detector.

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Analyst, 1975, **100**, 249–252.

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The Determination of Trace Amounts of Aluminium and Other Elements in Iron and Steel by Atomic-absorption Spectrometry with Carbon Furnace Atomisation

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A simple and rapid method is described for the determination of 0·0002–0·01 per cent. of acid-soluble aluminium and 0·0005–0·005 per cent. of acid-insoluble aluminium in iron and steel by using atomic-absorption spectrometry with carbon furnace atomisation. Samples are dissolved in nitric acid and analysed directly without pre-concentration. An initial investigation into the possibility of determining zinc, magnesium, manganese, vanadium, nickel and bismuth at the parts per million level in steel by a similar technique is also described and interferences are discussed.

Aluminium is present in cast iron and steel in several forms, which can be considered to be of two types according to their solubility in boiling mineral acid. Those forms which are either soluble or partially soluble in acid at normal pressures, such as elemental aluminium, are known as soluble aluminium, and other forms, including corundum (α-aluminium oxide) and aluminium combined with silica, are known as insoluble aluminium. The values obtained for soluble aluminium could be expected to vary with variations in dissolution procedure, type of acid, length of dissolution time, etc., but, within reasonable limits, acceptable agreement can be obtained from several different procedures. Agreement of results for insoluble aluminium is generally poorer owing to the lack of sensitivity of the conventional methods used for this determination. Aluminium is added to steel as an oxygen scavenger and the presence of excess of elemental aluminium indicates that sufficient has been added. Most of the aluminium oxide formed is removed in the slag but residual amounts are retained in the metal.

Small amounts of this element can have marked effects on the properties of both cast iron and steel. In cast iron, aluminium is important with regard to pinholing,[1] inoculation[2] and annealability,[3–5] whereas in steel it affects the yield point and ageing properties. There is, therefore, a well established need for analysis for both soluble and insoluble (or total) aluminium in cast iron and steel and methods involving emission spectrography, polarography, spectrophotometry and flame atomic-absorption spectrometry have been developed to fulfil this need. In many instances such methods require use of high sample masses (in order to determine the lowest concentration of aluminium found in cast iron and steel), followed by separation of the iron matrix prior to analysis. Physical methods, such as emission spectrography, are subject to matrix interferences and regular calibration against samples of known aluminium content and metallurgical history is required.

Probably the method used most frequently for the determination of soluble and total aluminium in cast iron and steel is that developed by Scholes and Smith,[6] which involves dissolution of the sample in dilute sulphuric acid, removal of the iron electrolytically and determination of the aluminium colorimetrically by using Eriochrome cyanine. The lowest concentration of aluminium at which this method can be applied is about 0·001 per cent.

More recently, several flame atomic-absorption spectrometric methods have been developed. These methods include a procedure[7] developed by the Strip Mills Divisional Chemical Analysis Committee of the British Steel Corporation by which small amounts of soluble and insoluble aluminium in high-silicon steels are determined. For soluble aluminium, a sample mass

(10 g) is dissolved in hydrochloric acid - nitric acid, the iron is removed by extraction into amyl acetate and the aluminium is determined by using a nitrous oxide - acetylene flame. For insoluble aluminium, 10 g of sample are dissolved in 10 per cent. sulphuric acid, the solution is filtered and the residue is treated with hydrofluoric acid - sulphuric acid and then fused with a sodium carbonate - borax mixture. The method is time consuming and the final concentrations of the solutions are very high, which leads to frequent blockage of the burner.

Headridge and Sowerbutts[8] have reported a flame atomic-absorption method for the determination of total aluminium in iron and steel within the range 0·001–0·14 per cent., which involves dissolution of the iron or steel in an open beaker and subsequent treatment in a PTFE-lined bomb at 200 °C. Iron is then extracted from the solution into isobutyl methyl ketone and the aluminium is pre-concentrated by extraction into acetylacetone. The final measurement is made in a nitrous oxide - acetylene flame.

Consideration of the methods mentioned indicates that the major problems in the determination of aluminium have been due to interferences (mainly from iron) and lack of sensitivity. The solution of these problems has led to lengthy procedures, which suffer from lack of precision owing to contamination and operator error. Methods with sufficient sensitivity and freedom from interference to permit direct determination of trace elements would therefore appear to be preferable. A direct-injection technique involving a nitrous oxide - acetylene flame has been proposed and with this technique it is possible to determine aluminium at the 0·001 per cent. concentration level.[9] Fernandez and Manning[10] have shown that carbon furnace atomisation improves the detection limit for most elements by a factor of 50 and for aluminium by a factor of 30 compared with flame atomisation, and we have recently reported[11–13] methods for the determination of lead in cast iron, steels,[11] copper and copper alloys[12] and rocks,[13] in which carbon furnace atomisation is used in order to obtain improved sensitivity. The development of a similar technique, reported in this paper, for the determination of 0·0002–0·01 per cent. of soluble aluminium and 0·0005–0·005 per cent. of insoluble aluminium in cast iron and steel allows analysis in this range to be accomplished without separation of the matrix or pre-concentration of the sample solution. The possibility of determining other trace elements in steel is also discussed on the basis of preliminary investigations into sensitivity and interference from iron.

Materials

Reagents

Reagents of the highest available purity were used throughout and solutions were prepared with distilled water obtained from a quartz still.

Stock aluminium solution, 100 p.p.m. of aluminium. Dissolve 0·1 g of analytical-reagent grade aluminium powder in 5 ml of AnalaR concentrated nitric acid, transfer the solution to a 1-l calibrated flask and dilute to the mark with water.

Fusion mixture. Mix 2 parts of AnalaR sodium carbonate with 1 part of AnalaR sodium tetraborate.

Stock manganese solution, 100 p.p.m. of manganese. Dissolve 0·405 g of analytical-reagent grade manganese(II) sulphate in 5 ml of 40 per cent. nitric acid (AnalaR) and dilute to 1 l with water.

Stock bismuth solution, 100 p.p.m. of bismuth. Dissolve 0·232 g of analytical-reagent grade bismuth(III) nitrate in 5 ml of 40 per cent. nitric acid (AnalaR) and dilute to 1 l with water.

Stock lead solution, 100 p.p.m. of lead. Dissolve 0·16 g of analytical-reagent grade lead nitrate in 5 ml of 40 per cent. nitric acid (AnalaR) and dilute to 1 l with water.

Stock magnesium solution, 100 p.p.m. of magnesium. Dissolve 1·015 g of analytical-reagent grade magnesium sulphate in 5 ml of 40 per cent. nitric acid (AnalaR) and dilute to 1 l with water.

Stock zinc solution, 100 p.p.m. of zinc. Dissolve 0·1 g of analytical-reagent grade zinc powder in 5 ml of 40 per cent. nitric acid (AnalaR) and dilute to 1 l with water.

Stock vanadium solution, 100 p.p.m. of vanadium. Dissolve 0·13 g of analytical-reagent grade ammonium metavanadate in 5 ml of 40 per cent. nitric acid (AnalaR) and dilute to 1 l with water.

Stock nickel solution, 100 p.p.m. of nickel. Dissolve 0·495 g of analytical-reagent grade nickel nitrate in 5 ml of 40 per cent. nitric acid (AnalaR) and dilute to 1 l with water.

Stock iron solution, 10 000 p.p.m. of iron. For interference studies. Dissolve 0·5 g of Specpure iron in 10 ml of 40 per cent. nitric acid (AnalaR), transfer the solution to a 50-ml calibrated flask and dilute to the mark with water.

Apparatus

The instrument used for all measurements was the Perkin-Elmer, Model 306, atomic-absorption spectrometer equipped with an HGA-72 heated-graphite atomiser and deuterium arc background corrector and coupled to an Electronik 194 strip-chart recorder. Perkin-Elmer Intensitron hollow-cathode lamps were used as sources. The design and operation of the HGA-72 are similar to those of the HGA-70 and have been described in detail elsewhere.[10,14] Samples were transferred to the centre of the carbon tube by means of 20- or 50-μl Eppendorf pipettes. The argon flow was 1·5 l min^{-1} at 40 lb in^{-2}.

Procedures for the Determination of Aluminium in Iron and Steel

In all procedures the aluminium line at 309·3 nm was used. The solution was dried for 40 s at setting 35 (100 °C), charred for 50 s at setting 350 (1660 °C) and atomised for 10 s at setting 999 (2660 °C). A lamp current of 25 mA and a band pass of 0·7 nm were used.

1. Soluble Aluminium

1.1. *Samples containing* 0·0002–0·0020 *per cent. of soluble aluminium*

1.1.1. *Preparation of calibration solutions.* Dilute 10 ml of stock aluminium solution to 1 l with water. This solution should be freshly prepared every day. Transfer 0, 2·0, 4·0, 6·0, 8·0 and 10·0 ml of this solution into 100-ml PTFE beakers each of which contains 0·5 g of Specpure iron and add 10 ml of 40 per cent. nitric acid. When the iron has dissolved, filter each solution through a Whatman No. 542 filter-paper that has previously been washed with acid and allow the filtrate to run into a 50-ml calibrated flask. Wash the filter-paper thoroughly with water, then dilute the solution in the flask to the mark with water. These solutions contain the equivalent of 0, 0·0004, 0·0008, 0·0012, 0·0016 and 0·0020 per cent. of soluble aluminium relative to the 0·5 g of iron used in the preparation of 50 ml of solution.

1.1.2. *Preparation of sample solutions.* Weigh 0·5 g of sample into a 100-ml PTFE beaker and dissolve in 10 ml of 40 per cent. nitric acid. Filter the solution through an acid-washed Whatman No. 542 filter-paper into a 50-ml calibrated flask, wash the paper thoroughly with water and dilute the solution in the flask to the mark with water.

1.1.3. *Instrument operation.* Sequentially inject aliquots of standard and sample into the graphite tube with a 50-μl pipette. Use a ×3 scale expansion on the recorder and the conditions given above under Apparatus. Derive the concentration of aluminium in the samples by interpolation from a calibration graph obtained from the standards.

1.2. *Samples containing more than* 0·0020 *per cent. of soluble aluminium*

For samples containing between 0·0020 and 0·0050 per cent. of aluminium proceed according to Procedure 1.1, using the calibration solutions necessary for the concentration range. Use a 20-μl pipette, ×1 scale expansion and the conditions given above.

For samples containing 0·0050–0·0080 per cent. of soluble aluminium proceed according to Procedure 1.1, using the calibration solutions necessary. Dilute the samples and standards by a factor of two prior to analysis. Use a 20-μl pipette and a ×1 scale expansion.

2. Insoluble Aluminium

2.1. *Samples containing* 0·0005–0·0020 *per cent. of insoluble aluminium*

2.1.1. *Preparation of calibration solutions.* Dilute 10 ml of stock aluminium solution to 1 l with water. Transfer 0, 2·5, 5·0, 7·5 and 10·0 ml of this solution into 50-ml calibrated flasks each of which contains 0·3 g of fusion mixture and 5 ml of 40 per cent. nitric acid. Dilute to the mark with water. These solutions contain the equivalent of 0, 0·0005, 0·0010, 0·0015 and 0·0020 per cent. of insoluble aluminium when 0·5 g of sample is used to prepare 50 ml of solution.

2.1.2. *Preparation of sample solution.* Transfer the filter-paper and residue (Procedure 1.1.2) to a 30-ml capacity platinum crucible and place the crucible at the door of a muffle furnace (temperature, 1400 °C) until all of the paper has dried. Push the crucible inside the

furnace and allow the paper to burn off for 15 min at 1400 °C. Add 0·3 g of fusion mixture and ignite in the furnace until a clear melt is obtained, taking care to ensure that all the residue is incorporated into the melt. Remove from the furnace, add 5 ml of 40 per cent. nitric acid and 5 ml of water and heat gently on a hot-plate in order to dissolve the salts. Finally, transfer the solution to a 50-ml calibrated flask and dilute to the mark with water. Carry out a parallel blank determination by transferring an acid-washed filter-paper to a platinum crucible and repeating the procedure.

2.1.3. *Instrument operation.* Proceed according to Procedure 1.1.3, using the same conditions.

2.2. *Samples containing* 0·0020–0·0050 *per cent. of insoluble aluminium*

2.2.1. *Preparation of calibration solutions.* Dilute 10 ml of stock aluminium solution to 100 ml with water. This solution should be freshly prepared every day. Transfer 0, 1·0, 1·5, 2·0 and 2·5 ml of this solution to a 50-ml calibrated flask containing 0·3 g of fusion mixture and 5 ml of 40 per cent. nitric acid. Dilute to the mark with distilled water. These solutions contain the equivalent of 0, 0·0020, 0·0030, 0·0040 and 0·0050 per cent. of insoluble aluminium when 0·5 g of sample is used to prepare 50 ml of solution.

2.2.2. *Preparation of sample solutions.* Proceed according to Procedure 2.1.2.

2.2.3. *Instrument operation.* Proceed according to Procedure 1.1.3 using a 20-μl pipette and a $\times 1$ scale expansion.

Investigations into the Determination of Other Elements in Iron and Steel and Possible Interference Effects

Bismuth. Dilute 1 ml of stock bismuth solution (100 p.p.m.) to 100 ml with water. Transfer 0 and 5 ml of this solution to 100-ml PTFE beakers each of which contains 0·5 g of Specpure iron and 10 ml of 40 per cent. nitric acid. When the iron has dissolved transfer the solution to a 50-ml calibrated flask and dilute to the mark with water. Use the conditions given in Table V.

Manganese, magnesium, zinc, lead, nickel, vanadium and aluminium. Prepare solutions as for bismuth using concentrations and conditions given in Table V.

Results and Discussion

Determination of Aluminium in Iron and Steel

Interference studies

It has been reported[11] that interferences due to molecular volatilisation of the analyte occur in the determination of lead, for which the HGA-70 carbon furnace is used, when the sample solutions are prepared in a chloride medium. Tests were carried out in order to discover if this effect also occurred with aluminium.

When a solution in distilled water of 0·1 p.p.m. of aluminium as chloride was injected into the graphite tube a reproducible signal was obtained (± 3 per cent.). When the same concentration of aluminium was present in 8 per cent. hydrochloric acid containing 10 000 p.p.m. of iron an erratic signal (± 30 per cent.) was obtained from which it was impossible to measure the aluminium signal. When the absorbance was measured at 256·8 nm (a wavelength of about one twelfth the sensitivity of the normal aluminium wavelength, 309·3 nm), a signal identical with that at 309·3 nm was obtained. An 8 per cent. solution of hydrochloric acid with 10 000 p.p.m. of iron that contained no aluminium gave the same erratic signal as a similar solution that also contained 0·1 p.p.m. of aluminium. Neither signal is therefore due to aluminium atoms. These measurements were carried out by using the deuterium arc background corrector and the signal obtained from both the aluminium plus iron solution and the pure iron solution was that for which the corrector could not completely compensate. It is apparent that in a strong chloride matrix two effects operate. The atomic-absorption signal for aluminium cannot be detected because, presumably, the aluminium is volatilised as the molecular chloride and is not subsequently atomised. An erratic signal is obtained during atomisation in the carbon furnace due to either absorption or scatter by smoke and/or molecular chlorides. It is not therefore feasible to analyse steels for aluminium by using a hydrochloric acid dissolution procedure.

In the determination of lead in steel and cast iron[11] no interference from the matrix was observed, provided that the samples were dissolved in oxy-acids such as nitric or perchloric acid. It has been postulated that the salts formed on drying such solutions are converted to oxides, which are then reduced to metal atoms by the graphite tube as the temperature rises.[15] Molecular volatilisation that occurs from chloride solutions is thus avoided. The interference from the iron matrix in nitrate media in the determination of aluminium was examined during atomisation [at 999 units (2660 °C) for 10 s] of a 50-μl solution containing 0·1 p.p.m. of aluminium and 10 000 p.p.m. of iron in 8 per cent. nitric acid. The results are shown in Table I. The values are given in each instance after the subtraction of any "blank" value. The blank from the 8 per cent. nitric acid solution was negligible, whereas that from the solution containing 8 per cent. of nitric acid and 10 000 p.p.m. of iron was 8 chart divisions or about 25 per cent. of the signal for 0·1 p.p.m. of aluminium. When no charring step was used addition of 8 per cent. nitric acid caused a depression of the signal of about 45 per cent. compared with that obtained for aluminium nitrate dissolved in distilled water, whereas with 8 per cent. nitric acid and 10 000 p.p.m. of iron the depression was reduced to 6 per cent., which is considered to be negligible. If, after drying, the solutions are charred at 1660 °C for 50 s, 8 per cent. nitric acid then causes a depression of 33 per cent. over the distilled water solution but the signal for the solution containing nitric acid plus 10 000 p.p.m. of iron is no different. Addition of nitric acid results in a depression of the signal over that obtained for the pure solution but the iron plus nitric acid has no effect. The iron acts as a releasing agent for the depression caused by nitric acid. It is therefore possible to analyse iron and steel for aluminium by direct dissolution in nitric acid but a charring step must be used.

It is important to note that charring of the solution results in an enhancement of the atomisation signal. It seems unlikely that the depressive effect of nitric acid occurs in the vapour phase. It is reasonable to suppose that when the acidic solution is charred at 1660 °C for 50 s, all of the nitric acid will be driven off and the absorption signals in the presence and the absence of nitric acid should then be the same if the interference occurred in the vapour phase. Some chemical change in the solid phase on charring must therefore be responsible for the increased signal.

TABLE I

EFFECT OF NITRIC ACID AND IRON ON THE ATOMISATION SIGNAL FROM 50 μl OF A 0·1 p.p.m. ALUMINIUM SOLUTION

Charring conditions: 50 s at 1660 °C.

Solution	Signal, chart divisions: No charring	With charring
$Al(NO_3)_3$ in distilled water	30	39
$Al(NO_3)_3$ in 8 per cent. nitric acid	16	26
$Al(NO_3)_3$ and 10 000 p.p.m. of iron in 8 per cent. nitric acid ..	28	38

The 10 000 p.p.m. of iron gave a small background signal at the aluminium wavelength even when the background corrector was used. No matter how carefully the deuterium arc background corrector was adjusted this small residual signal from the iron remained. This could have been due to aluminium impurity in the Specpure iron used to prepare the solutions or to a small signal from the smoke produced for which the corrector could not completely compensate. When the aluminium line at 256·8 nm was used, which is one twelfth as sensitive as the normal line at 309·3 nm, the signal from the iron remained the same as that at 309·3 nm, indicating that it was due to smoke and not to aluminium impurity. This can be understood by reference to Fig. 1, which shows the atomisation signal for aluminium and iron background as a function of time during atomisation. The smaller signal (A) is the signal from the iron matrix recorded using the background corrector, *i.e.*, that signal for which the deuterium arc cannot completely compensate. The signal (B) is that from the smoke recorded without the background corrector. It can be seen that the signal from the aluminium (C) is coincident with the corrected background signal and therefore when aluminium is atomised from a matrix consisting of 10 000 p.p.m. of iron a small blank signal is superimposed on the atomisation signal and must be subtracted. This effect did not occur with lead,[11] which atomises before the production of the blank signal (Fig. 1).

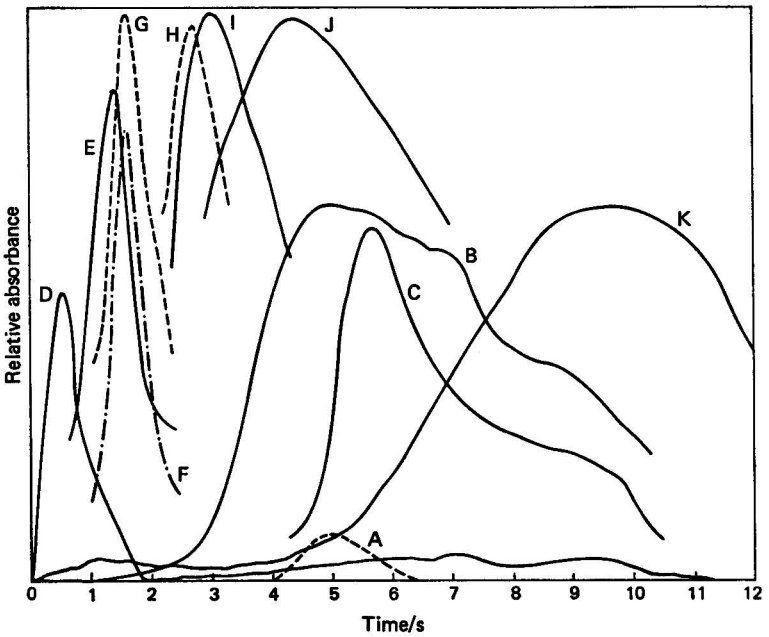

Fig. 1. Time dependence of absorption signals during atomisation cycle at 2470 °C. No charring step was used and the concentration of each element was varied in order to ensure that the recorder pen travelled approximately the same distance for each element. Signals measured at a chart speed of 2 cm s^{-1} with $\times 3$ scale expansion. Signals as follows: A, 10 000 p.p.m. of iron using background corrector; B, 10 000 p.p.m. of iron without background corrector; C, aluminium; D, nitric acid without background corrector; E, zinc; F, lead; G, bismuth; H, magnesium; I, manganese; J, nickel; and K, vanadium.

Results

The procedures described in the experimental section were applied to the determination of soluble and insoluble aluminium in iron and steels and the results are shown in Table II. The results shown are averages of three measurements on each separate solution and the individual results are for completely separate determinations. The determination of total aluminium was also carried out on BCS 317 and BCS 320 steel by adding the soluble and insoluble portions together, making up to 50 ml and injecting into the carbon furnace. Reasonably good agreement was obtained between the carbon furnace values and the certificate or other analytical values. As the values for total aluminium, obtained after combination of the two portions of soluble and insoluble aluminium, are in agreement with the standard values, this method can be used when total aluminium content only is required. The main advantage of the carbon furnace method is speed. Ten samples can be analysed for soluble and total aluminium in about 1·5 h, most of the time being taken up by the filtration. Samples containing amounts of aluminium that are higher than those covered in Table II could almost certainly be analysed by dilution of the sample solutions but this possibility has not been verified.

Reproducibility tests

(*a*) *The effect of charring on the determination of soluble aluminium.* In the determination of soluble aluminium, iron is present in the analytical solution whereas for insoluble aluminium no iron is present. With the type of background correction used the detector must distinguish between the smoke signal and the atomic-absorption signal from the aluminium. If the analyte signal occurs at the same time as the release of the bulk of the matrix, as is the case for aluminium and the iron matrix, then the reproducibility could be affected as the background corrector must then distinguish a small difference between two relatively large

TABLE II

DETERMINATION OF SOLUBLE AND INSOLUBLE ALUMINIUM (PER CENT.) IN IRON AND STEEL

	Results obtained by procedures described			Results obtained by other methods	
Sample	Soluble	Insoluble	Total (average)	Soluble	Total
BCS 317 high-silicon steel	0·000 34, 0·000 31, 0·000 45, 0·000 42, 0·000 50	0·0016, 0·0018, 0·0018, 0·0020, 0·0018	0·0022*	0·0003†	0·002†
Unisil high-silicon steel	0·000 55, 0·000 45, 0·000 60, 0·000 45, 0·000 55	0·0014, 0·0014, 0·0013, 0·0015, 0·0015	0·0019	0·0007†	0·0017
BCS 149/3 high-purity iron	0·000 47, 0·000 49, 0·000 50, 0·000 60, 0·000 47	0·000 70, 0·000 55, 0·000 58, 0·000 65, 0·000 70	0·0011	—	0·001–0·003 0·0033‡
BCS 260/3 high-purity iron	0·000 40, 0·000 40, 0·000 45, 0·000 47, 0·000 40	0·0010, 0·0012, 0·000 90, 0·0010, 0·0011	0·0015	—	0·001 0·0017‡
BCS 320 mild steel	0·0082, 0·0075, 0·0081, 0·0083, 0·0074	0·0052, 0·0043, 0·0056, 0·0046, 0·0052	0·013*	0·008	0·013
GKN 6 mild steel	0·000 27, 0·000 25, 0·000 29, 0·000 32, 0·000 43	0·0013, 0·000 85, 0·000 90, 0·0011, 0·0010	0·0013	—	0·002§
BCS 326 mild steel	0·0022, 0·0021, 0·0019, 0·0019, 0·0018	0·0020, 0·0017, 0·0019, 0·0021, 0·0015	0·0039	0·002–0·004	0·0050

* Analyses carried out after addition of soluble and insoluble portions gave values for the total aluminium content of a sample of BCS 317 of 0·0022 per cent. and 0·0021 per cent. and for BCS 320 of 0·0130 per cent. and 0·0135 per cent. (see text for explanation).

All results given for other methods are BCS standard values except:

† Results obtained by BSC study group method.[7]

‡ Results obtained by Headridge andd Sowerbutts.[8]

§ GKN standard value obtained using Eriochrome cyanine in a spectrophotometric method.

signals. If, however, some of the smoke is removed by charring at a temperature high enough to remove the iron matrix without significantly affecting the aluminium, then this may improve the reproducibility. This theory was tested by injecting a sample containing 0·1 p.p.m. of aluminium and 10 000 p.p.m. of iron into the carbon furnace and repeating the analysis ten times. This test was carried out with and without the introduction of a charring step and the results are shown in Table III. When charred for 50 s at 1660 °C

TABLE III

EFFECT OF CHARRING ON THE REPRODUCIBILITY OF THE SIGNALS FROM 0·1 p.p.m. OF ALUMINIUM IN THE PRESENCE OF 10 000 p.p.m. OF IRON

	Signal, chart divisions	
	No charring	With charring
	27	36
	21	40
	28	40
	22	34
	30	37
	22	39
	22	42
	29	37
	25	36
	28	31
Mean	25	37
Standard deviation	3·4	3·2
Relative standard deviation, per cent.	12·0	8·7

the relative standard deviation was 8·7 per cent. at a level equivalent to 0·0010 per cent. of aluminium in a steel and 12·0 per cent. at the same level when no charring step was used. Some of the iron is removed by charring, which improves the reproducibility.

(*b*) *Reproducibility of the analytical procedures.* The reproducibility was tested by carrying out ten complete analyses of each of two steel samples for both soluble and insoluble aluminium. The results are shown in Table IV.

TABLE IV

REPRODUCIBILITY TESTS ON THE DETERMINATION OF ALUMINIUM

	Instrument reproducibility, per cent. of aluminium BCS 320		Reproducibility of separate determinations, per cent. of aluminium			
			BCS 320		BCS 317	
	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble
	0·0074	0·0052	0·0075	0·0056	0·000 40	0·0020
	0·0074	0·0054	0·0080	0·0056	0·000 52	0·0016
	0·0082	0·0050	0·0085	0·0050	0·000 42	0·0015
	0·0082	0·0051	0·0085	0·0045	0·000 40	0·0016
	0·0075	0·0050	0·0075	0·0053	0·000 35	0·0014
	0·0074	0·0052	0·0070	0·0055	0·000 50	0·0015
	0·0074	0·0053	0·0070	0·0060	0·000 50	0·0017
	0·0084	0·0050	0·0084	0·0050	0·000 42	0·0018
	0·0084	0·0050	0·0080	0·0048	0·000 36	0·0014
	0·0080	0·0050	0·0074	0·0047	0·000 38	0·0015
Mean	0·0078	0·0051	0·0078	0·0052	0·000 43	0·0016
Certificate value* ..	0·008	0·005	0·008	0·005	0·0003	0·0017
Standard deviation ..	0·000 42	0·000 15	0·000 58	0·000 48	0·000 06	0·000 19
Relative standard deviation, per cent. ..	5·8	2·9	7·4	8·5	14·0	11·9
95 per cent. confidence limit, per cent. of aluminium	0·000 95	0·000 34	0·0012	0·0011	0·000 14	0·000 43

* Certificate values as given in Table II.

For soluble aluminium the relative standard deviation varies from 7·4 per cent. at the 0·0078 per cent. of aluminium level to 14·0 per cent. at the 0·00043 per cent. level. For insoluble aluminium the relative standard deviation varies from 8·5 per cent. at the 0·0052 per cent. level to 11·9 per cent. at the 0·0016 per cent. level. With soluble aluminium the major contribution to the standard deviation comes from the ability of the instrument to reproduce the signals during the atomisation stage and not from the dissolution procedure. This is demonstrated by ten separate analyses of a single solution. The relative standard deviation was 5·8 per cent. at the 0·0080 per cent. of aluminium level. This contribution is explained by consideration of the comments in section (*a*) of the discussion on reproducibility *i.e.*, it is due to the coincident release of the iron matrix. The contribution from the instrument to the standard deviation with insoluble aluminium is less than that from the sample preparation itself, the relative standard deviation of ten readings for one insoluble sample preparation being 2·9 per cent. at the 0·0050 per cent. level. The reproducibility of determinations of insoluble aluminium is not affected by background as no smoke is produced during the atomisation stage and therefore most of the contribution comes from the solution preparation. These results were all obtained using the HGA-72. It is likely that different instruments from the same or other manufacturers will give improved performance with regard to background correction, which could improve both the reproducibility and the detection limit of the method. The results in Table II indicate clearly the accuracy of the procedure that should be obtained with any carbon furnace atomiser.

The detection limit (2σ) and the sensitivity (1 per cent. absorption) for the lowest concentration range (Procedure 1, soluble aluminium) were found to be 0·000 12 and 0·000 049 per cent. of aluminium, respectively.

Determination of Other Elements in Iron and Steel

The importance of aluminium in iron and steel has already been discussed. Bismuth and lead are residual metals in steel, trace amounts being absorbed from the steelmaking materials. The problems associated with the presence of bismuth are similar to those associated with lead[11] but the effect can be more disastrous. A BISRA committee[16] found that as little as 0·0045 per cent. of bismuth in stainless steel reduced the ductility to zero.

Other elements such as zinc and magnesium are generally removed from the steel during its preparation simply because these metals are volatile and do not withstand the rigours of the steel-producing process. Trace amounts of these elements occasionally remain in the steel but very little is known about their effects on the quality of the steel. Any useful method of investigation of these effects can be facilitated by an accurate and rapid method of chemical analysis.

Having developed methods for the determination of trace amounts of lead[11] and aluminium in iron and steel by using carbon furnace atomic-absorption spectrometry, the use of this technique of trace analysis for other elements in steel was investigated. The optimum conditions for the analysis for some elements typically found in iron and steel are given in Table V and, as a typical analysis would involve the dissolution of the sample in nitric acid

TABLE V

DETERMINATION OF SOME ELEMENTS IN IRON AND STEEL

		Optimum conditions						
					Drying		Atomisation	
Element	Concentration of element, p.p.m.	Volume used/μl	Scale expansion	Wavelength/nm	Temperature/°C	Time/s	Temperature/°C	Time/s
Bismuth ..	0·1	50	×3	223·1	100	50	2230	10
Lead	0·1	50	×3	283·3	100	50	2230	10
Manganese ..	0·1	50	×1	279·5	100	50	2475	10
Magnesium ..	0·01	50	×1	285·2	100	50	2475	10
Zinc	0·01	50	×1	213·9	100	50	2230	10
Vanadium ..	0·8	50	×3	318·3	100	50	2660	10
Nickel ..	0·2	50	×3	232·0	100	50	2630	10
Aluminium ..	0·1	50	×3	309·3	100	50	2660	10

			Reproducibility and detection limit, per cent. of element	Interferences, per cent.	
Element	Highest possible charring temperature without loss of analyte/°C	Time of atomisation*/s		By 8 per cent. nitric acid	By nitric acid plus 10 000 p.p.m. of iron
Bismuth ..	420	1·6	0·000 12 0·000 24	+5	+5
Lead	520	1·6	0·000 07 0·000 14	−32	0
Manganese ..	1135	3·0	0·000 06 0·000 12	0	−12
Magnesium ..	1020	2·7	0·000 016 0·000 032	−32	−77
Zinc	420	1·5	0·000 009 0·000 018	−30	−30
Vanadium ..	1750	9·6	0·000 34 0·000 68	−58	0
Nickel ..	1245	4·2	0·000 17 0·000 34	−38	−31
Aluminium ..	1660	5·6	0·000 12 0·000 24	−33	0

* Time of atomisation defined as time for atomisation signal to reach a maximum from start of atomisation cycle.

and the matrix would be mostly iron, the interference of iron in the determination of these elements is included in the table. Aluminium and lead are included for comparison. As can be seen the magnitude of these interferences varies from −33 per cent. for nitric acid with aluminium to +5 per cent. for nitric acid with bismuth. The effect of nitric acid and 10 000 p.p.m. of iron varies from −77 per cent. for magnesium to +5 per cent. for bismuth.

The only serious effect is that of iron on the magnesium determination but, provided that the iron content is comparable in the standards and the samples, then, as the instrument is very sensitive to magnesium, there should be sufficient sensitivity to allow an accurate analysis to be made.

For reasons already discussed the effect of charring on the determination of aluminium improves the relative standard deviation from 12·0 to 8·7 per cent. at the 0·1 p.p.m. level. As can be seen from Table V the reproducibility is better for the more volatile elements when the iron matrix has no effect. In the case of bismuth the result is anomalous, the poor reproducibility being due in part to the fact that the intensity of the bismuth hollow-cathode source is low and therefore a high gain setting is required. In all instances except those of aluminium and vanadium it was not possible to reduce the amount of iron present by means of charring as at the temperature required for this purpose (setting above 300, *i.e.*, above 1500 °C) significant loss of the analyte occurred. If a charring step were used at a temperature low enough to ensure that no loss of analyte occurred, then for magnesium, bismuth, lead, zinc, nickel and manganese no appreciable removal of the iron would occur. As can be seen from Fig. 1, elements such as bismuth, zinc and lead are released from the matrix before the matrix itself produces any smoke. Thus, for these three elements, when they are determined in steel, there should be no contribution to the standard deviation from the iron matrix. As might be expected these elements can be determined with-

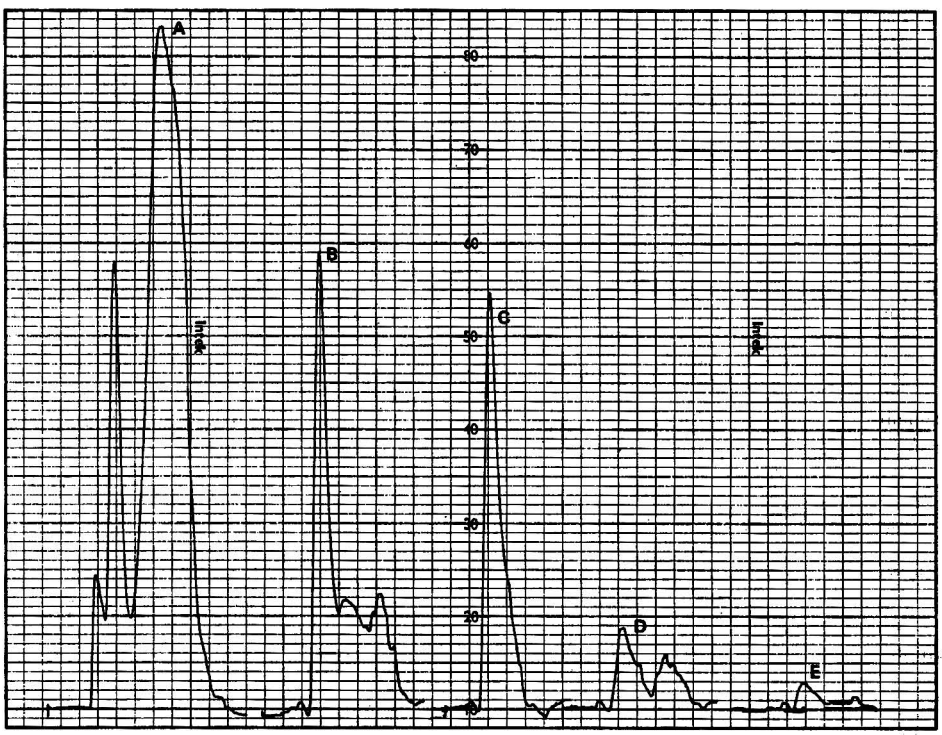

Fig. 2. Effect of iron and nitric acid background on the lead atomic-absorption signal: A, 0·1 p.p.m. of lead in 10 000 p.p.m. of iron and 8 per cent. nitric acid without background correction; B, as A but with background correction; C, as B but without iron; D, as B but without lead; and E, as B but without lead and iron.

out background correction simply by speeding up the chart recorder, as is demonstrated for 0·1 p.p.m. of lead in the presence of 10 000 p.p.m. of iron, with and without background correction when using a 50-μl pipette and a $\times 3$ scale expansion (Fig. 2). For lead plus iron in 8 per cent. nitric acid without background correction the first peak is due to nitric acid, as would be expected from Fig. 1. The second peak is due to lead and the large smoke

background can be seen immediately following. For lead plus iron in 8 per cent. nitric acid with background correction the nitric acid peak is no longer evident and the lead peak is identical with that obtained without background correction, which indicates that the background from the smoke does not contribute to the analyte peak. A tail from the smoke is evident just after the lead signal but it is greatly reduced. The signal, with background correction, from a 0·1 p.p.m. solution of lead in 8 per cent. nitric acid is also shown and it can be seen that if the small blank due to lead in the iron is added to this signal then it is identical with the signal from the lead plus iron. The blanks for 10 000 p.p.m. of iron in 8 per cent. nitric acid and for 8 per cent. nitric acid alone are also shown with background correction. Again, the residual smoke signal is evident after the lead peak in the iron plus acid blank solution.

Magnesium and manganese are just separable from the background but both include a contribution from the smoke and therefore some form of background correction is necessary. For aluminium and nickel the signal from the elements coincides with the release of the bulk of the matrix as smoke and some of the smoke signal is included in the aluminium and possibly the nickel signal, even with background correction. For magnesium, manganese and nickel a charring step can be used at a high enough temperature to remove a very small amount of the iron but this does not affect the reproducibility. For vanadium a considerable amount of iron can be removed by charring without loss of analyte but this does not affect the reproducibility.

By reference to Fig. 1 it can be seen that line background correction would not be effective for such elements as lead, bismuth, magnesium, manganese and zinc when these are atomised well before the matrix smoke appears. This type of correction would give rise to inaccurate results and could be used effectively only if atomisation of the analyte were coincident with the release of the matrix smoke.

Conclusion

The determination of various elements in iron and steel can be summarised as follows:

Element	Detection limit in steel (2σ), %	Comments
Lead	0·00014	No background correction is necessary and the reproducibility is good. Interference by iron + nitric acid is zero
Bismuth	0·00024	No background correction is necessary. Poor reproducibility probably due to low-intensity hollow-cathode lamp. Interference by iron + nitric acid is small
Zinc	0·000018	No background correction is necessary. Reproducibility very good. Interference by iron + nitric acid is fairly large
Magnesium	0·000032	Background correction advisable although it may be possible to analyse without correction if matrices of standard and sample are similar. Reproducibility good. Interference by iron + nitric acid is large
Manganese	0·00012	Background correction advisable although same conditions apply as for magnesium above. Reproducibility good. Interference by iron + nitric acid is fairly small
Nickel	0·00034	Background correction essential, reproducibility poor. Interference by iron + nitric acid is fairly large
Aluminium	0·00024	Background correction essential. Reproducibility poor although may be improved by use of high charring temperature to effect partial removal of iron. Interference by iron + nitric acid is zero
Vanadium	0·00068	Background correction essential. Reproducibility poor. Interference by iron + nitric acid is zero
Antimony[17]	0·00007	No background correction is necessary. Reproducibility good. Interference by iron + nitric acid + hydrochloric acid is zero

From the few results obtained it appears that there should be no problems associated with the determination of bismuth, lead,[11] zinc, magnesium, manganese, nickel, aluminium and vanadium in steel, and, in general, analysis for these elements by this technique is much faster and likely to be more precise than analysis by most other techniques. These conclusions are supported by Frech[17] in his method for the determination of antimony in steel by dissolution of the sample in aqua regia and direct injection into the heated carbon furnace.

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A Kinetic Theory of Atomisation for Non-flame Atomic-absorption Spectrometry with a Graphite Furnace

Part II.* Analytical Applications of Kinetic Information for Copper

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Kinetic information for the atomisation of copper in a heated graphite furnace has been used to derive analytical parameters that are encountered in atomic-absorption determinations. Equations have been derived to describe quantitatively pre-atomisation heating losses, the advantages to be gained from operating under stopped gas flow conditions and the relative merits of signal peak height and signal integration measurements.

In Part I,[1] the kinetics and a possible mechanism of atomisation for copper in a graphite furnace, used as an atom source in atomic-absorption spectrometry, were described. The rate of change of copper atoms in the furnace was given by equation (1).

$$\frac{d[Cu]}{dt} = k_1[Cu]_0 e^{-k_1 t} - k_2[Cu] \quad \ldots \quad (1)$$

while the measured absorbance value for copper at time t was given by equation (2).

$$\text{Absorbance (copper)} = \frac{k_1}{k_2 - k_1} p\,[Cu]_0 (e^{-k_1 t} - e^{-k_2 t}) \quad \ldots \quad (2)$$

where [Cu] is the amount of copper atoms in the furnace at time t, $[Cu]_0$ is the initial amount of copper present, p is a constant relating measured absorbance with the amount of copper atoms in the furnace, k_1 is the first-order rate constant for the formation of copper atoms by reduction of copper oxide with carbon and k_2 is the first-order rate constant for the removal of copper atoms by the inert gas flowing through the furnace.

In this paper the kinetic approach to atomisation is developed by using this kinetic information together with extrapolated kinetic values for copper in order to evaluate the experimentally observed phenomena of pre-atomisation heating losses, the absorbance signal enhancement by stopped gas flow and the advantage of using signal integration compared with the signal peak height for calibration purposes.

Experimental

A Perkin-Elmer HGA70 graphite furnace fitted to a Perkin-Elmer, Model 103, atomic-absorption spectrometer with a Model 165 recorder was used for atomic-absorption measurements. A signal integrator, which enabled the integration period to be varied to any value between 3 and 50 s, was used.

For atomic-absorption measurements in the stopped gas flow mode a three-way tap was inserted into the argon feed line to the graphite furnace. Immediately after the atomisation stage began, the argon flow was rapidly switched from the furnace to the atmosphere and when the peak absorbance had passed the gas flow was switched back through the furnace.

Other experimental conditions have been described in Part I.[1]

An optical pyrometer was used to measure the temperatures of the graphite furnace as the nominal values for the temperatures supplied by the manufacturer of the furnace were not always reliable, particularly for the ashing programmes. Large temperature variations have been observed, depending on the condition of the graphite tubes and of the graphite

* For details of Part I of this series, see reference list, p. 233.

end support cones. For normal experimental work, however, the nominal temperature values are usually acceptable.

Results and Discussion

Pre-atomisation Heating Losses

It is now well established that copper,[2] among many other elements, is easily lost during the ashing stage of an analytical determination when using a graphite furnace atomic-absorption system; however, no quantitative explanation of these occurrences has yet been proposed. Examination of the suggested reaction mechanism for the atomisation of copper[1] and the rate equation (equation 3) for the rate of formation of copper atoms in the graphite furnace shows that quantitative predictions can be made for the losses of copper at various temperatures and for various lengths of ashing time. These predictions can be verified readily by experimental observation.

The rate of atomisation of copper was shown[1] to follow the simple first-order kinetic equation

$$\frac{\mathrm{d}[\mathrm{Cu}]_{\text{formation}}}{\mathrm{d}t} = k_1([\mathrm{Cu}]_0 - [\mathrm{Cu}]_t) \qquad (3)$$

where $[\mathrm{Cu}]_t$ is the total amount of copper vaporised after time t.

Hence by integration of this equation a value of $[\mathrm{Cu}]_t$ can be obtained:

$$[\mathrm{Cu}]_t = [\mathrm{Cu}]_0 (1 - \mathrm{e}^{-k_1 t}) \qquad (4)$$

Equation (4) can be used in several ways to predict the amount of copper lost during the ashing stage of an analytical determination: determination of (*a*) the variation in the amount of copper lost at various ashing temperatures with a fixed ashing time; (*b*) the variation in the amount of copper lost for various ashing times at a fixed ashing temperature; and (*c*) the simple but useful value $t_{\frac{1}{2}}$, the time at which 50 per cent. of the initial amount of copper present is lost.

Substitution of $[\mathrm{Cu}]_t = [\mathrm{Cu}]_0/2$ at $t = t_{\frac{1}{2}}$ (the half-life) into equation (4) immediately produces the well known kinetic expression for $t_{\frac{1}{2}}$:

$$t_{\frac{1}{2}} = \frac{\ln 2}{k_1} \qquad (5)$$

Fig. 1 shows the extrapolated variation of $\log_{10} k_1$ with the reciprocal of the absolute temperature using the information reported in Part I.[1] Applying this information to equation (4) enables the theoretical percentage loss of copper at various temperatures and times (equivalent to experimental ashing conditions) to be calculated. The percentage of copper determined during the atomisation stage is then equal to 100 minus the percentage lost during the ashing stage. Figs. 2 and 3 show these theoretically predicted graphs and compare them with some experimentally observed values.

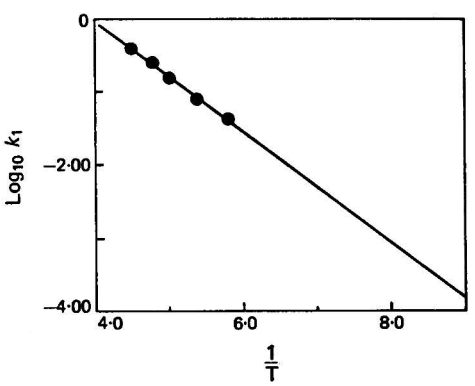

Fig. 1. Extrapolation of the graph of $\log_{10} k_1$ *versus* $1/T$(K) (results from reference 1).

Very good agreement is obtained in both instances. The experimental results reported here also compare well with those given in an earlier publication,[2] although there is a discrepancy in the actual temperatures reported because in that publication only the instrument manufacturer's nominal temperature values were quoted rather than experimentally measured temperatures.

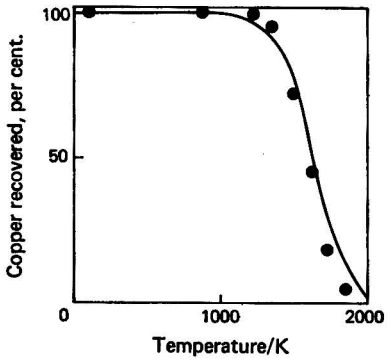

Fig. 2. Variation of the percentage of copper recovered with increasing ashing temperature: 20 μl of a 0·1 μg ml^{-1} aqueous copper standard, using a 30-s drying period at 373 K, a 30-s ashing period and a 10-s atomisation period at a setting of 9 V. Continuous line, theoretically predicted values; and ●, experimental values.

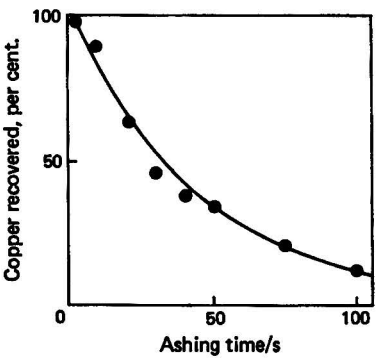

Fig. 3. Variation of the percentage of copper recovered with increasing ashing time: 20 μl of a 0·1 μg ml^{-1} aqueous copper standard, using a 30-s drying period at 373 K, an ashing period at 1625 K and a 10-s atomisation period at a setting of 9 V. Continuous line, theoretically predicted values; and ●, experimental values.

Stopped Gas Flow Operation

Kahn and Slavin[3] originally proposed operation of an HGA70 graphite furnace with the flow of inert gas stopped in order to obtain increased sensitivity in atomic-absorption determinations. They inserted a solenoid valve, which could be operated during the atomisation stage of a determination by a push-button, into the gas purge line. When the solenoid valve was activated the gas flow was vented to the atmosphere.

The idealised situation of stopped gas flow operation would be given by setting $k_2 = 0$ in equation (1), *i.e.*, when there is no removal of copper atoms from the graphite furnace. However, this situation is impossible to achieve as the atom population will be diminished by natural diffusion effects and by expansion of the gas within the furnace as the temperature is raised.

Nevertheless, equation (2) can be differentiated to obtain the peak absorbance value for copper, *i.e.*,

$$\text{Absorbance (copper}_{\text{max.}}) = p\,[\text{Cu}]_0 \left(\frac{k_2}{k_1}\right)^{\frac{k_2}{k_1 - k_2}} \quad .. \quad .. \quad .. \quad (6)$$

A typical atomisation temperature for a copper determination would be about 2600 K so that by using extrapolated values for k_1 and k_2 (from Figs. 1 and 4) of 1·20 and 1·17, respectively, at this temperature it is possible to obtain a value for the peak absorbance of $p\,[\text{Cu}]_0$ 0·37. The extrapolated values of k_2 shown in Fig. 4 are based on results reported in Part I.

Because under stopped gas flow conditions k_2 tends to zero, then from equation (6) the peak absorbance value tends to $p\,[\text{Cu}]_0$. This shows, therefore, that under these conditions the maximum improvement in the absorbance signal attainable by stopped gas flow is by a factor of 2·7, which compares well with an average value of 1·7 obtained with the simple three-way tap used in this investigation and the value of 2 obtained by Kahn and Slavin in their work.[3] Remembering that the value of 2·7 is the theoretically predicted maximum improvement in peak height, it is clear that the best improvement in absorbance signal has probably already been achieved by current instrumentation using stopped gas flow operation.

At lower atomisation temperatures the improvement effected in absorbance peak height by using stopped gas flow will be greater but the actual measured values will clearly be less than those obtained at the higher temperatures.

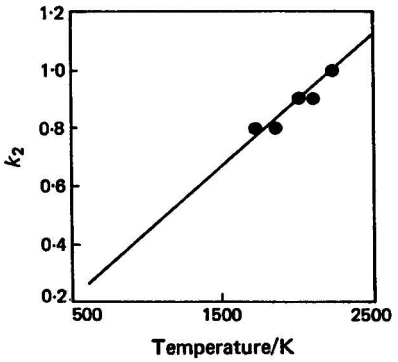

Fig. 4. Extrapolation of the graph of k_2 *versus* T (K) (results from reference 1).

Calibration by Signal Peak Height or Signal Integration

Stopped gas flow operation is in reality a simple method of signal integration whereby the atomised copper is allowed to accumulate in the graphite furnace. Electronic signal integration is, however, far simpler to operate.

Equation (2), which describes the amount of copper atoms in the graphite furnace at time t, can be differentiated in order to obtain the peak absorbance signal (equation 6) and integrated so as to obtain the integrated absorbance value (equation 7) between times $t = 0$ and $t = t$:

$$[\text{Absorbance (copper)}]_{t=0}^{t=t} = p\,[\text{Cu}]_0 \frac{k_1}{k_2 - k_1}\left(\frac{e^{-k_2 t}}{k_2} - \frac{e^{-k_1 t}}{k_1}\right)_{t=0}^{t=t} \quad \ldots \quad (7)$$

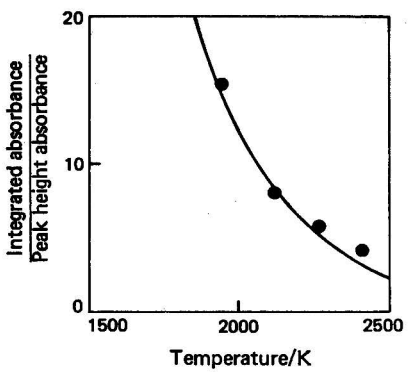

Fig. 5. Variation of the integrated absorbance to peak height absorbance ratio with temperature: 20 μl of a 0·2 μg ml^{-1} aqueous copper standard, using a 30-s drying period at 373 K, a 30-s ashing period at 700 K and a 50-s atomisation period for signal integration purposes. Continuous line, theoretically predicted values; and ●, experimental values.

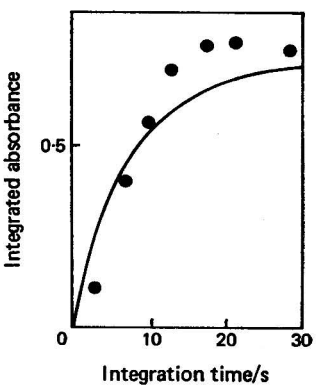

Fig. 6. Variation of the integrated absorbance value with integration time: 20 μl of a 0·2 μg ml^{-1} aqueous copper standard, using a 30-s drying period at 373 K, a 30-s ashing period at 700 K and an atomisation period at 2000 K. Continuous line, theoretically predicted values; and ●, experimental values.

By using equations (6) and (7) and extrapolated values of k_1 and k_2 it is possible to obtain the theoretically predicted values of the ratio of integrated absorbance ($t = \infty$) to peak height absorbance at all temperatures (Fig. 5). Also included in Fig. 5 are the experimentally observed values for this ratio. By using equation (7) alone it is possible to obtain the theoretically predicted variation in the integrated absorbance value with increasing integration time for a fixed atomisation temperature. This variation for an atomisation temperature of 2000 K is shown in Fig. 6 together with some experimentally observed values. In both instances good agreement is obtained between the theoretically predicted values and the experimental values.

From the results shown in Fig. 5 the greatest advantage of using an integrated signal measurement system is clearly under conditions when small peak height values are obtained due to a slow rate of atomisation (in this instance at a lower temperature); as the atomisation temperature increases the advantages of integration rapidly disappear. Fig. 6 illustrates that under the conditions used here 75 per cent. of the integrated signal for copper is achieved within 10 s and little further advantage is to be gained by longer integration periods. Obviously, at slower rates of atomisation (lower temperatures) longer integration periods would be advantageous and for faster rates of atomisation (higher temperatures) shorter integration periods could be used. This is demonstrated in Fig. 7, which shows the variation in the predicted time required to reach 75 per cent. of the integrated absorbance signal as a function of temperature.

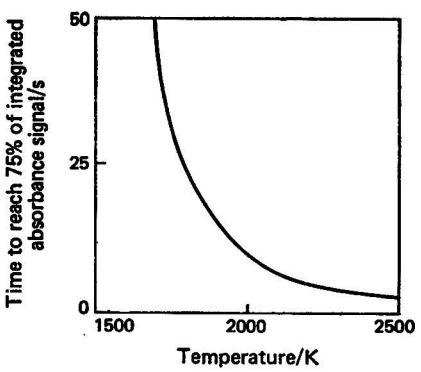

Fig. 7. Variation in the theoretically predicted time required to reach 75% of the integrated absorbance signal as a function of temperature.

The work described above has shown that the rate equation derived for the atomisation of copper in a graphite furnace[1] is valid and that it can be used to predict experimentally observable parameters. When kinetic information becomes available for other elements it should ultimately be possible to predict the ideal analytical conditions for the determination of many elements by non-flame atomic-absorption spectrometry.

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NOTE—Reference 1 is to Part I of this series.

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Atomic-absorption Spectrophotometric Determination of Lead in Beverages and Fruit Juices and of Lead Extracted by Their Action on Glazed Ceramic Surfaces

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A rapid procedure for the determination of lead in beverages and fruit juices by directly aspirating them into an atomic-absorption spectrophotometer is described. Concentrations of lead down to 0·1 p.p.m. can easily be determined. The standard deviation in the range 0·5–2·0 p.p.m. of lead is about 0·04 p.p.m. The accuracy of the method is good; although its sensitivity is slightly lower than that with methods in which condensation procedures are used, the proposed method is simpler and more rapid. Interferences caused by the different matrices of the samples are negligible in most instances investigated.

The method has been applied to samples of beverages and fruit juices that had been kept in glazed ceramic cups for 0·5 h in an attempt to compare the amount of lead extracted by these products with that extracted by 4 per cent. acetic acid solution, the reagent used for testing ceramic ware for extractable lead.

The cumulative poison lead is probably the most serious metallic contaminant of food. Monier-Williams[1] has estimated that the daily intake of lead by a normal healthy individual is about 0·4 mg, 0·22 mg being derived from food, 0·10 mg from water and 0·08 mg from inhaled dust. Small amounts of lead occur naturally in many foods, but food and beverages can become contamined with lead from processing materials, storage containers and glazed ceramics. Inorganic lead salts have been commonly used for glazing ceramic ware. Although toxic amounts of lead can be leached from such products under certain conditions, the seriousness of this problem has only recently been recognised.[2]

The determination of lead in liquid products by flame atomic-absorption spectrophotometry usually requires the sample either to be ashed or extracted with an organic solvent.

This paper presents a study of the determination of lead in beverages and fruit juices by their direct aspiration into an atomic-absorption spectrophotometer. The sensitivity of the method is lower than with methods that involve the use of condensation procedures, but it has the advantages of greater simplicity and speed and is comparable in accuracy and precision. As a check on the safety limits set by various official regulations, the method was applied to beverages and fruit juices that had been allowed to stand in ceramic cups for 0·5 h and the amount of lead leached by them from the cups was compared with that leached by the 4 per cent. acetic acid test solution.[2]

Experimental

Reagents and Apparatus

Analytical-reagent grade reagents were used and normal precautions for trace analysis were taken throughout.

Lead nitrate stock solution for atomic-absorption spectrophotometry (Riedel-de Haën) was used for the standard solutions. Working standards were prepared with water, water - ethanol mixtures and 4 per cent. acetic acid solution.

Standards and samples were analysed on an Instrumental Laboratories Inc., Model 353, atomic-absorption spectrophotometer, with an air - acetylene flame. The operating conditions were as described in the procedure and in the operator's manuals supplied with the instrument for determining lead.

Procedure

For orange juice and coca cola, de-gas carbonated samples before analysis. After measuring the atomic absorption, measure the aspiration rate of water and sample and correct the result obtained for any decrease in the aspiration rate of the sample.

For instant coffee use 2·7 g in 200 ml of water and for tea, 1 bag per 200 ml of water.

All samples with added lead were analysed for their lead content, before the addition, by the dry-ashing - atomic-absorption method.[3,4]

Ceramic cups were cleaned with detergent, rinsed with water, dried, filled with test solution, and subsequently with liquid food products, and were then covered and left to stand at room temperature. Before sampling for atomic-absorption measurement the solution or sample was stirred by hand.

Results and Discussion

The following samples: infusions of instant coffee and tea, orange juice, coca cola and dry wine, were analysed for added lead content by direct aspiration into an atomic-absorption spectrophotometer and their absorptions measured at 217·0 and 283·3 nm. The calibration graph was linear in the range 0·1–20 p.p.m. of lead for all samples examined except wine, which furnished a linear calibration graph up to only 5 p.p.m. of lead. In order to investigate the effect of the matrices of the products on the absorption, both the absorption and aspiration rates of the above samples were compared with those of water containing the same amount of added lead; the results obtained are given in Table I, from which it can be seen that the beverages instant coffee and tea do not affect the absorption of lead.

TABLE I

ABSORPTION AND ASPIRATION RATE OF LIQUID FOOD PRODUCTS AND WATER CONTAINING EQUAL AMOUNTS OF ADDED LEAD

Sample	Absorption (arbitrary units)		Aspiration rate/ml min^{-1}
	Lead added, 10 p.p.m.	Lead added, 5 p.p.m.	
Water	1200	610	5·3
Orange juice	990	510	4·5
Orange juice - water (1 + 1)	1080	560	4·9
Coca cola	1080	560	4·9
Coca cola - water (1 + 1)	1150	590	5·1
Instant coffee	1200	615	5·3
Tea	1200	610	5·3
Wine (dry)	—	610	3·7
Wine - water (1 + 1)	—	620	4·5

This is also true for orange juice and coca cola, because, compared with water, the decrease in lead absorption is caused mainly (96 per cent.) by the decrease in the aspiration rate. With wine it is easily seen that the decrease in the aspiration rate is compensated for by an equal enhancement of the lead absorption by the matrices of the samples. Ethanol is known to

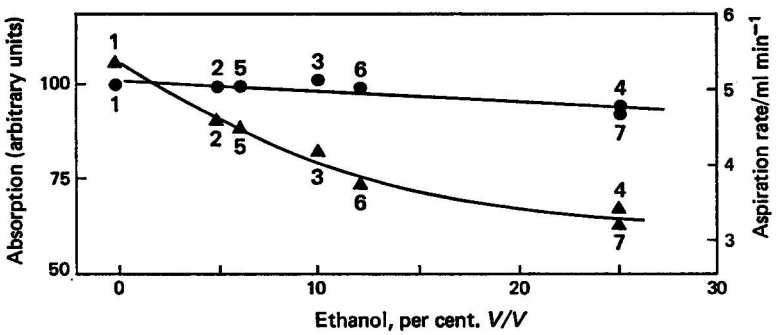

Fig. 1. Absorption (●) and aspiration rate (▲) of the following mixtures with 5 p.p.m. of added lead: 1, water; 2, 3 and 4, water - ethanol; 5, wine - water (1 + 1); 6, wine; 7, wine - water - ethanol (50 + 31 + 19).

enhance the absorption of iron, manganese, zinc and copper in wine[5]; the results shown in Fig. 1 indicate that ethanol also exerts an enhancing effect on the absorption of lead.

Thus, soluble lead in instant coffee and tea can be determined by direct aspiration of their infusions into an atomic-absorption spectrophotometer and reference to lead standards in water. This also holds true for ready-to-drink fruit juices (orange, lemon and grapefruit) and coca cola, but with these products the results have to be corrected for the decrease in aspiration rate compared with the water aspiration rate. Soluble lead in wine was determined by the method of additions,[6] as ethanol was found to enhance its absorption. Dilution of the samples with water (1 + 1) is sometimes preferable (provided that the dilution does not cause the lead content to fall below the sensitivity range of the method) because with direct aspiration of the samples the burner becomes dirty and needs to be cleaned frequently.

The method was also applied to samples of beverages and fruit juices that had been kept for 0·5 h in glazed ceramic cups that had previously been checked for leachable lead by the 4 per cent. acetic acid test solution[2]; the results obtained are presented in Table II.

TABLE II

DETERMINATION OF LEAD (p.p.m.) IN BEVERAGES AND FRUIT JUICES (200 ml) THAT HAD BEEN LEFT TO STAND FOR 0·5 h IN GLAZED CERAMIC CUPS (ABOUT 250 ml)

Values given are average values of the quoted number of identical units of ceramic products. Values in parentheses indicate ranges.

No. of cups	Acetic acid solution (4 per cent.) 0·5 h	Acetic acid solution (4 per cent.) 24 h	Orange juice	Coffee infusion*	Tea infusion*	Wine (dry)
4	0·5 (0·3–0·7)	3·3 (0·5–7·1)	0·4 (0·1–0·6)	0·3 (0·1–0·6)	0·3 (0·2–0·5)	0·2 (0·1–0·6)
12	1·2 (0·3–2·2)	4·5 (2·0–8·5)	1·2 (0·3–2·5)	1·4 (0·1–2·4)	1·1 (0·1–2·0)	1·0 (0·3–1·5)
6	2·4 (0·2–4·0)	7·2 (2·1–12·8)	1·4 (0·3–2·7)	2·0 (0·2–3·5)	1·6 (0·1–3·4)	1·5 (0·4–3·7)
6	5·1 (0·8–8·7)	15·3 (9·2–22·3)	5·0 (2·0–9·2)	3·0 (0·8–7·0)	3·0 (1·2–5·8)	2·5 (1·5–5·0)
4	9·3 (3·2–16·2)	34·5 (21·2–40·0)	6·0 (2·1–11·0)	3·3 (1·2–6·4)	3·8 (2·0–6·2)	3·5 (1·0–5·4)
3	41·5 (33·5–53·5)	93·2 (69·5–147·0)	20·4 (14·2–29·0)	8·5 (4·2–15·2)	9·5 (3·1–15·0)	8·0 (4·8–16·4)

* Initial temperature 80 °C.

The results given in Table II show that lead is leached not only by orange juice (total acidity 1 per cent., as citric acid) but also, to a considerable extent, by beverages such as coffee, tea and wine, for which ceramic cups are often used. In order to check whether these results are higher than is usual because of the pre-treatment of the cups with the acetic acid solution, six new identical cups were left to stand for 0·5 h containing orange juice and were then subjected to the acetic acid test. The results (average values) obtained were: 1·5 p.p.m. of lead in the orange juice and 5·4 p.p.m. in the leaching solution.

Table III presents the results obtained from the analysis of samples of fruit juices; the results for direct aspiration are compared with those obtained by the extraction method[3] and/or the dry-ashing method.[3,4]

It can be seen that there is good agreement between the results by the three methods. All direct aspiration values are the average of three separate determinations.

The precision was checked both at 217·0 and 283·3 nm, the results for which are presented in Table IV.

The above results show good precision at 217·0 nm and probably better precision at 283·3 nm.

The precision and accuracy of the determination of lead in liquid food products by direct aspiration into an atomic-absorption spectrophotometer are comparable with those of other atomic-absorption methods. The lower sensitivity of the method when compared with that of methods in which condensation procedures are used is, of course, a disadvantage, which

TABLE III

COMPARISON OF ATOMIC-ABSORPTION DETERMINATION OF LEAD (p.p.m.) BY DIRECT ASPIRATION, EXTRACTION AND DRY ASHING

Sample	Direct aspiration	Extraction	Dry ashing
Squeezed orange	0·26	0·24	0·24
Squeezed lemon	0·26	0·22	0·24
Orange juice*	0·29	0·30	0·27
Grapefruit juice*	0·80	1·20	—
Grapefruit juice	0·52	0·53	—
Lemon juice*	1·90	2·20	2·00

* Canned products.

is surely compensated for by its simplicity and speed, given that the limits for lead in beverages and juices set by food regulations are within the range of its sensitivity. There is a need in food analytical laboratories for fast and accurate methods of analysis, as the number of determinations that have to be performed on a single product increases daily.

TABLE IV

PRECISION OF THE DETERMINATION OF LEAD (p.p.m.) IN A BEVERAGE AND FRUIT JUICES BY DIRECT ASPIRATION INTO AN ATOMIC-ABSORPTION SPECTROPHOTOMETER

	Grapefruit juice		Lemon juice		Instant coffee infusion (lead added)	
	217·0 nm	283·3 nm	217·0 nm	283·3 nm	217·0 nm	283·3 nm
	0·46	0·47	1·89	1·88	2·20	2·01
	0·56	0·48	1·97	1·90	2·16	2·01
	0·48	0·47	1·86	1·87	2·08	2·00
	0·55	0·47	1·93	1·86	2·08	1·85
	0·55	0·44	1·91	1·93	2·16	1·98
	0·45	0·44	1·86	1·89	2·17	1·85
	0·52	0·45	1·94	1·93	2·18	1·87
	0·52	0·47	1·92	1·93	2·22	2·00
	0·51	0·48	1·93	1·87	2·05	1·97
	0·56	0·49	1·85	1·86	2·14	2·02
Mean	0·52	0·47	1·91	1·89	2·14	1·96
Standard deviation	0·041	0·018	0·040	0·029	0·055	0·070
Coefficient of variation, per cent.	7·9	3·8	2·1	1·5	2·6	3·6

Conclusion

The recommended limit for leachable lead in ceramic ware by the Food and Drug Administration is 7 p.p.m. of lead.[2] Examination of Table II shows that beverages that remain for 0·5 h in such ceramic products would contain about 1·5 p.p.m. of lead, which value is high considering British Standards limits for lead in non-alcoholic beverages (0·2 p.p.m.), fruit juices (0·5 p.p.m.) and wines (1·0 p.p.m.).[7]

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Colorimetric Determination of Vitamin D in Some Oily Pharmaceutical Preparations

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A colorimetric method for the determination of vitamin D, based upon the use of anisaldehyde - sulphuric acid as a colour reagent, has been developed. The method enables 0·1–0·5 mg of calciferol to be determined with a mean percentage recovery of 100·2 ± 1·44 per cent., and it avoids the difficulties met with in the antimony(III) chloride colour reaction.

Both methods, as applied to oily injections of vitamin D, are compared, and a statistical analysis of the results reveals that the proposed method is the more precise, and has an accuracy equal to that of the antimony(III) chloride method.

Several methods have been proposed for the determination of vitamin D, including biological,[1,2] titrimetric,[3,4] ultraviolet spectrophotometric,[5–8] infrared spectrophotometric,[9,10] polarographic[11,12] and gas-chromatographic methods.[13–17] The literature also refers to many colorimetric methods,[18–32] of which that involving the use of antimony(III) chloride, first introduced by Brockmann and Chen[18] and modified by Nield *et al.*,[19] is widely used. The method involves the rapid measurement, at wavelengths of 500 and 550 nm, of the orange - yellow colour produced on mixing antimony(III) chloride reagent with a solution of the vitamin in chloroform; the absorbance difference (A_{500}–A_{550}) is proportional to the concentration of the vitamin. However, in spite of the high sensitivity of this last method, it suffers from a number of disadvantages.[32]

Several colour reactions based upon the use of aldehyde - sulphuric acid reagents[23–26] were put forward as possible methods for the determination of vitamin D, but the instability of the colours produced, the lengthy procedures to be followed and the strictly controlled conditions to be adopted represent disadvantages with these methods.

The present investigation is concerned with the study of a colour reaction that is based upon the use of anisaldehyde and sulphuric acid for the determination of vitamin D; the method is easy to automate for routine analysis.

Experimental

Reagents

Calciferol, 1 *mg* ≡ 40 000 *i.u.* (*BDH Chemicals Ltd.*).
Absolute methanol, Prolabo grade.
Diethyl ether, peroxide-free.
Sulphuric acid, analytical-reagent grade.
Hydroquinone (*E. Merck*).
Nitrogen, oxygen-free.
Potassium hydroxide solution, 0·5 N.
Anisaldehyde (*E. Merck*), 1 *per cent.* V/V *solution in methanol.*
Sulphuric acid, 10 *per cent.* V/V *in methanol.*
Potassium hydroxide solution in ethanol, 0·5 N.
Antimony(III) chloride reagent solution, B.P. 1968.

Apparatus

Unicam SP600 spectrophotometer.

Procedure

(a) General procedure

Into a 25-ml calibrated flask introduce 2 ml of a solution of calciferol in methanol (containing 0·4–2·0 mg per 100 ml), 0·6 ml of anisaldehyde reagent and 1·25 ml of analytical-reagent grade sulphuric acid, which is added dropwise with constant agitation. Allow the mixture to stand for 1 min, then dilute to volume with 10 per cent. *V/V* sulphuric acid in methanol and set it aside for 20 min. Measure the absorbance of the greenish-blue colour at 685 nm, using a 1-cm glass cell, against a blank solution similarly treated. Calculate the concentration of calciferol by reference to a calibration graph.

(b) For injections

To an accurately weighed amount of the oily solution (equivalent to about 0·3 mg of calciferol), add 0·1 g of hydroquinone, 25 ml of 0·5 N potassium hydroxide solution in ethanol and boil the mixture under reflux for 20 min. Cool, then add 50 ml of water and extract with three 30-ml amounts of peroxide-free ether. Wash the combined ethereal extracts with 20 ml of water, then with 20 ml of 0·5 N aqueous potassium hydroxide and finally with successive volumes, each of 20 ml, of water until the aqueous washings are no longer alkaline to phenolphthalein. Filter the ethereal solution through a small plug of cotton-wool, wash the plug with two 10-ml volumes of peroxide-free ether and evaporate the combined ethereal filtrate and washings to dryness on a water-bath, at 50 °C, under an atmosphere of oxygen-free nitrogen. Then dissolve the residue in 2 ml of methanol and apply the general procedure mentioned above.

Results

The results obtained by analysing five samples of chemically pure calciferol by use of the proposed anisaldehyde - sulphuric acid method and by the antimony(III) chloride method are given in Tables I and II.

TABLE I

DETERMINATION OF VITAMIN D USING THE ANISALDEHYDE - SULPHURIC ACID COLOUR REACTION

Sample number	Taken*/ mg	Absorbance (1-cm layer)	Found/ mg	Recovery, per cent.
1	0·1	0·190	0·0990	99·0
2	0·2	0·380	0·1980	99·0
3	0·3	0·580	0·3022	100·7
4	0·4	0·780	0·4065	101·6
5	0·5	0·966	0·5034	100·7

Mean = 100·2 ± 1·44 per cent. ($P = 0·05$)

* Final volume made up to 25 ml.

TABLE II

DETERMINATION OF VITAMIN D BY USING THE ANTIMONY(III) CHLORIDE COLOUR REACTION

Sample number	Taken*/ mg	Absorbance (1-cm layer)	Found/ mg	Recovery, per cent.
1	0·02	0·301	0·01996	99·8
2	0·03	0·442	0·02932	97·7
3	0·04	0·587	0·03893	97·3
4	0·05	0·800	0·05305	106·1
5	0·06	0·910	0·06035	100·6

Mean = 100·3 ± 4·38 per cent. ($P = 0·05$)

* Final volume made up to 10 ml.

Table III shows the results obtained by determining vitamin D in five samples of oily injections, collected at random from the local market, by using both the anisaldehyde - sulphuric acid and the antimony(III) chloride methods.

TABLE III

DETERMINATION OF VITAMIN D IN OILY INJECTIONS BY USING ANISALDEHYDE - SULPHURIC ACID AND ANTIMONY(III) CHLORIDE COLOUR REACTIONS AFTER SAPONIFICATION

Sample number	Label on ampoules	Labelled concentration/ mg ml^{-1}	Found by use of— antimony(III) chloride method/ mg ml^{-1}	Found by use of— anisaldehyde - sulphuric acid method/mg ml^{-1}
1	Devarol (Memphis Co.) B.N.* 10725	15	14·42	14·24
2	Devarol (Memphis Co.) B.N. 10296	15	14·02	13·99
3	D-Viton (Kahira Co.) B.N. 6550	15	15·26	14·73
4	D-Viton (Kahira Co.) B.N. 1144	15	15·13	14·60
5	Calciferol (Adco) B.N. 34	15	14·28	13·63

* Batch number.

Discussion

The antimony(III) chloride method for the determination of vitamin D suffers from a number of disadvantages, these being mainly that the reagent is extremely corrosive and that it absorbs moisture from the atmosphere, thus forming a mixture of oxychlorides and leaving a film of antimony oxychloride on the absorption cells. Also, the colour produced is unstable, non-specific and its intensity is found to vary from batch to batch of the reagent and from day to day.[32]

Schaltegger[24] reported a colour reaction between calciferol and five aromatic aldehydes, *viz.*, anisaldehyde, vanillin, *p*-cuminaldehyde, furfural and 4-hydroxy-1-naphthaldehyde, in the presence of sulphuric acid and in an anhydrous benzene medium. He also reported that benzene and other reagents need preliminary and lengthy treatment in order to achieve the necessary state of purity. Schaltegger tried to simplify the reagent by using only one aldehyde, but he found that an error of 20 per cent. was introduced, whereas the use of the five aldehydes reduced the average error to 8 per cent.

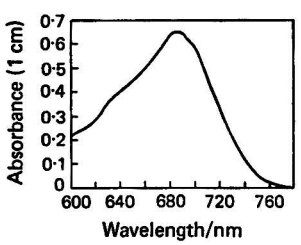

Fig. 1. Absorption graph for the colour reaction of calciferol with a 0·3 mg per 25 ml solution of anisaldehyde in sulphuric acid.

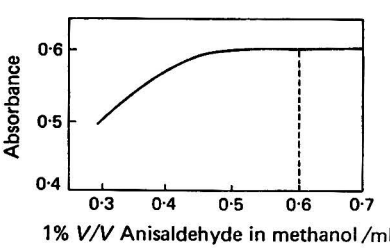

Fig. 2. Effect of anisaldehyde on the colour reaction of calciferol at a wavelength of 685 nm.

The proposed method makes use of the reaction with anisaldehyde alone, in the presence of sulphuric acid, to produce a greenish-blue colour that has maximum absorption at about 685 nm (Fig. 1). The optimum conditions for colour development were found to be: 0·6 ml of a 1 per cent. solution of anisaldehyde in methanol (Fig. 2); 1·25 ml of analytical-reagent

grade sulphuric acid (Fig. 3); and 20 min standing time after adding the sulphuric acid for the colour to reach its maximum (Fig. 4). The maximum colour is stable for 35 min, after which it starts to fade. The colour is reproducible and obeys Beer's law over a range of 0·1 to 0·5 mg of calciferol.

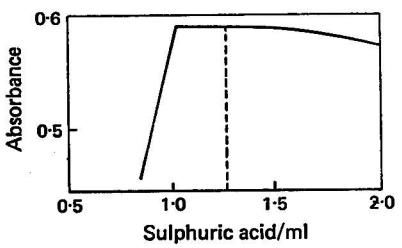

Fig. 3. Effect of sulphuric acid on the colour reaction of calciferol at a wavelength of 685 nm.

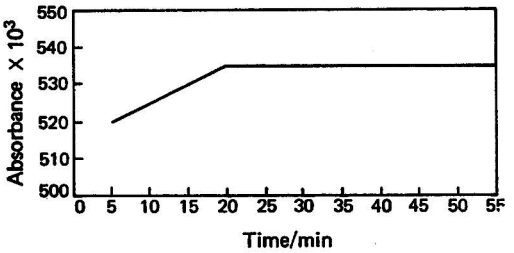

Fig. 4. Stability of the colour formed by reaction between calciferol and anisaldehyde - sulphuric acid at a wavelength of 685 nm.

Applying the proposed method to the determination of pure ergocalciferol, and comparing the results obtained with those from the antimony(III) chloride method (Tables I and II), it is evident that the mean recoveries for the two methods are 100·2 ± 1·44 per cent. and 100·3 ± 4·38 per cent., respectively. On comparing the variances and the mean percentage recoveries of both methods by the variance ratio and the *t*-test (Table IV), a significant difference between the precisions of the two methods is revealed. Thus, although both methods are considered to be accurate, the anisaldehyde - sulphuric acid method is the more precise.

TABLE IV

COMPARISON BETWEEN VARIANCES AND MEAN PERCENTAGE RECOVERIES OBTAINED BY USING THE ANISALDEHYDE - SULPHURIC ACID AND THE ANTIMONY(III) CHLORIDE COLOUR REACTIONS FOR THE DETERMINATION OF VITAMIN D

	Anisaldehyde - sulphuric acid method	Antimony(III) chloride method
Mean recovery, per cent. (*P* = 0·05) ..	100·2 ± 1·44	100·3 ± 4·38
N	5	5
Variance	1·335	12·435
t	$t_{(calc.)} = 0·0597$	$t_{0·95} = 2·31$ Degrees of freedom = 8
F	$F_{(calc.)} = 9·315$	$F_{0·95} = 6·39$

Subjecting the results given in Table III to paired comparison,[33] the Student's *t*-value is calculated to be 1·699 (theoretical $t_{0·975} = 2·571$). Then, with $P = 0·05$, no difference exists between the anisaldehyde - sulphuric acid method and the antimony(III) chloride method when applied to pharmaceutical preparations, which is further confirmation for the above conclusion concerning the relative precisions of the methods. However, with reference to the variances, fiducial limits and variance ratios in Table IV, it can be concluded that the proposed anisaldehyde - sulphuric acid method is as accurate as, but more precise than, the antimony(III) chloride method; the latter method is more sensitive to interferences from the unsaponifiable portion of the oil.

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A Thin-layer Chromatographic Test for the Identification of Some Drugs: Its Application to Steroids, Tetracyclines, Penicillins and Cephalosporins

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The identity test described is based on the thin-layer chromatographic separation of the complex mixtures of substances formed by thermal degradation of small amounts of many organic substances applied as spots to thin-layer plates. Subsequent development of the plate, and the application of spray reagents when necessary, yields characteristic patterns that are suitable for comparison under 254- and 366-nm ultraviolet light with authentic specimens that have been similarly treated. The test has been found to give good discrimination within the groups of steroids, and the tetracycline, penicillin and cephalosporin antibiotics described in the European and British Pharmacopoeias. For the cephalosporin group, separation of the thermal degradation products by paper electrophoresis is an alternative that gives slightly better results.

The technique is flexible in that there is a wide choice of thin-layer chromatographic support media, heating conditions, developing solvents and spray reagents, which facilitates its application to different classes of compounds.

The British Pharmacopoeia 1973[1] and the European Pharmacopoeia 1971[2] contain many examples of colorimetric and other chemical tests for the identification of individual compounds. Many of the official substances fall into well defined groups wherein individual substances may differ from each other only in the nature of the chemical groups attached to a common structural nucleus, or in configuration. Under such circumstances the development of a specific chemical test for each substance becomes an almost impossible task. Additional evidence of identity must therefore be sought from the application of instrumental techniques, such as spectroscopy or some forms of chromatography.

The method of thin-layer chromatography has been adopted as one of the official identity tests for certain classes of official compounds, notably the steroids and the tetracycline group of antibiotics. However, a method that relies on a single chromatographic system and a single R_F value, in comparison with that of reference compounds run on the same thin-layer chromatographic plate, can afford only limited discrimination. For a large group of compounds it may be necessary to use more than one solvent system in order to establish identification with reasonable certainty.

We therefore sought an identity test that would retain the efficiency of separation obtainable with thin-layer chromatography while increasing the discrimination between closely related compounds. The proposed test was initially developed[3] for the identification of individual members of the large group of steroids included in the British Pharmacopoeia, but it has since been applied successfully to the official tetracycline, penicillin and cephalosporin groups of antibiotics. It is based on the observation that most complex organic compounds, when applied to chromatographic plates which are subsequently heated in air under suitable conditions, break down into complex mixtures. Subsequent development of the plate with a selected solvent system separates the decomposition products to form a pattern characteristic of each substance.

Identification of Steroids

Method

Apply as separate spots to a suitable fluorescent silica gel plate (*e.g.*, Antec silica gel 254 glass plates, without binder*, Merck Kieselgel 60 F254 plates, 0·25 mm thick, or Adsorbosil-5

* These plates are no longer commercially available.

Prekotes, Applied Science Laboratories Inc.) 200 μg of the test and reference steroids dissolved separately in 10-μl amounts of a suitable volatile solvent, such as dioxan, and heat the loaded plate for 18 h at 110 °C. Allow it to cool and develop it in a closed tank with butyl acetate or another suitable solvent (see below). Remove the plate from the tank, allow most of the solvent to evaporate from it and spray it evenly with a 20 per cent. *m/V* solution of anhydrous zinc chloride in methyl alcohol. Heat for 20 min in an oven at 110 °C, remove it from the oven and immediately cover it with a glass plate in order to minimise contact with air. Examine the chromatographic plate (with the covering plate removed) under ultraviolet light at 254 nm, 366 nm and at both wavelengths simultaneously. Observation, and photographic recording if required, must be carried out immediately as colour degradation is rapid in moist air.

Results and Discussion

The test described was applied to the steroids listed in Table I, which include most of those in current therapeutic use and, in some instances, closely related degradation products (see Figs. 1–4). Unequivocal identification was possible in all instances, including differentiation between the "difficult "pairs megestrol and chlormadin one acetates, norethisterone and norethynodrel and fluocinolone and triamcinolone acetonides, although the last pair are not well separated in the particular example shown in Fig. 4.

TABLE I

STEROIDS EXAMINED

Steroid No.	Name	Derivative	Steroid No.	Name	Derivative
1	Beclomethasone	Dipropionate	21	Hydrocortisone	—
2	Betamethasone	—	22		Acetate
3		17-Valerate	23		Hydrogen succinate
4		21-Valerate	24		Sodium succinate
5		21-Acetate-17-iso-butyrate	25		Disodium phosphate
			26	Bishydrocortisone	Phosphate
6		Sodium phosphate	27	Hydroxyprogesterone	Hexanoate
7		Disodium phosphate	28	Megestrol	Acetate
8	Chlormadinone	Acetate	29	Methylprednisolone	—
9	Cortisone	Acetate	30	Norethisterone	—
10	Deoxycortone	Acetate	31	Norethynodrel	—
11		Pivalate	32	Prednisolone	—
12	Dexamethasone	—	33		Acetate
13	Dimethisterone	—	34		Sodium phosphate
14	Dydrogesterone	—	35	Prednisone	—
15	Ethinyloestradiol	—	36		Acetate
16	Ethynodiol	Diacetate	37	Progesterone	—
17	Fludrocortisone	Acetate	38	Testosterone	Propionate
18	Fluocinolone	Acetonide	39	Triamcinolone	—
19	Fluocortolone	Hexanoate	40		Acetonide
20		Pivalate			

As reference samples are run beside the sample for identification purposes, the make of chromatographic plate used is not critical. Other coating materials were tried and, of these, alumina appeared to be the most promising; it gives different, but equally useful, patterns. For steroids, one developing solvent, butyl acetate, is generally satisfactory for positive identification but added confirmation, if needed, can be obtained by development with dichloroethane - methyl acetate - water (2 + 1 + 1, lower layer).

Several variables were examined, including time and severity of heating, and the composition of the spray reagent, in order to establish operating conditions that were not critical. Overnight heating and a temperature of 110 °C were found to be the most convenient conditions, although a shorter period (*e.g.*, 2 h) is satisfactory for some steroids. Heating for 30 min at 170 °C is practicable but gives completely different breakdown patterns from those at 110 °C.

The zinc chloride reagent was found to give the most variety of colour, although for some steroids antimony(III) chloride solution (10 per cent. *m/V* in chloroform) gave better results. Whichever reagent is used the plate must be saturated in one spraying, as re-spraying causes loss of some of the fainter spots.

The possibility that commercial steroids may contain trace amounts of manufacturing impurities or degradation products that interfere with the test requires consideration. In practice, this does not appear to be a problem. If necessary, however, the main component can first be separated by applying the unknown to the plate near to one corner and developing with a suitable solvent system. After allowing the plate to dry, the reference substance is spotted on to the line of development (between the origin and the solvent front), close to the main component, but at a convenient point free from interfering components. The plate is then heated at 110 °C and the test completed, developing at right-angles to the first direction of development.

Application to Formulated Products

Although the test was originally intended for application to drug substances it has been used successfully for some formulated preparations containing a steroid as the active agent.

In general, simple extraction (*e.g.*, from tablets) with an organic solvent will give an extract sufficiently free from excipients to be spotted directly on to a chromatographic plate. For creams containing steroids, a clean-up consisting in passing a solution of the sample through a short silica gel column is often effective, or the bulk of the excipients can be frozen out of the solution because there is no need for quantitative recovery. If an extract sufficiently free from excipients cannot be obtained by these means, if appreciable breakdown of the active agent is suspected, or if there is more than one active agent, the two-dimensional procedure referred to above should be adaptable to the particular circumstances. Fig. 5 shows the identification of the main component (dimethisterone) in Secrodyl tablets.

Identification of Tetracycline Antibiotics

The European Pharmacopoeia 1971 (Volume II and Supplement 1973)[2] includes monographs for the hydrochlorides of tetracycline and three of its derivatives (chlortetracycline, demeclocycline, and oxytetracycline) and for oxytetracycline dihydrate. A further three are described in the British Pharmacopoeia, doxycycline and methacycline hydrochlorides, together with lymecycline, a water-soluble combination of tetracycline, lysine and formaldehyde.

The official identification test for all of these compounds is based on characteristic R_F values in comparison with those of reference samples that have been similarly treated in a thin-layer chromatographic system; the essential details of the test are common to both pharmacopoeias. In practice, the test is not very satisfactory; preparation of the necessary plates is lengthy, the correct degree of activity is not always attained and, in the test of the European Pharmacopoeia, efficient separation must be demonstrated on the same plate with a mixture of four compounds. A shortened and simplified version of the identity test described above for steroids gives satisfactory identification of tetracycline and its official derivatives, except that differentiation between oxytetracycline and its hydrochloride is not possible as the sample is dissolved in methyl alcohol - hydrochloric acid.

Lymecycline gives a pattern similar to that of its main component, tetracycline, but is readily differentiated by spraying with a 0·3 per cent. m/V solution of ninhydrin in methyl alcohol containing 5 per cent. V/V of γ-collidine (2,4,6-trimethylpyridine) and heating for 30 min at 100 °C. The lysine component gives a purple spot at the origin in the system used.

Method

Separately dissolve about 2 mg of the sample to be identified and the reference specimen(s) in 0·25-ml volumes of a mixture of 0·1 N hydrochloric acid and methyl alcohol (1 + 4). Apply 10 μl of each solution as a separate spot on the starting line of a silica gel chromatographic plate, preferably without binder (see above). Heat the plate for 1 h at 100 °C, allow it to cool, and develop the chromatogram in n-propyl alcohol - n-butyl acetate - acetone - water (2 + 2 + 2 + 1) until the solvent front is approximately 10 cm from the starting line. Remove the residual solvent from the plate in a stream of warm air and examine the chromatogram under ultraviolet light at a wavelength of 366 nm (Fig. 6).

If the plate is allowed to stand in daylight and in air at room temperature the patterns become directly visible, although examination under ultraviolet light is to be preferred.

Identification of Penicillins

A modification of the proposed identity test is applicable to the official penicillins (ampicillin, amoxycillin, benzathine penicillin, benzylpenicillin, carbenicillin, cloxacillin, methicillin, phenethicillin, phenoxymethylpenicillin, procaine penicillin and propacillin). Satisfactory identification of these compounds is attainable (see Fig. 7).

Method

Separately dissolve 5 mg of the unknown and reference samples in 0·1-ml amounts of 10 per cent. *V/V* ammonia solution. Spot 5-μl amounts on the starting line of a suitable fluorescent silica gel plate and heat the plate for 1·5 h at 150 °C. Allow it to cool and develop the chromatogram in a closed container, running it for a distance of about 15 cm with chloroform - acetone - glacial acetic acid (5 + 5 + 1). Remove the plate and examine it under ultraviolet light (254 and 366 nm).

Identification of Cephalosporins

Three cephalosporin antibiotics (cephaloridine, sodium cephalothin and cephalexin) are included in the British Pharmacopoeia 1973 and a further three (cephazoline, cephaloglycine and cephradine) are of commercial interest. The members of this group vary widely in their solubility in water and other solvents, but it was found that solutions of the desired concentration could be obtained in 5 per cent. *m/V* sodium hydrogen carbonate solution.

The thin-layer chromatographic test for penicillins can also be applied to cephalosporins, except that in this instance heating is effected for 1 h at 150 °C and the developed plate is sprayed with a freshly prepared mixture of equal volumes of a 0·135 per cent. *m/V* aqueous solution of platinum(VI) chloride and a 1·1 per cent. *m/V* aqueous solution of potassium iodide and then examined under ultraviolet and visible light. However, differentiation between the very closely related pair cephalexin and cephradine is small. For this reason the possibilities of carrying out the separation by electrophoresis rather than by thin-layer chromatography were explored. It was found that suitable patterns could be obtained with the electrophoresis conditions described in the B.P. 1973 under the test for related substances in the monograph for cephaloridine (see Fig. 8).

Fig. 1. 1, Betamethasone 17-valerate; 2, progesterone; 3, testosterone propionate; 4, ethynodiol diacetate; 5, hydroxyprogesterone hexanoate; 6, dydrogesterone; 7, fluocortolone hexanoate; 8, fluocortolone pivalate. Antec silica gel plate, without binder, sprayed with zinc chloride reagent and viewed under 254-nm light.

Fig. 2. As Fig. 1, viewed under 254- and 366-nm light.

Fig. 3. As Fig. 1, viewed under 366-nm light.

Fig. 4. 1, Betamethasone 17-valerate; 2, megestrol acetate; 3, chlormadinone acetate; 4, triamcinolone acetonide; 5, fluocinolone acetonide; 6, norethisterone; 7, norethynodrel; 8, betamethasone 17-valerate. Zinc chloride spray, on Antec silica gel plate, viewed under 254- and 366-nm light.

Fig. 5. Extract of Secrodyl tablets spotted at bottom right. First development (right to left) yields 1, 2, 4 (dimethisterone) and 6. Standards (3, dimethisterone and 5, ethinyloestradiol) then added. After heating to decompose, second development vertical. Ethinyloestradiol below detection limit at loading used. Zinc chloride spray, on Antec silica gel plate, viewed under 254- and 366-nm light.

Fig. 6. 1, Tetracycline; 2, lymecycline; 3, chlortetracycline; 4, demeclocycline; 5, oxytetracycline; 6, doxycycline; 7, methacycline. Antec silica gel plate, viewed under 366-nm light.

Fig. 7. 1, Propicillin; 2, cloxacillin; 3, phenethicillin; 4, methicillin; 5, ampicillin; 6, amoxycillin; 7, carbenicillin; 8, benzylpenicillin; 9, phenoxymethylpenicillin; 10, procaine penicillin; 11, benzathine penicillin. Merck silica gel plate, no spray, viewed under 254- and 366-nm light.

Fig. 8. 1, Cephradine; 2, cephaloglycine; 3, cephalexin; 4, cephazolin; 5, cephalothin; 6, cephaloridine. Electrophoretogram on Whatman 3MM paper, cathode at top. Sprayed with iodoplatinate reagent and viewed under visible light.

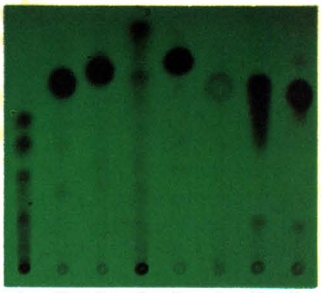

Fig. 1

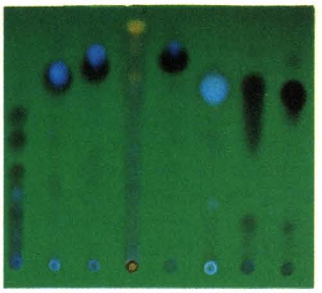

Fig. 2

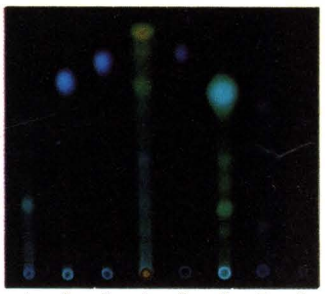

Fig. 3

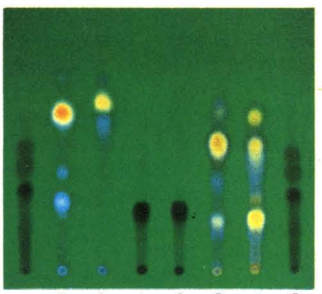

Fig. 4

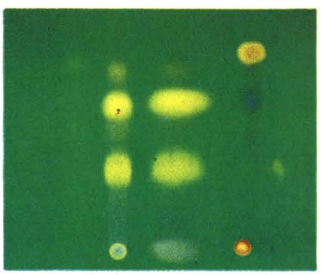

Fig. 5

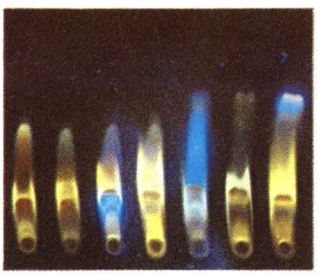

Fig. 6

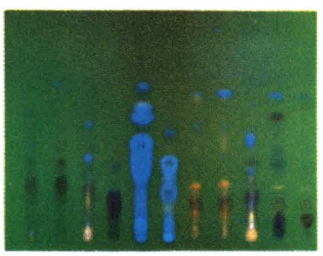

Fig. 7

Fig. 8

Paper-electrophoresis Method

Dissolve 5 mg each of the reference and unknown samples in 0·1-ml amounts of 5 per cent. *m/V* sodium hydrogen carbonate solution. Spot 5-μl amounts of the resulting solutions on to Whatman 3MM paper (35 cm long and of sufficient width for the number of samples to be tested) along a line drawn across the paper such that, when placed in the electrophoresis chamber, the line will be 7·5 cm from the anode compartment. Allow a 2·5-cm gap between each spot and 2 cm clear at each edge of the paper. Place the paper in an oven at 100–110 °C for 5–10 min, then carry out electrophoresis as described in the test for related substances under Cephaloridine in the British Pharmacopoeia 1973, using a buffer prepared by diluting a mixture of 5 ml of formic acid, 25 ml of glacial acetic acid and 30 ml of acetone to 1 l with water. Apply a potential of 20 V cm^{-1} for 2 h. Finally, switch off the current, spray the paper with iodoplatinate reagent (see above) and examine it immediately. If a permanent record is required, photograph the wet paper without delay.

Discussion

The proposed thin-layer chromatographic test appears to be suitable for general application to the identification of complex organic substances and has shown good discrimination within the main classes of compounds studied. For the cephalosporin group of antibiotics, separation by electrophoresis of the thermal degradation products on paper gives slightly better resolution.

Trace amounts of impurities or degradation products in the reference sample do not usually cause difficulties in matching patterns. When the only available reference material is of low purity, the two-dimensional clean-up technique for test samples can be applied instead to the reference. If both the sample under test and the reference require clean-up the following technique can be used.

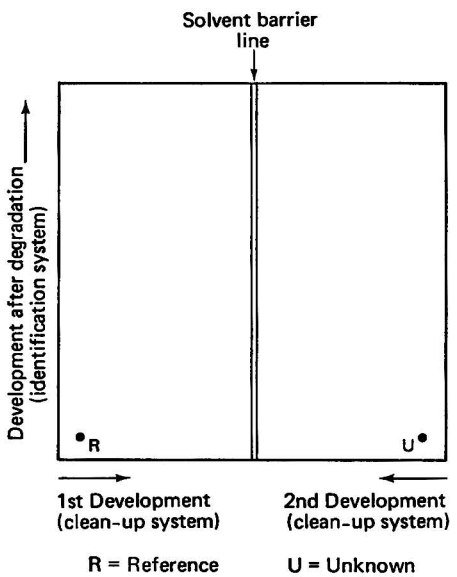

Fig. 9. Plate for separate two-dimensional thin-layer chromatogram of sample and reference.

A 20-cm square chromatographic plate is divided into two equal rectangles with a central line scored through the coating medium to serve as a solvent barrier. With the line vertical the unknown and reference solutions are spotted separately near the two bottom corners of the plate (see Fig. 9). Both spots are developed in sequence towards the central line until, in each instance, the clean-up solvent system reaches the line. After the plate has dried, thermal degradation is carried out and the plate is then developed at right-angles to the previous directions with the identification solvent system.

The test can be considered to belong to the group of techniques, which includes pyrolysis gas - liquid chromatography, activation analysis and mass spectrometry, that depend on the behaviour of fragments of the substance of interest. There are clear advantages in possessing a single, simple identity test that is applicable to groups of similar compounds but does not require complex instrumentation. The technique is flexible in that numerous combinations of thin-layer chromatographic support medium, time and temperature of heating, developing solvent and, if necessary, spray reagent are possible for application to different classes of compounds.

The accompanying figures are reproduced for illustrative purposes only. The patterns obtained are influenced by small differences in experimental conditions and those shown are not necessarily suitable for use as reference patterns.

We thank Beecham Research Laboratories for samples of some penicillins.

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Fungicide Residues

Part IV.* Determination of Residues of Carboxin in Grain by Gas Chromatography

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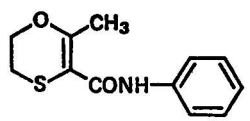
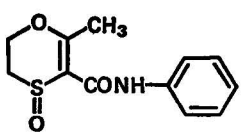
A simple method for determining residues of carboxin in grain is presented. The carboxin is extracted from the sample with acetone in a Soxhlet extraction apparatus and, after concentration of the extract, is determined by gas - liquid chromatography using a nitrogen-selective detector. The presence of carboxin is confirmed by the use of a sulphur flame-photometric detector.

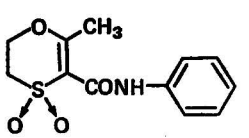
Carboxin (2,3-dihydro-6-methyl-5-phenylcarbamoyl-1,4-oxathiin, **I**) is a systemic fungicide used for seed treatment of cereals against smuts and bunts. Carboxin is included in the list of Approved Products for Farmers and Growers[1] for use with organomercury as a seed dressing in order to control loose smut and other seed-borne diseases of barley and wheat. It is used at the rate of 3 oz of active ingredient per 100 lb of seed, which corresponds to about 200 mg kg^{-1} on the grain.



I



II



III

In plants, carboxin is resistant to hydrolysis but has a tendency to oxidise to the sulphoxide (**II**) and the sulphone (**III**, oxycarboxin).[2–4] The latter is also used as a fungicide for the control of rusts on cereals.

Lane[5] developed a method for the determination of carboxin residues in plants that was based on hydrolysis and colorimetric determination with 4-dimethylaminobenzaldehyde of the aniline thus liberated. Sisken and Newell[6] modified this method for use on grains and other seeds and used gas chromatography with a microcoulometric nitrogen detector for the determination of the liberated aniline. Neither of these methods will distinguish between carboxin, carboxin sulphoxide, oxycarboxin or other carboxanilides. Chin, Stone and Smith[2] used gas chromatography with a microcoulometric sulphur detector for the determination of carboxin in their studies on plant metabolism. A bioassay technique has been described by Solel and Pinkas[7] for the determination of carboxin in lemon seedlings, but few laboratories are equipped to carry out this type of analysis.

A method has been developed for the direct determination of extractable residues of carboxin in grain by using gas chromatography with a nitrogen-selective detector. An attempt to determine carboxin sulphoxide and oxycarboxin simultaneously was unsuccessful because the sulphoxide gave a peak of very short retention time in a region where peaks from other co-extractives appeared. This peak has a retention time corresponding to that of aniline and may be caused by aniline formed from the decomposition of the sulphoxide; Bowman and Beroza[8] have reported that sulphoxides decompose on gas-chromatographic columns at high temperatures. The response to oxycarboxin varied by ± 25 per cent. but gas chromatography can be used for the qualitative detection of oxycarboxin. The presence of carboxin and oxycarboxin can be confirmed by the use of a sulphur flame-photometric detector.

* For Part III of this series, see *Analyst*, 1973, **98**, 172.

Experimental

Samples of barley and wheat were obtained from various sources, the pesticide treatment of certain of these being known. The grain was ground, extracted with acetone for 4 h in a Soxhlet extraction apparatus and the extract concentrated in a Kuderna-Danish evaporator. The carboxin content was determined by gas - liquid chromatography using a 5 per cent. OV-17 column (column 1) with a rubidium chloride thermionic detector. For confirmation, a 3 per cent. OV-225 column (column 2) with a sulphur flame-photometric detector was used for carboxin and a 5 per cent. OV-101 column for oxycarboxin.[8] Relative retention times of other fungicides and insecticides used on grain are given in Table I. Although retention times are quoted for organochlorine compounds, the response of the rubidium chloride thermionic detector to these compounds is of the order of one twentieth of its response to organonitrogen compounds.

TABLE I

RELATIVE RETENTION TIMES OF CARBOXIN AND OTHER PESTICIDES

Compound	Retention time on column 1 (carboxin = 1·00)	Retention time on column 2 (carboxin = 1·00)
Chloraniformethan	0·10	—
Tridemorph	0·13	—
Demeton-*S*-methyl	0·16	—
Thiometon	0·19	—
γ-BHC	0·21	—
Dimethoate	0·25	0·33
Formothion	0·34	0·47
Ethirimol	0·35	—
Malathion	0·38	0·33
Dieldrin	0·70	—
Carboxin	1·00*	1·00†
pp'-DDT	1·18	—
Oxycarboxin	2·46	—

* Carboxin retention time = 9 min.
† Carboxin retention time = 6 min.

Reagents

Acetone. Laboratory-reagent grade; check its suitability for gas chromatography before use.

Carboxin standard solution. Dissolve 20·0 mg of carboxin in acetone and dilute to 100 ml with acetone. Further dilute this solution as necessary with acetone.

Apparatus

Grinder. A small coffee grinder.

Soxhlet extraction apparatus. This consisted of a 250-ml flat-bottomed flask, a Soxhlet extractor of 60-ml capacity (Quickfit No. Ex 5/55) and a double-surface condenser.

Extraction thimbles. These were of single thickness, 28 mm i.d. × 80 mm long.

Evaporator. A 500-ml capacity Kuderna-Danish evaporator was used. In order to reduce the final volume, if necessary, a glass micro-Snyder column was used.

Gas chromatograph. Column 1 consisted of 5 per cent. OV-17 on Gas-Chrom Q (80–100 mesh) in a 150 × 0·4 cm i.d. glass column contained in a Pye 104 gas chromatograph that was fitted with a rubidium chloride thermionic detector (column temperature 230 °C, detector temperature 350 °C and injector temperature 240 °C). Nitrogen was used as the carrier gas at a flow-rate of 50 ml min^{-1} and the flame gases were hydrogen at 25 ml min^{-1} and air at 180 ml min^{-1}. Column 2 consisted of 3 per cent. OV-225 on Gas-Chrom Q (80–100 mesh) in a 150 × 0·4 cm i.d. glass column contained in a Pye 104 gas chromatograph that was fitted with a United Analysts flame-photometric detector operated in the sulphur mode (column temperature 240 °C and injector temperature 240 °C). Nitrogen was used as the carrier gas at a flow-rate of 45 ml min^{-1} and the flame gases were hydrogen at 80 ml min^{-1} and oxygen at 7 ml min^{-1}.

Procedure

Grind a 20-g sample of the grain for 30 s and transfer it to an extraction thimble. Place the thimble in the Soxhlet extraction apparatus and extract the sample with acetone for 4 h,

using about 200 ml of acetone. Adjust the rate of heating so that the solvent cycles about twenty-five times per hour. Next, decant the acetone extract into the Kuderna-Danish evaporator and then concentrate it to a volume of 4 ml on a steam-bath. The sensitivity of the method can be increased by reducing the volume to 2 ml by use of a micro-Snyder column. Then, inject 2 μl of the sample solution on to column 1 and compare the peak height with those obtained for 2-μl injections of standard solutions. A calibration graph, prepared by injecting 2-μl volumes of standard solutions and plotting the resultant peak heights against the masses of carboxin, showed that the rubidium chloride detector response was linear over the range 2·0–40 ng. With a signal to noise ratio of 3:1 the limit of detection was 500 pg of carboxin.

Inject 5 μl of the sample solution on to column 2 for confirmatory purposes. The flame-photometric detector gives a response proportional to the square of the concentration; the limit of detection was about 5 ng of carboxin.

Results and Discussion

Carboxin is soluble in a number of organic solvents. Acetone, methanol and ethyl acetate were used for the extraction of carboxin from grain and it was found that acetone was the most suitable solvent. Methanol extracted more interfering co-extractives than acetone, and with ethyl acetate, an additional peak was observed in the gas chromatogram at a retention time of 0·7 relative to carboxin. This peak appeared only when solutions had been stored in daylight and it increased in size on storage, whereas the peak for carboxin decreased. Although ethyl acetate was the best solvent for extraction purposes, as less co-extracted material was obtained, it was considered unsuitable because of the photochemical breakdown of carboxin. Acetone extracts of grain containing carboxin could be kept for 5 days without any apparent detrimental effect. A Soxhlet extraction apparatus was used because blending the sample with the solvent was not as efficient.

TABLE II

RECOVERY OF CARBOXIN FROM GRAIN USING COLUMN 1

Grain	Carboxin added/mg kg^{-1}	Carboxin recovered, per cent. Mean	Range
Barley, sample 1	1·0	73 (3)*	69 – 76
Barley, sample 2	1·0	78 (3)	76 – 82
Barley, sample 3	1·0	80 (3)	77 – 84
Barley, sample 1	10·0	78 (4)	74 – 82
Wheat	0·5	73 (4)	70 – 80
Wheat	1·0	75 (8)	68 – 82
Wheat	4·0	78 (4)	74 – 81

* Number of determinations in parentheses.

The recovery of carboxin from samples of barley and wheat was checked by adding known volumes of a standard solution to 20-g portions of the whole grain and allowing the solvent to evaporate at room temperature in a draught of air. The samples were then treated as

TABLE III

RELATIVE RETENTION TIMES OF VARIOUS PESTICIDES ON OV-101

Compound	Retention time on 5 per cent. OV-101 column (carboxin = 1·00)
Demeton-*S*-methyl	0·25
Thiometon	0·30
Dimethoate	0·31
Formothion	0·39
Malathion	0·53
Carboxin	1·00*
Oxycarboxin	1·70

*Carboxin retention time $\approx$ 5·0 min.

described under Procedure. The results obtained by using column 1 for the gas-chromatographic determination are shown in Table II, while the retention times of various pesticides on the column of OV-101 are given in Table III.

No carboxin was found to be present in the samples of barley and wheat examined. Phenylmercury acetate and ethylmercury chloride, which are used in carboxin formulations, did not interfere. An increase in the standing current of the detector and a loss of resolution may be experienced after repeated injections of the uncleaned extracts on to column 1. It is recommended that sample and standard solutions be injected alternately and that the first 10 cm of the column packing be replaced after 50 sample injections.

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A Comparison of the Extraction of Mercury from Sediments by Using Hydrochloric - Nitric Acid, Sulphuric - Nitric Acid and Hydrofluoric Acid - Aqua Regia Mixtures

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A study of the extraction of mercury from various sediments with hydrochloric - nitric acid, sulphuric - nitric acid and hydrofluoric acid - aqua regia mixtures is reported. The mercury in the extract is detected by using an automated cold-vapour atomic-absorption technique. Although greater extraction of mercury from sediments is possible with a hydrofluoric acid - aqua regia mixture than is possible with hydrochloric - nitric acid and sulphuric - nitric acid mixtures, the results, for the purposes of environmental surveys, are equivalent. The lower detection limit, better precision and simpler application of the methods involving the last two mixtures make them preferable. The hydrofluoric acid - aqua regia extractions were performed in a PTFE bomb.

A critical study of the extraction of mercury from sediments was initiated because some discrepant values for hydrofluoric acid extractable mercury were revealed in a recent study on sediment samples. It was thought that these values occur because the hydrofluoric acid treatment resulted in complete dissolution of silica and thus additional mercury was released. Several opinions on this matter have already been expressed in the literature. For instance, Head and Nicholson[1] have indicated that for geological samples hydrofluoric acid would be necessary for complete recovery of mercury. Melton *et al.*[2] have shown that for soils, the use of hydrofluoric acid fails to extract additional mercury. Iskandar *et al.*[3] have studied sediments extensively by using a sulphuric - nitric acid digestion procedure and they describe a method for determination of total mercury content. A variety of other extraction methods have also been studied. Hatch and Ott[4] used sulphuric acid and hydrogen peroxide for rock samples. Cranston and Bunkley[5] used sulphuric and nitric acids for sediments. Jonasson *et al.*[6] used nitric and hydrochloric acids for rocks, Klein[7] used nitric acid and potassium permanganate for soils. Muscat *et al.*[8] used sulphuric and nitric acids with potassium permanganate and potassium persulphate.

Of the various methods available, three were chosen in order to compare the extractable mercury when using identical sub-samples of sediments. The extraction mixtures were (*a*), hydrochloric - nitric acid (1 + 9), (*b*), sulphuric - nitric acid (2 + 1) and (*c*), hydrofluoric acid - aqua regia (6 + 1). All extracts were digested with solutions of potassium permanganate and potassium persulphate subsequent to acid leaching. As will be shown in the experimental section, these three extraction procedures are adaptable to the automated cold-vapour technique for the determination of mercury reported by Goulden and Afghan.[9,10] The first method (using hydrochloric - nitric acid) is an extraction system that is widely used for geological samples.[6] Small changes in the ratio of hydrochloric to nitric acid are not critical. An excessive amount of hydrochloric acid should, however, be avoided as generation of chlorine[11] would lead to the loss of volatile mercury chlorides.[12,13] A noteworthy feature of the hydrochloric - nitric acid digestion mixture is its ability to decompose cinnabar, and thus it is suitable for geological application.[6] The second method (using sulphuric - nitric acid) has been described by Iskandar *et al.*[3] The method is suitable for the analysis of sediments as the oxidising character of the extraction mixture is adequate for the destruction of organic matter.[14] The third method is the hydrofluoric acid bomb method described by Buckley and Cranston.[15] The main feature of the method is that it is a sealed system, capable of high digestion temperatures and complete dissolution of the sediment can be effected. Although used for the determination of heavy metals,[16–19] and of mercury in ores,[20] it is a method that has not yet been reported for the determination of mercury in sediments.

Experimental

Apparatus

Digestion of samples in sulphuric - nitric acid (2 + 1) or hydrochloric - nitric acid (1 + 9) was performed in 100-ml calibrated flasks in a temperature-controlled shaker bath at 50–60 °C (Model 75, Precision Scientific Co.). For samples digested with hydrofluoric acid - aqua regia (6 + 1) the Parr 4745 acid-digestion bomb (Parr Instrument Co., Moline, Ill. 61265, U.S.A.) was used for the decomposition.

The equipment used for the analysis consisted of (*a*), an automatic sampler (Technicon Auto-Analyzer II with 20–1/5 cam), (*b*), a proportionating pump (Carlo Erba, Model 08–59–10202), (*c*), Technicon AutoAnalyzer tubing of specified dimensions (Fig. 1), (*d*), a gas separator as used by Goulden and Afghan,[9,10] (*e*), a mercury monitor (Pharmacia Fine Chemicals) and (*f*), a strip-chart recorder (Hewlett-Packard, Model 7101B).

The system (Fig. 1) is similar to that used by Goulden and Afghan[9,10] and that used by Bailey and Lo[21] except that no heated digestion bath is employed, and only one reagent line is used [for tin(II) sulphate - sulphuric acid].

Reagents

High-purity certified reagents were used for all analyses. (Obtained from Fisher Scientific Co., Fairlawn, New Jersey 07410, U.S.A.)

Sulphuric acid, 36 N.
Nitric acid, 16 N.
Hydrochloric acid, 12 N.
Aqua regia [*hydrochloric - nitric acid* (3 + 1)].
Hydrofluoric acid, 48 *per cent.*
Boric acid. Analytical-reagent grade.
Potassium permanganate solution, 6 *per cent.* m/V.
Potassium persulphate solution, 5 *per cent.* m/V.
Tin(II) sulphate solution, 10 *per cent.* m/V *in* 2 N *sulphuric acid.*
Hydroxylammonium sulphate (6 *per cent.* m/V) - *sodium chloride* (6 *per cent.* m/V) *solution.*
Standard mercury solutions. Standards were prepared by serial dilution of a 1000 mg l^{-1} mercury stock solution [mercury(II) chloride, Fisher Scientific]. All standard solutions were prepared so as to contain all reagents added to samples.

Procedure

All sediment samples were air dried at room temperature and crushed to a mesh size of lower than 200 (ASTM, 75 μm).

A. Sulphuric - nitric acid digestion

Representative samples of about 0·1–1 g of powdered sediments were weighed into 100-ml calibrated flasks. Fifteen millilitres of sulphuric - nitric acid (2 + 1) were added and samples were digested for 2 h in a shaking water-bath at 50–60 °C. After digestion, the flasks were allowed to cool and 10 ml of potassium permanganate solution were added carefully while cooling the flasks in an ice - water bath. After half an hour 5 ml of potassium persulphate solution were added, with gentle stirring. Solutions were left to digest at room temperature overnight.

B. Hydrochloric - nitric acid digestion

The procedure given in A was followed except that 15 ml of hydrochloric - nitric acid (1 + 9) were used instead of 15 ml of sulphuric - nitric acid (2 + 1).

C. Hydrofluoric acid - aqua regia digestion

Representative samples of about 0·1–0·5 g of powdered sediments were weighed into the PTFE bombs and 6 ml of hydrofluoric acid and 1 ml of aqua regia were added. The sealed bombs were then heated at 110 °C for 2 h in an oven. After digestion the mixture was transferred into a 125-ml polypropylene wide-mouthed bottle containing 4·8 g of boric acid. The mixtures were shaken and treated with potassium permanganate and potassium persulphate as in A above.

The solutions were then transferred into 100-ml calibrated flasks (the boric acid reacts with hydrofluoric acid to form hydrofluoroboric acid[16] and so does not attack the glass).

Whichever digestion procedure had been used, 10 ml of hydroxylammonium sulphate - sodium chloride solution were added to the flasks in order to reduce the precipitated manganese(IV) oxide and the excess of oxidant. This solution is a mild reducing agent and does not reduce the mercury to the elemental state, which was tested by monitoring the concentration of mercury in such solutions for up to 3 d. No change in the mercury concentration was detected. The above method provides clear solutions, which can be analysed by an automatic sampler using the automated system shown in Fig. 1. The advantages of and reasons for using hydroxylammonium sulphate - sodium chloride solution are discussed in depth by Agemian and Chau.[22]

Solutions were made up to volume and an aliquot centrifuged at 2500 rev min^{-1} for 5 min. Aliquots of the clear supernatants were transferred into glass sample cups and placed in the automatic sampler. The manifold in Fig. 1 was used for the automated analysis of all the solutions. A cam designed for 20 samples per hour and a 1:5 sample to wash ratio was used in the automatic sampler.

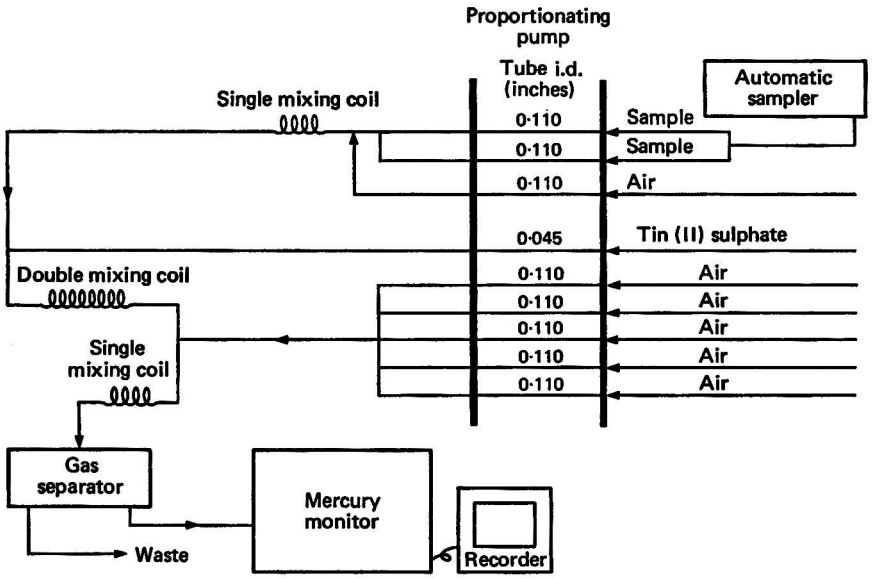

Fig. 1. Mercury manifold.

Results and Discussion

As stated above, all sediments used in this study were air dried and crushed. Crushing the dried sediments to 200 mesh (ASTM, 75 μm) produced homogeneous samples as is shown by the good repeatability of most results. Even though losses may occur in the sample preparation,[3,23,24] this does not prevent a careful comparison as the same dried sample was employed for all extractions.

Shown in Fig. 2 are the calibration graphs for the range 0 to 4 $\mu g\ l^{-1}$ of mercury. All four calibration graphs are linear up to about 6 $\mu g\ l^{-1}$ of mercury. Blank solutions consisting of reagents only (no sediment) gave rise to a signal corresponding to 0·5 $\mu g\ l^{-1}$ of mercury for the hydrofluoric acid - aqua regia blank and 0·25 $\mu g\ l^{-1}$ of mercury for the sulphuric - nitric acid blank. The blank for hydrochloric - nitric acid (not shown) was the same as for sulphuric - nitric acid. The different blank readings reflect the background mercury in the reagents employed (refer to the experimental section for details). In fact, these blank readings can be used to calculate the detection limits for the methods. For example, when 0·500-g test portions of sediment are taken for the hydrofluoric acid - aqua regia extraction, a detection limit of about 100 $\mu g\ kg^{-1}$ of mercury is found and if a 1·000-g portion is taken for the other two extraction methods a detection limit of about 25 $\mu g\ kg^{-1}$ of mercury is obtained. The latter value is well below the mercury content of the sediments normally analysed in this

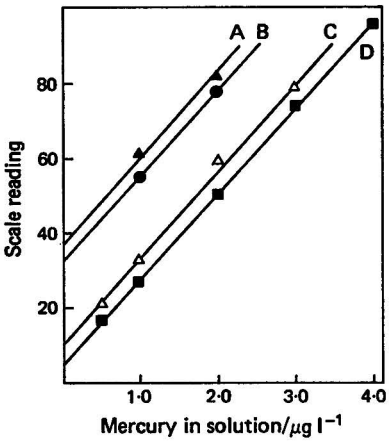

Fig. 2. Calibration graphs for the determination of mercury by the automated cold-vapour technique. All solutions contain 10 per cent. of 6 per cent. potassium permanganate, 5 per cent. of 5 per cent. potassium persulphate and 10 per cent. of 6 per cent. hydroxylammonium sulphate - sodium chloride solutions in addition to the acids used for digestion of the sediments. A, Standard additions graph on a sediment extract containing 6 per cent. of 48 per cent. hydrofluoric acid and 1 per cent. of aqua regia. B, Standard additions graph on a sediment extract containing 15 per cent. of a sulphuric acid - nitric acid mixture (2 + 1). C, Curve for standard solutions containing 6 per cent. of 48 per cent. hydrofluoric acid and 1 per cent. of aqua regia. D, Graph for standard solutions containing 15 per cent. of a sulphuric acid - nitric acid mixture (2 + 1).

laboratory. A statistical assessment of the hydrofluoric acid - aqua regia blank revealed a coefficient of variation of 13 per cent. for the blank reading. The other two extraction procedures have a smaller coefficient of variation.

In order to determine the recovery for the hydrofluoric acid - aqua regia and sulphuric - nitric acid extraction procedures, a standard addition analysis was performed on one of the sediments. The data for the hydrochloric - nitric acid extraction method are not shown because they were similar to those for the sulphuric - nitric acid extraction method. The results of this study are shown graphically in Fig. 2. Recovery and precision data are given in Tables I and II. The hydrofluoric acid - aqua regia method is clearly less precise than the other two.

TABLE I

RECOVERY DATA

All mercury levels are means.

Extraction mixture	Number of trials	Unspiked sediment*/ μg kg⁻¹	Amount of mercury added/ μg kg⁻¹	Amount of mercury found/ μg kg⁻¹	Recovery, per cent.	Coefficient of variation, per cent.
H_2SO_4 - HNO_3	6	597	0	597	—	2·1
	6	597	500	1115	102	1·9
	6	597	1000	1627	102	2·3
HF - aqua regia	4	575	0	575	—	9·4
	6	575	500	1132	105	9·6
	6	575	1000	1566	99	5·0

* The sediment used for this study is sample 14 (see Table III).

TABLE II

COMPARISON OF PRECISIONS OF THE THREE METHODS

All data were calculated on ten replicate samples.

Sample number*	H_2SO_4 - HNO_3 Mercury/ $\mu g\ kg^{-1}$	H_2SO_4 - HNO_3 Coefficient of variation, per cent.	HCl - HNO_3 Mercury/ $\mu g\ kg^{-1}$	HCl - HNO_3 Coefficient of variation, per cent.	HF - aqua regia Mercury/ $\mu g\ kg^{-1}$	HF - aqua regia Coefficient of variation, per cent.
15	64	14	96	13	110	17
5	2000	2·7	2100	1·7	2100	5·7

* See Table III.

All methods gave almost 100 per cent. recovery. It is clear that for all three methods the coefficient of variation increases as the concentration of mercury in the sample decreases. Also, the data in Table II confirm that the sulphuric - nitric acid and hydrochloric - nitric acid extraction methods have essentially the same precision and that it is better than that of the hydrofluoric acid - aqua regia extraction method. It is worth noting that the automated analysis procedure has been shown to have better precision than the manually operated methods.[25] Thus, the major variation in precision is not governed by the analyst but by the extraction system, which is confirmed by the fact that standard solutions of mercury in the three extraction mixtures studied could be prepared and processed through the entire method with less than a 2 per cent. coefficient of variation. (These data are not shown.)

In order to check the three methods for consistency a series of sediments was analysed in duplicate. The average results for 15 such samples are given in Table III. The feature to note in these results is the higher values obtained with the hydrofluoric acid - aqua regia extraction method. The sulphuric - nitric acid and hydrochloric - nitric acid extraction methods give comparable results. These data indicate that some mercury may be associated with the silica matrix of the sediment as the basic difference between the sulphuric - nitric acid and hydrochloric - nitric acid extraction methods compared with the hydrofluoric acid - aqua regia extraction method is the ability of the latter to effect complete dissolution of the silicate lattice.

The analysis of environmental and geological samples for mercury content requires reliable, rapid and precise techniques. A number of surveys have recently been reported.[12,13,26–30] Irrespective of how or in what form mercury is introduced into lake or stream sediments it eventually becomes associated with fine-grained inorganic or organic particles.[27] Mercury in sediments has been shown by many workers[13,16,29] to be adsorbed on iron oxides, on sulphide surfaces and on clay particles, in the form of inorganic phosphorus complexes, in humate complexes and as methyl- and dimethylmercury produced by microbial action. Furthermore,

TABLE III

COMPARISON OF RESULTS ($\mu g\ kg^{-1}$ OF MERCURY) FOR THE THREE METHODS

	Sample	Method: HCl - HNO_3	Method: H_2SO_4 - HNO_3	Method: HF - aqua regia
1.	Chert (mineral)	—	1850	2100
2.	Andesite (mineral)	—	440	750
3.	Soil, B horizon	—	130	240
4.	Soil, A horizon	—	140	190
5.	Soil (Ontario)	2000	2100	2100
6.	Soil (Ontario)	900	970	1150
7.	Soil (Ontario)	240	250	270
8.	Silty clay (L.H.*)	—	135	285
9.	Sand (L.H.*)	—	65	120
10.	Silty clay (L.H.*)	—	48	95
11.	Silt (L.O.†)	320	360	390
12.	Silty clay (L.O.†)	1000	1000	1000
13.	Silty clay (L.O.†)	1600	1600	1500
14.	Silty clay (L.O.†)	600	600	600
15.	Silty clay (L.H.*)	96	64	110

* Lake Huron sediment.
† Lake Ontario sediment.

it is recognised[23,29] that there exists a high correlation between the mercury content and the organic matter content of lake sediments.

The results in Table III tend to confirm these facts as the difference between the results obtained by hydrofluoric acid - aqua regia extraction and those obtained by the other two methods are not very significant from an environmental point of view. This conclusion correlates with the approach taken by workers concerned with environmental problems who are chiefly concerned with gross variations. In fact, it has been stated[27] that errors (20 per cent.) due to loss of mercury in the drying or crushing of sediments are not a serious matter. Thus, of the three extraction methods studied the sulphuric - nitric acid and hydrochloric - nitric acid extraction methods are preferable to the more rigorous hydrofluoric acid - aqua regia extraction method because of their lower detection limit, better precision, easier analytical handling and processing and suitability to environmental work. Although the first two extraction methods have very similar recovery and precision (Tables II and III) and are thus analytically equivalent, the hydrochloric - nitric acid method has the disadvantage that when hydrochloric acid is used, on addition of potassium permanganate to oxidise the organomercury in the sample, violent frothing of the solution with evolution of chlorine gas makes the analysis very cumbersome. Therefore, the sulphuric - nitric acid method is recommended.

Conclusions

Three different extraction procedures used for the determination of mercury in sediments (100–2000 $\mu g\ kg^{-1}$ of mercury) have been compared. The sulphuric - nitric acid and hydrochloric - nitric acid extractions have similar precision and give identical results. The hydrofluoric acid - aqua regia extraction performed in a sealed PTFE bomb has less adequate precision and liberates a slightly larger amount of mercury than the other methods. Although the hydrofluoric acid - aqua regia method gives significantly larger values, the results are not sufficiently different to affect the outcome of any environmental survey.

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Determination of Trace Amounts of Phosphorus in Trichloro- and Tetrachlorosilane

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A method is described for the determination of phosphorus below the parts per million level in halosilanes. The sample is hydrolysed in an enclosed vessel and the silicic acid formed is volatilised off. Phosphorus is extracted as molybdophosphate into chloroform - n-butanol mixture. The molybdenum associated with the extracted phosphorus is determined by the thiocyanate method.

During the preparation of semiconductor grade silicon the problem of determining the phosphorus content of halosilanes arose. Two methods of determining phosphorus in these materials have been reported. Lancaster and Everingham[1] separated phosphorus halides from tetrachlorosilane by extraction with small amounts of concentrated sulphuric acid, while Lipshits and Kiseleva[2] hydrolysed the material in open polythene beakers and volatilised off the silica before determining the phosphorus as molybdovanadophosphate. We found that the former method gave incomplete extraction of the phosphorus and that the latter resulted in loss of phosphorus. In order to carry out determinations at the parts per billion (10^9) level the use of either a large sample size or of long cell path lengths for absorbance measurements has been recommended. The sensitivity of the method for the determination of phosphorus is considerably improved by extracting it as molybdophosphate and then ascertaining the molybdenum content by spectrophotometry[3,4] or by atomic-absorption spectrometry.[5] The sensitivity of the molybdenum determination[4] is improved when the molybdenum is extracted as its thiocyanate into butyl acetate.[6] However, this method is less sensitive than that in which 2-amino-4-chlorobenzenethiol is used to complex the molybdenum.[3]

This paper describes an improved procedure based on the use of a closed extraction system for the hydrolysis of the material, followed by the removal of the silica by volatilisation, extraction of molybdophosphate and determination of the phosphorus indirectly[6] from the amount of molybdenum extracted.

Experimental

Reagents

Hydrofluoric acid. Analytical-reagent grade acid was distilled in PTFE apparatus, when the phosphorus content was reduced from about 60 p.p.b. to less than the 2 p.p.b. level.

Standard phosphorus solutions, aqueous. Dissolve 0·4263 g of anhydrous diammonium hydrogen orthophosphate in water and make the volume up to 1 l. Suitably dilute this solution to give a working solution containing 1 μg ml^{-1} of phosphorus.

Standard phosphorus solution, non-aqueous. Suitably dilute, in stages, phosphorus oxychloride ($POCl_3$, 99·99 per cent. pure; supplied by the Chemistry Division, B.A.R.C.) with carbon tetrachloride to give a working solution containing 1 μg ml^{-1} of phosphorus.

Solvent mixture. Chloroform - butan-1-ol (4 + 1 V/V), both reagents being analytical-reagent grade.

All other reagents and concentrations used in the spectrophotometric determination of molybdenum were identical with those previously described.[6]

Apparatus

The apparatus recommended by Lancaster and Everingham[1] for the extraction of phosphorus was used for the hydrolysis of halosilanes, and consists of a conical flask, a distillation head and a separating funnel, fitted together with ground-glass joints as shown in Fig. 1. Absorbance measurements were made with a Beckman spectrophotometer, Model DU, using a matched set of 1-cm cells.

Standard solutions of phosphorus oxychloride in carbon tetrachloride were analysed, following the hydrolysis step (omitting the volatilisation of the silica) in the closed apparatus, by the spectrophotometric method. The procedure is given in detail below. The results (1–4) given in Table I show that the volatile phosphorus oxychloride is hydrolysed without loss. Samples of trichloro- and tetrachlorosilane with standard additions of phosphorus

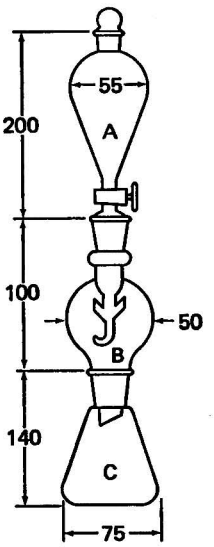

Fig. 1. Hydrolysis assembly. A, Separating funnel, 60 ml, 24/28; B, distillation head, 24/40; and C, Erlenmeyer flask, 150 ml, 24/28. All dimensions are in millimetres.

oxychloride were also analysed by following this procedure and the results obtained are included in the table. The recovery of added phosphorus was found to be satisfactory.

Procedure

Cool separately the various units of the apparatus, demineralised water and the sample vial containing trichloro- or tetrachlorosilane in water cooled with ice. Transfer 5 ml (7·0 g) of trichlorosilane (with a measuring cylinder) into the conical flask, kept in water cooled with ice, and immediately connect the distillation head and separating funnel. Introduce 75 ml of demineralised water into the separating funnel and allow it to drain slowly into the flask. Close the stopcock and allow the flask to stand in water cooled with ice for 20 min. Remove the separating funnel, wash it with demineralised water and run the washings through the distillation head into the conical flask.

Transfer the apparatus to a hot-plate and allow the mixture to boil for 15 min, then remove the distillation head, wash it with water and add the washings to the mixture in the conical flask. Transfer a small portion of the slurry and washings in the flask into a 100-ml platinum dish, add hydrofluoric acid, dropwise, until a clear solution is obtained and evaporate to very small volume, then repeat the procedure with further small portions until all of the slurry has been transferred. Finally, wash the conical flask with water, transfer the washings into the dish and evaporate the solution to small volume. Add 0·5 ml of 60 per cent. perchloric acid and evaporate to fumes. Cool the dish, add 10 ml of 2 N hydrochloric acid and warm it in order to dissolve the residue. Cool the solution, transfer it into a 50-ml separating

funnel, then add 10 ml of ammonium molybdate solution and 5 ml of water, mix, and extract the molybdophosphate twice with 10 ml of solvent mixture, shaking the funnel each time for 2 min.

Transfer the organic phase into another clean separating funnel (a different set of separating funnels should be used for high concentrations of ammonium molybdate). Add 10 ml of 2 N hydrochloric acid, shake the funnel for 1 min and transfer the organic phase into a beaker. Reject the aqueous phase and clean the funnel by washing it with 1 + 1 ammonia solution and then with water. Place the organic phase into the clean separating funnel and back-extract it first with 10 ml of 2 N ammonia solution and then with 10 ml of 2 N hydrochloric acid. Make the combined extracts up to a known volume, if necessary, and determine the molybdenum content in the whole volume (or in an aliquot) by the thiocyanate method.[6] Take the standards through the molybdophosphate extraction and thiocyanate determination steps of the procedure. Calculate the phosphorus content from a calibration graph.

Discussion

The present study was carried out in a dust-free room.

The absorbance for 1 μg of phosphorus in 10 ml of final organic phase is about 0·54 unit, when measured against the corresponding reagent blank for the procedure. The absorbance

TABLE I

DETERMINATION OF TRACE AMOUNTS OF PHOSPHORUS

Sample No.	Sample	Phosphorus added, p.p.m.	Phosphorus, p.p.m. Expected	Phosphorus, p.p.m. Obtained	Difference, p.p.m.
1	Standard $POCl_3$	—	0·10	0·10	—
				0·12	0·02
				0·11	0·01
2	Standard $POCl_3$	—	0·20	0·20	—
				0·18	−0·02
3	Standard $POCl_3$	—	1·0	1·07	0·07
				1·01	0·01
4	Standard $POCl_3$	—	2·0	1·98	−0·02
5	Trichlorosilane (1)	—	—	1·24	—
		1·0	2·24	2·38	0·06
		2·0	3·24	3·33	0·09
6	Trichlorosilane (2)	—	—	0·24	—
		0·1	0·34	0·36	0·02
		0·2	0·44	0·46	0·02
7	Tetrachlorosilane (1)	—	—	0·12	—
		0·1	0·22	0·24	0·02
		0·2	0·32	0·32	—
8	Tetrachlorosilane (2)	—	—	0·10	—
		0·1	0·20	0·22	0·02
		0·2	0·30	0·38	0·08
9	Trichlorosilane (3)	—	—	0·06	—

of the blank against the solvent mixture varies between 0·03 and 0·04, which corresponds to 8–9 p.p.b. of phosphorus on the 7-g sample basis. The coefficient of variation at the 1·7 and 0·09 p.p.m. levels of phosphorus (six determinations on a single sample) was found to be 7·6 and 17 per cent., respectively.

When halosilanes were hydrolysed in open beakers[2] loss of phosphorus was observed and when extraction of phosphorus with concentrated sulphuric acid was attempted (as recommended by Lancaster and Everingham[1]) it was found to be incomplete even after two extractions.

The sensitivity of the above spectrophotometric method is greater by about two orders of magnitude than that of the direct molybdovanadophosphate method followed by earlier workers,[1,2] and hence it is possible to reduce the sample size.

The authors are grateful to Dr. M. Sankar Das, Head of the Analytical Chemistry Division and Shri T. K. S. Murthy, Head of the Ore Extraction Section for their keen interest and helpful discussions during the progress of the work.

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A Method for Determining 2-Aminoethanephosphonic Acid in Rumen Contents

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The work described is an attempt to develop a reliable method for determining AEP when mixed with related substances from micro-organisms in the rumen. Hydrochloric acid hydrolysates of rumen contents, rumen bacteria, rumen ciliate protozoa and clarified rumen contents were applied to a column (10 × 1 cm) of Dowex 50–X8, eluted with 0·6 N hydrochloric acid and 2·4-ml fractions collected. Inorganic phosphorus was separated in column fractions 2–10 and AEP appeared in fractions 11–23.

When fractions containing AEP were spotted on to Whatman No. 1 filter-paper strips the developed chromatograms showed six ninhydrin-positive spots in addition to that of AEP, which was the slowest acid to migrate (R_F = 0·3). The substance giving spot No. 4, another phosphonic acid (R_F = 0·59), was present in ciliate protozoa and ciliate-free fractions of rumen contents, whereas AEP was confined to protozoa. A highly significant correlation was found between the AEP concentrations and protozoal counts in samples of rumen contents collected at different intervals. It is therefore suggested that the concentration of AEP can be used as a marker of the protozoal growth in the rumen.

2-Aminoethanephosphonic acid (AEP) was the first nitrogenous compound with a covalent carbon to phosphorus bond to be isolated from biological material. It has been isolated from rumen ciliate protozoa, a free-living protozoon (*Tetrahymena pyriformis*),[1–3] and the sea anemone (*Anthopleura elegantissa*).[4] This acid, with a related compound, 2-amino-3-phosphonopropionic acid, was later detected in the ciliate *Tetrahymena pyriformis*.[5] AEP is ninhydrin-positive and has a relative molecular mass of 125.[1] Rosenberg[3] found that AEP was present in protozoal protein but it is not present in bacterial or dietary protein.[6,7]

Abou Akkada *et al.*[6] therefore suggested the use of AEP as a marker to measure rumen protozoal population and noted that *Isotricha* sp. contained larger amounts of AEP than *Entodinium* sp. Methods of AEP assay were based on the resistance of the carbon to phosphorus bond to hydrolysis during 24 h of exposure to 6 N hydrochloric acid at 110 °C and the subsequent separation of AEP from orthophosphate by chromatography on either Dowex 50-H or Dowex 1 (acetate).[3] However, the substances isolated by these methods appeared to include AEP and related compounds with carbon to phosphorus bonds, which were found in the rumen protozoal cells.[6,7] The present work is therefore an attempt to find a reliable method for determining AEP, free from related substances, in the rumen micro-organisms of sheep. This work is a continuation of a study previously carried out by the senior author at the Rowett Research Institute in 1970.

Experimental

Source of Rumen Contents

Rumen contents were obtained from a sheep fitted with a permanent rumen canula and fed on a concentrate mixture [cottonseed cake and rice bran (1 + 1)] and Berseem (*Trifolium alexandrium*). The rumen contents were prepared and treated as previously described.[8]

Solutions

Magnesium nitrate solution, 10 per cent. in ethanol. This was prepared by dissolving 10 g of magnesium nitrate hexahydrate in ethanol and diluting the solution to 100 ml with more ethanol.

Ninhydrin solution, 0·1 per cent. Ninhydrin (0·1 g) was dissolved in ethanol (75 per cent. *V*/*V*) and the final volume made up to 100 ml.

Ammonium molybdate solution, 5 per cent. Ammonium molybdate (5 g) was dissolved in water and the solution diluted to a volume of 100 ml.

Formaldehyde solution, 10 per cent. Formaldehyde solution (40 per cent. *m/V*) (formalin) was diluted, 25 ml being mixed with 75 ml of water.

Fractionation of Rumen Contents

The contents of the rumen were strained through two layers of cheesecloth and left at 38 °C for 1 h; the surface layer was then discarded and the remainder centrifuged at 5000 rev min^{-1} for 15 min. The supernatant contained bacteria only and the residue consisted of protozoa.[6,7] The supernatant was then centrifuged at 15 000 rev min^{-1} for 30 min; the new residue was referred to as the bacterial fraction and the remainder as clarified rumen liquor. The new residue was repeatedly washed (three to five times) by re-suspension in citrate buffer (pH 6·5) and centrifugation, then dried at 105 °C, ground and kept in a vacuum desiccator. The clarified rumen liquor was concentrated over a steam-bath and saved for analysis. Portions (0·1 g) of the various fractions of rumen contents (protozoal and bacterial fractions and clarified rumen liquor) were hydrolysed in 5 ml of 6 N hydrochloric acid in a sealed test-tube at 105 °C for 48 h.

Portions (25 g) of whole rumen contents were hydrolysed in 125 ml of 6 N hydrochloric acid for 48 h in a Kjeldahl flask that was fitted with a ground-glass joint and connected to a Liebig condenser. The hydrolysate was filtered while hot through Whatman No. 42 filter-paper and the residue was washed three times with boiling 0·5 N hydrochloric acid, the washings being added to the filtrate; then boiling water was added in order to give a final volume of about 150 ml. This filtrate was evaporated to dryness on a boiling water bath and the residue washed with water and dried three times. The residue left after evaporation was transferred into a 10-ml calibrated flask with 0·6 N hydrochloric acid and the solution was diluted to the mark with the same acid.

Separation of the Acid

Portions (5 ml) of acid hydrolysate were introduced on to a column (10 cm long × 1 cm diameter) of Dowex 50-X8 and eluted with 0·6 N hydrochloric acid containing 1 per cent. Brij-35 detergent. The eluate was collected at room temperature in 2·4-ml fractions at the rate of approximately 45 ml h^{-1}. The fractions containing AEP, as indicated by the elution of a standard solution of AEP with 0·6 N hydrochloric acid on a control column, were pooled together and dried over a steam-bath by directing a small jet of air on to the surface of the liquid.

The residue was diluted with 0·5 ml of water and 20 μl of diluate were spotted on to strips of Whatman No. 1 filter-paper with a micropipette. A single-dimensional chromatogram with butan-1-ol - acetic acid - water (3 + 1 + 1) as solvent was prepared. The spots were made visible by use of ninhydrin solution, then the developed spots given by AEP were cut out and placed in a 50-ml test-tube. Ashing of the contents of the tube was conducted by adding 10 ml of concentrated nitric acid and heating nearly to dryness over a micro-Kjeldahl burner, followed by the addition of 0·1 ml of ethanolic magnesium nitrate solution and then heating on a direct, strong flame. Next, 4·1 ml of water, 0·5 ml of 10 N sulphuric acid, 0·2 ml of ammonium molybdate and 0·2 ml of Fiske and Subbarow reagent (sodium hydrogen sulphite, sodium sulphite and 1-amino-2-naphthol-4-sulphonic acid) were added; the mixture was then heated for 7 min and the colour was measured on a colorimeter at 830 nm.[9]

Relationship Between Protozoal Counts and AEP

AEP was determined in a number of rumen samples collected at 0, 3, 6, 9 and 12 h after feeding. The contents of the rumen (30 g) were strained through one layer of cheesecloth, the fibrous residue was washed with 40 ml of saline solution, then the washings were added to the strained rumen contents and the total mixture was diluted with an equal volume of 10 per cent. formaldehyde solution. A number of 5-ml portions of strained rumen contents diluted with formaldehyde solution were mixed with 5 ml of Lugol iodine solution and the protozoa in the fixed samples were counted under a microscope using a 0·2-mm depth of Eosinophil counter (Scientific Products, Kansas City, U.S.A.).

An absorbance calibration graph for 2-aminoethanephosphonic acid, obtained by subjecting samples of AEP to single-dimensional filter-paper chromatography, is a straight-line graph,

passing through the origin. The 10-cm Dowex 50-X8 column is capable of separating inorganic phosphorus from the phosphonic acid fraction, as is clearly demonstrated in Fig. 1.

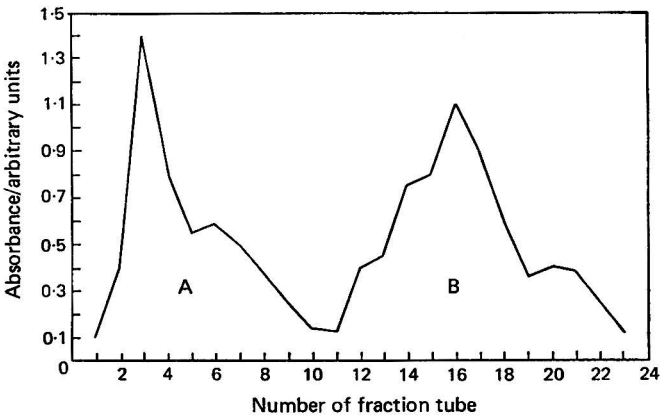

Fig. 1. Separation of inorganic phosphate and 2-aminoethanephosphonic acid on column. A, Potassium dihydrogen orthophosphate; B, 2-aminoethanephosphonic acid.

While inorganic phosphorus was separated in fractions 2–10, AEP appeared in fractions 11–23; Fig. 2 shows that sodium glycerophosphate (another form) is also obtained in fractions 2–10. Recoveries of AEP added to rumen contents, separated on a Dowex column and chromatographed on a filter-paper, are shown in Table I. The values for AEP in hydrolysed samples of rumen contents and rumen liquor are given in Table II as milligrams of AEP per 100 g of rumen contents or 100 ml of rumen liquor.

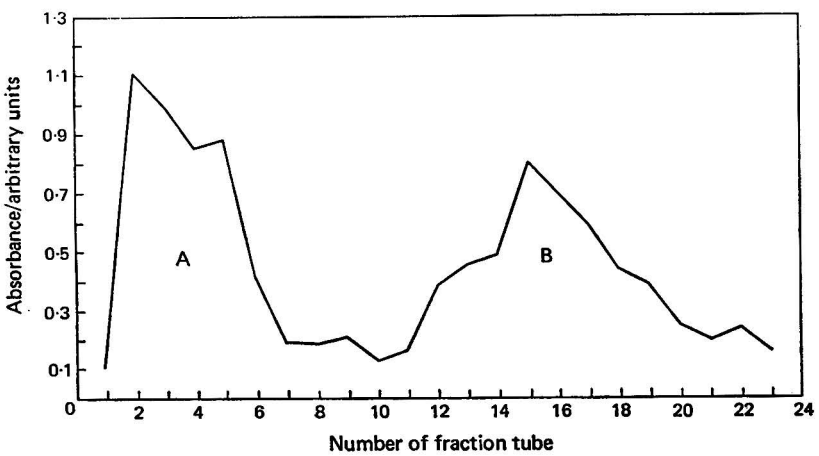

Fig. 2. Distribution of a mixture of sodium β-glycerophosphate and ciliatine (AEP) on column. A, Sodium β-glycerophosphate; B, 2-aminoethanephosphonic acid.

When portions of different fractions of rumen contents were run through the Dowex column and the fractions containing the AEP were concentrated and spotted on to a filter-paper strip, the developed chromatogram showed six more ninhydrin-positive spots (A, B, C, D, E and F) other than that due to AEP, which was the slowest amino-acid to migrate ($R_F = 0{\cdot}3$) (Fig. 3). Spot C, which had an R_F value of 0·64, gave a positive Fiske and Subbarrow phosphorus test. The other amino-acids gave negative reactions for phosphorus. The bacterial

TABLE I

RECOVERY OF AEP ADDED TO RUMEN CONTENTS

Experiment No.	AEP added/mg	AEP recovered/mg	Recovery, per cent.
1	62	63	101·5
2	76	72	94·7
3	83	80	96·2
4	93	90	96·6

fractions (residues from centrifugation at 15 000 rev min^{-1}) contained no AEP, but included the amino-acid that gave spot C, which was found to be present in all fractions.

The relationship between protozoal count and AEP values in samples of rumen contents that were collected at different intervals after feeding is shown in Fig. 4. Highly significant correlation values, $r = 0{\cdot}68$ and 0·72, were obtained in two different experiments.

TABLE II

CONCENTRATION OF AEP IN RUMEN SAMPLES
(AS MILLIGRAMS OF AEP PER 100 ml OF RUMEN CONTENT OR RUMEN LIQUOR)

Time of sampling	Sample	AEP concentration, per cent.
Before feeding	50 ml RL	1·28
Before feeding	25 ml RL	0·92
Before feeding	25 g RC	1·36
2 h after feeding	25 g RC	1·58

RL = rumen liquor, RC = rumen contents.

Discussion

A method was devised to determine 2-aminoethanephosphonic acid in whole rumen contents to an accuracy of ±5 per cent. The method depends on the determination of phosphorus

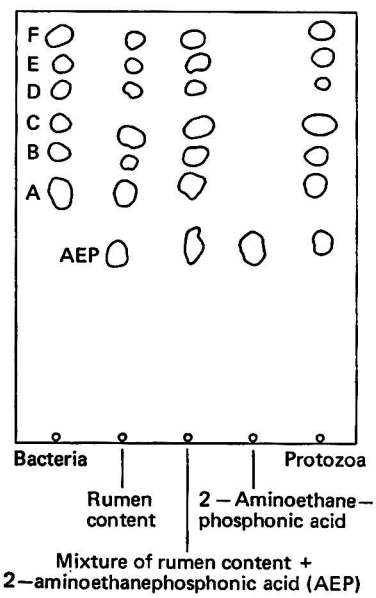

Fig. 3. Single-dimensional chromatogram: solvent, butan-1-ol - acetic acid - water (8 + 3 + 3). A–F represent six ninhydrin-positive spots.

for assessing the AEP content rather than the application of ninhydrin, as was used by other workers.[1,6,7]

It has been indicated that the C—P link is a very chemically resistant bond and that AEP is resistant to hydrolysis in 8 N hydrochloric acid at 105 °C for 24 h[4,10] or in 6 N hydrochloric acid for 48 h.[3,11] It is not, therefore, possible to liberate phosphorus from AEP until it has been oxidised by ashing in nitric acid and magnesium nitrate with strong heating. A modification of the method described by Bartlett[12] for wet ashing was used, as is described above. The method described for AEP determination is a simple one and the analysis requires a relatively shorter period of time as the column used is only 10 cm long. The AEP fraction has been found to contain six other amino-acids, which have been identified on a one-dimensional chromatogram on filter-paper. One of the six amino-acids was found to contain phosphorus. Because the phosphonic acid to ester link in this amino-acid is readily hydrolysed by dilute acid to give inorganic phosphorus, the compound identified under the present conditions is probably another aminophosphonic acid. Work is being carried out at present to identify this second acid, which is found in all fractions, including the bacterial fraction.

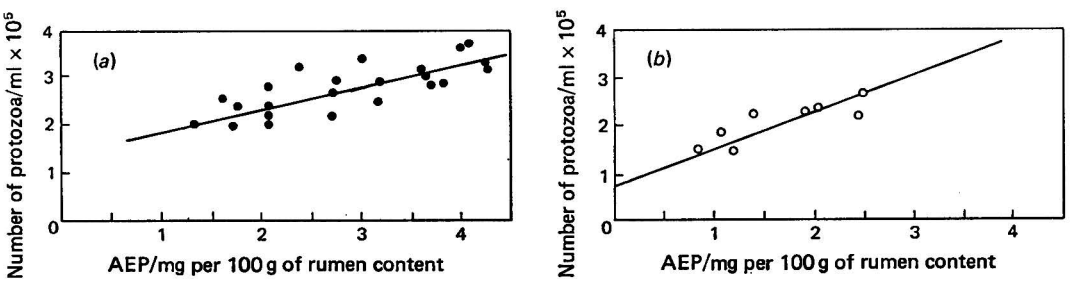

Fig. 4. Correlation between total number of protozoa per millilitre and concentration of 2-aminoethanephosphonic acid. $R^2 = b \Sigma (X - \bar{x}) (Y - \bar{y}) / \Sigma (Y - \bar{y})^2$, where X = independent variable (total number of protozoa per millilitre), $\bar{x}$ = mean of independent variable, Y = dependent variable (concentration of 2-aminoethanephosphonic acid), $\bar{y}$ = mean of dependent variable and b = regression coefficient. (*a*), Experiment 1, $r = 0{\cdot}72$; (*b*), experiment 2, $r = 0{\cdot}68$.

It is possible that flagellates not separated from the bacterial fraction by centrifugation are the source of this amino-acid; its presence in the AEP fraction necessitated the separation of the amino-acids on Whatman No. 1 filter-paper in order to determine the AEP quantitatively. This step was found to be necessary by Abou Akkada *et al.*,[6] although they used a 100 cm long Dowex 50-X8 column. After making the spots on the filter-paper visible with ninhydrin, the AEP spots were cut out carefully and ashed as described above. The results indicated a highly significant correlation between the AEP concentration in 100 g of rumen contents and the total protozoal counts ($r = 0{\cdot}68$ and $0{\cdot}72$; $P < 0{\cdot}01$). This low, but significant, value is probably indicative of the variable content of ciliatine in different species of protozoa. Abou Akkada *et al.*[6] found that *Isotricha* contains a higher concentration of AEP than *Entodenia*.

AEP is found as an integral part of lipoprotein in ciliates and is also found in the free state.[2,3] The variables indicated above may affect the accuracy of the method when AEP is used as a marker for protozoa. This method of AEP determination was developed to facilitate the use of AEP as a marker for monitoring changes in the protozoa content of the rumen. The concentration of AEP has been compared with ciliate protozoal counts. Work is under way to test the possibility of using AEP as a marker for measuring the concentration of ciliate protozoa in the rumen.

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Determination of the "Potential Surface-active Hydroxyl Content" of Fatty Alcohols and Their Ethoxylates

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A quantitative sulphation procedure for hydroxyl groups has been developed, which, when used in conjunction with an established method for determining anionic surfactants, gives a rapid analytical method for the determination of the "potential surface-active content" of alcohols or their ethylene oxide derivatives. With this procedure it is possible to discriminate between potentially surface-active and other hydroxyl groups.

The "surface-active content" of anionic surface-active agents is readily measured by titration with a cationic surface-active agent.[1,2] Thus, if an alcohol or its ethoxylate could be sulphated quantitatively and then subjected to the same titration, it would be possible to measure the hydroxyl groups linked to hydrocarbon chains, which in anionic surfactants are classified as "surface active," by the method described elsewhere.[1,2] Such a procedure would be particularly useful for ethoxylated fatty alcohols, which generally contain polyglycols that do not yield anionic surfactants on sulphation, as the normal method of measuring the hydroxyl value, *i.e.*, by acetylation,[3] includes the measurement of hydroxyl groups of these polyglycols. With alcohols, the measurement of those hydroxyl groups which can be converted into anionic surfactants is important.

Experimental

Reagents

De-ionised water or water of equivalent quality was used throughout.
Sulphur trioxide. Stabilised "Sulphan" (Hardman and Holden Limited).
Chlorosulphonic acid. Technical grade.
Carbon tetrachloride. AnalaR.
Hyamine 1622 *cationic surface-active agent.*
Dimidium bromide - disulphine blue (mixed acid indicator solution).
Chloroform.
Monoethanolamine. Shell Chemicals Limited.

Development of the Method

Sulphation of alcohols and their ethoxylates is often accompanied by oxidative side reactions and it was decided to use a solvent to overcome this disadvantage. The preferred choice of solvent would be one that could be used in both sulphation and titration stages. The determination of anionic surfactants consists of a two-phase titration using chloroform and water. Chloroform normally contains up to 2 per cent. of ethanol as a stabiliser and would thus give too high a hydroxyl group content in the sulphation stage. The use of carbon tetrachloride in the two-phase titration was investigated for the instance when the anionic surfactant was the sodium salt of a C_{12}–C_{14} alkyl ether sulphate containing 3 mol of ethylene oxide.

The normal titrand consists of a mixture of 15 ml of chloroform, 10 ml of aqueous anionic surfactant solution, 20 ml of water and 10 ml of mixed acid indicator solution, and the results obtained are given in Table I.

From these results it can be seen that dilution of the sample in the titrand has no effect on the titration value and end-point clarity when a 2:1 V/V ratio of carbon tetrachloride to chloroform is used.

The following factors were investigated in order to ascertain their effect upon the results: (*a*), molar ratio of sulphating agent to sample, using both sulphur trioxide and chlorosulphonic acid; (*b*), time allowed for sulphation; and (*c*), water content of the sample.

TABLE I

EFFECT OF TITRAND COMPOSITION ON RESULT AND END-POINT CLARITY

Titrand composition/ml					
Chloroform	Carbon tetrachloride	Water†	Indicator solution	Titration/ml	End-point clarity
*15	0	30	10	10·3	Good
0	15	30	10	10·2	Poor‡
15	15	60	20	10·2	Good
10	20	60	20	10·3	Good
5	10	30	10	10·3	Good

* Standard method.
† Includes 10 ml of aqueous sample.
‡ End-point clarity was poor in that there was considerable emulsification and very little colour change, the end-point being detected by a minimum emulsification technique.

The procedure adopted was to add 10 ml of the sulphating agent carefully dissolved in carbon tetrachloride to 10 ml of the sample also dissolved in carbon tetrachloride (for the concentrations see Tables II and III), allowing sulphation to occur before completing the titrand by adding 10 ml of chloroform, 60 ml of water and 20 ml of mixed acid indicator solution. The sulphating agent solution was prepared by introducing the required amount dropwise into a calibrated flask partly filled with solvent and then diluting to the mark with more solvent.

The results obtained were then expressed in a manner similar to that used for the normal hydroxyl value, *i.e.*, in milligrams of potassium hydroxide equivalent to 1 g of sample,* and called the "surface-active hydroxyl value."

Preliminary work was carried out on a sample of C_{12}–C_{15} alcohol that had been made to react with 3 mol of ethylene oxide. The hydroxyl value* of the sample was 167·0 mg-KOH g^{-1} (ref. 3) and the polyglycol content determined by liquid chromatography was 1·0 per cent.[4]

Table II shows the results obtained with sulphur trioxide as sulphating agent and Tables III and IV those obtained with chlorosulphonic acid as the sulphating agent. Unless otherwise stated a sulphation time of 5 min was used.

TABLE II

EFFECT OF MOLAR RATIO OF SULPHUR TRIOXIDE TO SAMPLE ON SURFACE-ACTIVE HYDROXYL VALUE

Concentration of sample was 2·9 mM.

Sulphur trioxide concentration in carbon tetrachloride/mM	Molar ratio of sulphur trioxide to sample	Surface-active hydroxyl value/ mg-KOH g^{-1}
6	2·2	108
10	3·5	161
12	4·4	164
15	5·2	157
18	6·6	145
36*	6·6	Visible oxidation

* 5 ml of the sulphur trioxide solution were used.

These results show that: (*a*) the molar ratio of sulphating agent to sample needs to be within the range 3·5–4·5 : 1·0; (*b*) little or no difference in the results, under the preferred conditions, is obtained if the sulphation time is between 2 and 10 min, there being evidence to suggest that sulphation is incomplete in less than 2 min and that some degradation occurs after more than 10 min; and (*c*) up to 4 per cent. of water in the sample can be tolerated (the normal water content with which alcohols and alcohol ethoxylates are sold is below 1 per cent.).

* In this paper, the hydroxyl value is represented by the expression mg-KOH g^{-1}.

Although there appears to be little to choose between sulphur trioxide and chlorosulphonic acid as sulphating agents the latter is more convenient to handle. If, however, compounds other than alcohols or alcohol ethoxylates are to be analysed, for example, alkylbenzenes and olefines, then sulphur trioxide would need to be used.

TABLE III

EFFECT OF MOLAR RATIO OF CHLOROSULPHONIC ACID TO SAMPLE ON SURFACE-ACTIVE HYDROXYL VALUE

Concentration of sample was 2·8 mM.

Chlorosulphonic acid concentration in carbon tetrachloride/ mM	Molar ratio of chlorosulphonic acid to sample	Surface-active hydroxyl value/ mg-KOH g^{-1}
4·2	1·5	58
4·5	1·6	60
5·6	2·0	90
7·5	2·7	130
10·0	3·5	166
11·7	4·2	165
14·0	5·0	154
17·0	6·0	125

Some spurious results were obtained during preliminary work, which were caused by aqueous hydrolysis during the addition of chloroform, water and mixed indicator when making up the titrand. This defect was overcome by the addition of one drop of monoethanolamine after sulphation and paying particular attention to achieving efficient mixing after addition of the indicator solution.

TABLE IV

EFFECT OF SULPHATION TIME AND WATER CONTENT OF THE SAMPLE ON SURFACE-ACTIVE HYDROXYL VALUE

10 ml of a 3·0 mM solution of sample and 10 ml of a 12·0 mM solution of chlorosulphonic acid were used.

Sulphation time/ min	Water content of sample, per cent. *m/m*	Surface-active hydroxyl value/ mg-KOH g^{-1}
1	0·1	161
2	0·1	162
5	0·1	165
10	0·1	165
30	0·1	162
60	0·1	157
5	2·0	165*
5	3·0	165*
5	4·0	165*

* Dilution factor of the added water allowed for in the calculation.

Method

The reagents used are as given under Experimental.

A 12 mM solution of chlorosulphonic acid is prepared by adding 1·15 ± 0·15 mmol of chlorosulphonic acid dropwise to a 100-ml calibrated flask partly filled with AnalaR carbon tetrachloride, which is then made up to the mark with the same solvent.

Procedure

A 0·7 ± 0·1 mmol amount of the sample to be analysed is accurately weighed and diluted to 250 ml in a calibrated flask with AnalaR carbon tetrachloride; 10 ml of this solution are then pipetted into a clean, dry 200-ml stoppered measuring cylinder. Into this solution are pipetted, with swirling, 10 ml of a freshly prepared sulphating agent solution. The cylinder is then stoppered and allowed to stand for 5 min. One drop of monoethanolamine is added and the solution shaken for 30 s. A mixture of 20 ml of mixed acid indicator, 60 ml of de-ionised water and 10 ml of chloroform is added and the whole immediately shaken thoroughly for 1 min. The titrand is titrated with a standard aqueous 2·5 mM solution of cationic surface-

active agent until a colour change from pink to blue in the non-aqueous layer is observed.

The surface-active hydroxyl value is described as the number of milligrams of potassium hydroxide equivalent to the hydroxyl groups contained in 1 g of sample which, on sulphation, form anionic surfactants.

$$\text{Surface-active hydroxyl value} = \frac{3{\cdot}505 \times V}{W} \text{ mg-KOH g}^{-1}$$

where V ml is the volume of 2·5 mM cationic surface-active agent required to reach the end-point and W g the amount of sample.

A range of samples has been analysed by this procedure and the results are given in Table V.

TABLE V

SAMPLES ANALYSED BY THE DEVELOPED PROCEDURE

Sample	Hydroxyl value by acetylation/ mg-KOH g^{-1}	Polyglycol content, per cent. *m/m*	Surface-active hydroxyl value/ mg-KOH g^{-1}
C_{12}-C_{13} alcohol	286·0	0·0	286·0
C_{13}-C_{15} alcohol	266·5	0·0	266·0
C_{12}-C_{15} alcohol + 2 mol of ethylene oxide	188·0	1·0	183·5
C_{12}-C_{15} alcohol + 3 mol of ethylene oxide	167·0	1·0	164·0
C_{12}-C_{15} alcohol + 9 mol of ethylene oxide	93·4	1·0	91·5

Applications

Non-ionic Surfactants

These products can now be analysed for:

(*i*) Total hydroxyl value, by acetylation.[3]
(*ii*) Polyglycol content, by liquid chromatography.[4]
(*iii*) Ethylene oxide content, by infrared spectrophotometric analysis[5] or hydriodic acid cleavage.[2]
(*iv*) Surface-active hydroxyl value, by the developed procedure.

From the above results it should now be possible to calculate the following values.

(*a*) *The actual hydroxyl value of the potential surface-active alcohol ethoxylate*

Actual hydroxyl value of alcohol ethoxylate component =

$$\frac{\text{Surface-active hydroxyl value} \times 100}{100 - \text{polyglycol (\%)}} \text{ mg-KOH g}^{-1}$$

This value has been calculated for a sample of C_{13} alcohol + 4 mol of ethylene oxide containing different amounts of polyethylene glycol of mean relative molecular mass 300 (Table VI). A surface-active hydroxyl value was also determined on the polyglycol, the value obtained being 0.

TABLE VI

ACTUAL HYDROXYL VALUE FROM SURFACE-ACTIVE HYDROXYL VALUE AND POLYGLYCOL CONTENT

Polyglycol content, per cent.	Surface-active hydroxyl value/ mg-KOH g^{-1}	Actual hydroxyl value of surface-active component/mg-KOH g^{-1}
1·0	145·4	147·0
5·8	138·5	147·0
10·5	131·4	146·8
100	0·0	—

(*b*) *The hydroxyl value of the polyglycol component*

Hydroxyl value of polyglycol =

$$\frac{(\text{Acetylation hydroxyl value} - \text{surface-active hydroxyl value}) \times 100}{\text{Polyglycol (\%)}} \text{ mg-KOH g}^{-1}$$

This value has been calculated for a C_{13} alcohol + 4 mol of ethylene oxide to which

approximately 5 and 10 per cent. *m/m* of polyethylene glycol 300 was added, this polyglycol having a hydroxyl value by acetylation of 300 mg-KOH g^{-1} (Table VII).

TABLE VII

HYDROXYL VALUE OF POLYGLYCOL

Acetylation hydroxyl value/ mg-KOH g^{-1}	Surface-active hydroxyl value/ mg-KOH g^{-1}	Polyglycol content, per cent. *m/m*	Hydroxyl value of polyglycol/ mg-KOH g^{-1}
148·5	145·4	1·0	310
159·0	138·5	5·8	353
165·0	131·4	10·4	325

The calculated value is only an indication of the true value as small errors in determining the polyglycol content are magnified in the calculation.

(*c*) *The mean relative molecular mass of the parent alcohol*

Mean relative molecular mass of parent alcohol =

$$M\left\{1-\left[\frac{\text{Ethylene oxide (\%) in product} - \text{polyglycol (\%)}\ [(P-18)/P]}{100-\text{polyglycol (\%)}}\right]\right\}$$

where M is the mean relative molecular mass of the alcohol ethoxylate portion determined in (*a*) and P the mean relative molecular mass of the polyglycol portion determined in (*b*).

This value has been calculated for a C_{13} alcohol + 4 mol of ethylene oxide with different levels of polyglycol added. The mean relative molecular mass of the alcohol was 199, as determined by acetylation (Table VIII).

TABLE VIII

MEAN RELATIVE MOLECULAR MASS OF PARENT ALCOHOL

Ethylene oxide content, per cent.	Polyglycol content, per cent. *m/m*	Mean relative molecular mass of parent alcohol
54·5	1·0	206
55·3	10·5	200

The ethylene oxide content was determined by infrared spectroscopy,[5] using an Optika CV4 double-beam near infrared - ultraviolet spectrophotometer. The Siggia method using hydriodic acid can also be used to determine the ethylene oxide content.

Alcohols

When used in conjunction with the hydroxyl value obtained by acetylation the method will give an indication as to the potential surface activity of a mixture of alcohols. A range of alcohols have been analysed and their potential surface activities calculated (Table IX).

$$\text{Potential surface activity, per cent.} = \frac{\text{Surface-active hydroxyl value}}{\text{Acetylation hydroxyl value}} \times 100$$

TABLE IX

POTENTIAL SURFACE ACTIVITY OF ALCOHOLS

Alcohol sample	Acetylation hydroxyl value/ mg-KOH g^{-1}	Surface-active hydroxyl value/ mg-KOH g^{-1}	Potential surface activity, per cent.
C_{12}-C_{13}	286·0	287·0	100
C_9-C_{11}	349·0	338·0	97
C_7-C_9	440·0	315·0	72
C_{12}-C_{13}/C_9-C_{11} (90 : 10) ..	289·5	289·5	100
C_{12}-C_{13}/C_7-C_9 (90 : 10) ..	301·5	283·7	94

Conclusions

A quantitative analytical technique has been developed that enables fatty alcohols and their ethoxylates to be sulphated and that, when allied with the standard titration used for determining anionic surfactants,[1,2] can be used to measure the potential surface-active hydroxyl content of these compounds.

The method has proved to be rapid compared with the standard acetylation process[3] and also shows the often desired selectivity when dealing with potential surface-active agents. This selectivity can be used to discriminate between potentially surface-active and non-surface-active hydroxyl groups, which, for example, could depend on the chain length of an alcohol or the presence of polyethylene glycols in a fatty alcohol ethoxylate.

For non-ionic surface-active agents, using readily available techniques, it is now possible to measure the actual hydroxyl values of the potential surface-active material. Although it should be possible to calculate the hydroxyl value of any polyethylene glycol present, its generally low level limits the accuracy of the calculation. The mean relative molecular mass of the parent alcohol can also be calculated.

The authors are indebted to the Directors of Lankro Chemicals Limited for permission to publish this work and also to Mr. M. C. Rocca for technical assistance.

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Sulphonated Alizarin Fluorine Blue

Part II. Fundamental Solution Chemistry and an Improved Isolation Procedure

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Sulphonated alizarin fluorine blue {3-[*NN*-di(carboxymethyl)aminomethyl]-1,2-dihydroxyanthraquinone-5-sulphonic acid}, or its monopotassium salt, shows promise as a general reagent for metals and as a positive absorptiometric reagent for fluoride. An improved purification procedure for the reagent is described. The acid-dissociation constants are $pK_1 = 0{\cdot}5$; $pK_2 = 1{\cdot}4$; $pK_3 = 2{\cdot}5$; $pK_4 = 5{\cdot}8$; $pK_5 = 10{\cdot}0$; and $pK_6 = 12{\cdot}3$. Detailed pH - absorption spectrum graphs and pH - absorbance graphs are given, together with the distribution diagram and the likely ionisation scheme. The following solubilities at 20 °C have been deduced: H_5A, 4×10^{-5} M; $K^+.H_4A^-$, $5{\cdot}6 \times 10^{-3}$ M; and $K_2^+.H_3A^{2-}$, $>4{\cdot}4 \times 10^{-2}$ M.

Sulphonated alizarin fluorine blue {potassium 3-[*NN*-di(carboxymethyl)aminomethyl]-1,2-dihydroxyanthraquinone-5-sulphonate, AFBS} shows promise as a general reagent for metals and as a positive absorptiometric reagent for fluoride. The synthesis of the compound and a tentative account of its use for the determination of fluoride have recently been described in this journal.[1] Since then an improved purification procedure has been evolved and the dissociation constants and solubility properties of the reagent have been measured.

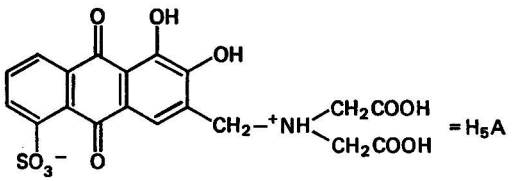
Improved Isolation and Purification of AFBS

The reagent is synthesised by means of a Mannich condensation between sodium alizarin-5-sulphonate, formaldehyde and iminodiacetic acid, using potassium hydroxide as a base to render the mixture soluble. The product is isolated by fractional crystallisation of the potassium salt at pH 3 and, unless great care is exercised, it may be contaminated with starting materials and potassium chloride. It has been found that the passage of a fairly dilute solution of the crude product through a strongly acidic cation exchanger in the hydrogen form results in the slow precipitation of the zwitterion, H_5A, free from iminodiacetic acid and potassium chloride. Supersaturation occurs readily; this prevents precipitation in the resin column and is of great value in the acid - base potentiometric titration to be described. The ion-exchange treatment will not readily remove residual potassium alizarin-5-sulphonate, but this is not a problem as the starting material can be eliminated by careful initial precipitation at pH 3·5.

Procedure

Prepare about 1·5 g of crude AFBS (potassium salt) as described in the earlier paper.[1] Test for the absence of sulphonated alizarin by means of electrophoresis[1] (note that at pH 3·5–4 product solubility is very high while that of potassium alizarin-5-sulphonate is minimal). Dissolve the crude product in 400 ml of water and pass the solution through a column of Amberlite IR-120 or similar resin in the hydrogen form that is at least 50 ml in volume. Wash the column with water until the eluate has only a pale yellow colour. Allow the solution to stand for 24 h or longer if possible, decant it and reject the cloudy supernatant liquid, then filter off the remaining orange - yellow solid. Wash the product well with water, then with acetone and diethyl ether; dry it at 80 °C under vacuum in the presence of phosphorus(V) oxide.

The product is the zwitterion:



Elemental analysis

Required for $C_{19}H_{15}O_{11}NS$: C, 49·03; H, 3·25; N, 3·01; S, 6·89 per cent. Found: C, 49·28; H, 3·46; N, 2·79; S, 6·89 per cent.

The synthesis was attempted with lithium hydroxide, sodium hydroxide, barium hydroxide and tetramethylammonium hydroxide as the basic agent, but none proved superior to potassium hydroxide.

Production of pure monopotassium salt from the zwitterion

Dissolve 1 g of the above product in 40 ml of water containing 0·31 g of potassium hydroxide (mole ratio of 2·5). Reduce the pH of the solution to 2·8 with 1 M hydrochloric acid, then allow the solution to stand overnight. Filter off the precipitate, wash it sparingly with water followed by acetone and ether, then dry it under vacuum at 50 °C over phosphorus(V) oxide. Required for $C_{19}H_{14}O_{11}NSK$: C, 45·32; H, 2·80; N, 2·78 per cent. Found: C, 45·98; H, 3·37; N, 2·72 per cent.

The acid-dissociation constants of AFBS

Under very acidic conditions AFBS can take up six protons to give a form that will be represented by H_6A^+; pK values for these protons were determined by spectrophotometric and pH-titration methods.

Experimental

Solutions

Sulphonated alizarin fluorine blue, 5×10^{-4} M. Triturate 0·1163 g of AFBS zwitterion with 5 ml of 0·1 M sodium hydroxide solution (mole ratio of 2). When the solid has dissolved dilute the solution to about 400 ml, add 0·1 M hydrochloric acid dropwise until an orange solution is obtained, then dilute to 500 ml.

Potassium nitrate solution, 1 M.

Potassium chloride solution, 1 M.

Composite buffer solution. Dissolve 2·1 g of citric acid, 1·42 g of anhydrous disodium hydrogen orthophosphate and 1·21 g of trishydroxymethylaminomethane in water. Add 10 ml of 0·02 M EDTA solution and dilute to a volume of 1 l. This buffer is 0·01 M in each of the first three components.

Ammonia buffer solution. A solution 0·02 M in ammonia and in ammonium chloride.

Diethylamine buffer solution. A solution 0·04 M in diethylamine and 0·02 M in hydrochloric acid.

Procedure and Results

The ionisation $H_3A^{2-} \rightleftharpoons H^+ + H_2A^{3-}$ causes the clear yellow to red transition and is virtually unaffected by the presence of other ionised forms. It was studied by use of the following procedure. Into twelve 50-ml beakers pipette 5 ml of each of 5×10^{-4} M AFBS solution, 1 M potassium nitrate solution and composite buffer solution and dilute the mixtures to about 40 ml. Adjust the pH of each solution to the required value, ranging from pH 3·08 to pH 7·91, by using dilute hydrochloric acid or ammonia solution, then transfer the solutions to 50-ml calibrated flasks and dilute them to the mark. Take absorbance measurements for the contents of each flask, then immediately check the pH value. The pH - absorption spectrum graphs are shown in Fig. 1 and indicate a clear isosbestic point at a wavelength of 457 nm. The graph of absorbance *versus* pH, shown in Fig. 2, is of perfect sigmoidal form and plotting $\log \frac{A - A_{min.}}{A_{max.} - A}$ against pH, where $A_{min.}$ is the asymptotic absorbance value at low pH, $A_{max.}$ is the asymptotic value at high pH and A is any intermediate absorbance.

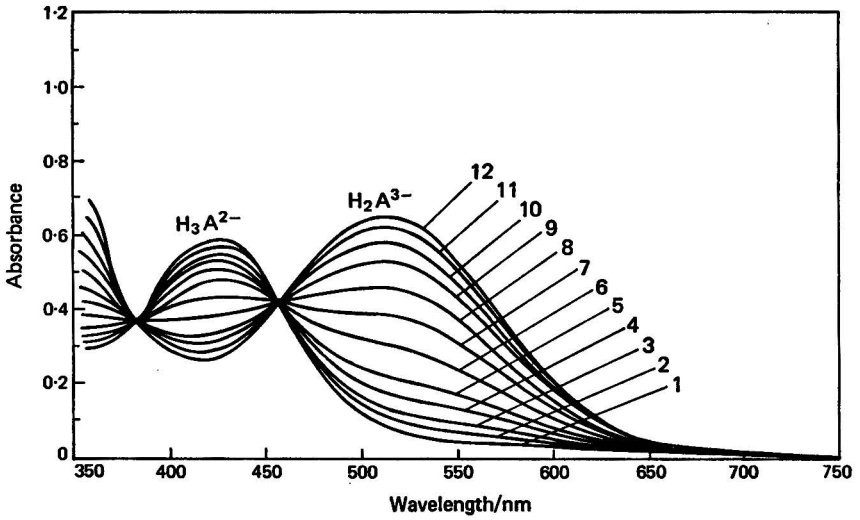


Fig. 1. Change in the absorption spectrum of AFBS with pH between pH values 3 and 8. $C_R = 5 \times 10^{-5}$ M, $\mu = 0{\cdot}1$ (KNO_3), 2-cm cells, 5 ml of composite buffer per 50 ml. 1, pH 3·08; 2, pH 4·50; 3, pH 4·75; 4, pH 5·00; 5, pH 5·22; 6, pH 5·50; 7, pH 5·75; 8, pH 6·00; 9, pH 6·29; 10, pH 6·50; 11, pH 7·00; 12, pH 7·91.

value at suitable pH, yields a straight-line graph of slope 1·00, thus demonstrating a one-proton ionisation.[2] The pH-axis intercept is at pH 5·75 and therefore $pK_4 = 5{\cdot}75$.

The ionisations $H_2A^{3-} \rightleftharpoons H^+ + HA^{4-}$ and $HA^{4-} \rightleftharpoons H^+ + A^{5-}$ overlap to some extent. The former, giving a colour change only from red to wine-red, was studied in the manner previously described but using the ammonia and diethylamine buffers (5 ml) where appropriate. At high pH values 0·1 M diethylamine solution is also required. The pH - spectrum graphs shown in Fig. 3 cover the $H_2A^{3-} \rightleftharpoons HA^{4-}$ ionisation. The isosbestic point is diffuse because of the overlap mentioned above. As will be shown later the proportion of HA^{4-} species reaches a maximum at pH 11·2.

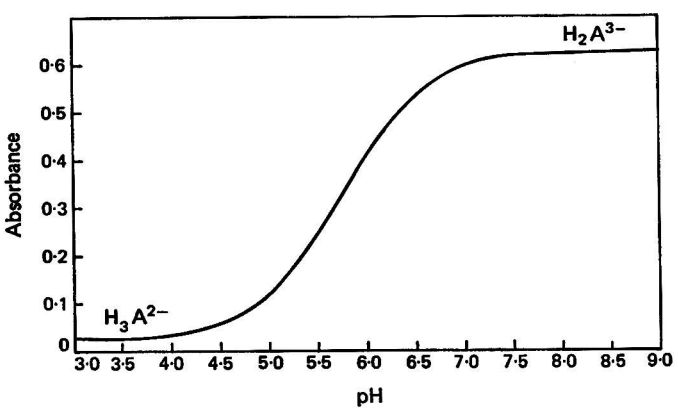


Fig. 2. Variation of absorbance with pH for AFBS in the pH range 3–8. Solution conditions as for Fig. 1. Wavelength 520 nm.

Fig. 4 shows the pH - spectrum graphs for the final $HA^{4-} \rightleftharpoons A^{5-}$ ionisation; pH values were fixed by using diethylamine buffer or potassium hydroxide solution as appropriate. The ionic strength was adjusted to 0·1 with potassium chloride when possible. The isosbestic point is again diffuse owing to overlap.

Very alkaline solutions of AFBS are unstable. The decomposition is complex, but some

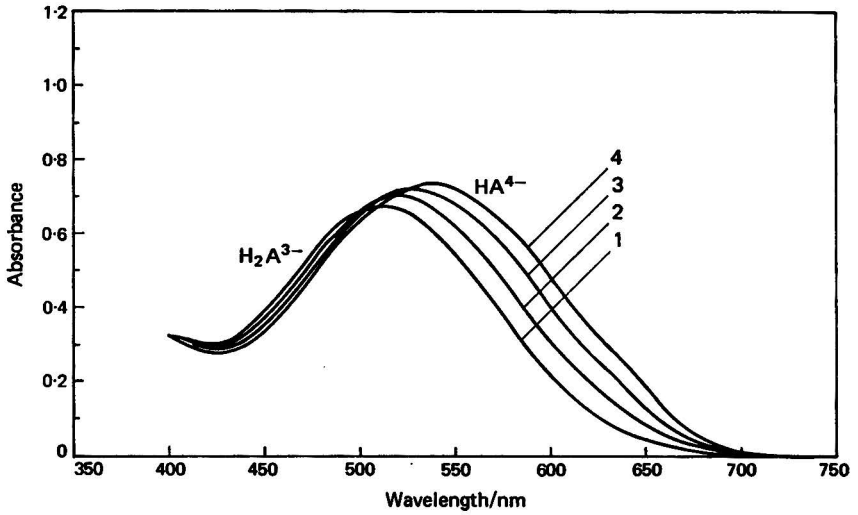


Fig. 3. Change in the absorption spectrum of AFBS with pH in the pH range 8·5 –11·5. $C_R = 5 \times 10^{-5}$ M, $\mu = 0{\cdot}1$ (KCl), 2-cm cells, 10 ml of ammonia or diethylamine buffer per 50 ml. 1, pH = 8·42; 2, pH = 10·19; 3, pH = 10·93; 4, pH = 11·36.

indication of the nature of this complexity is given by Fig. 5 and its accompanying details. Hence, solutions with a pH of greater than 12 must be examined immediately after preparation, although the problem is not severe.

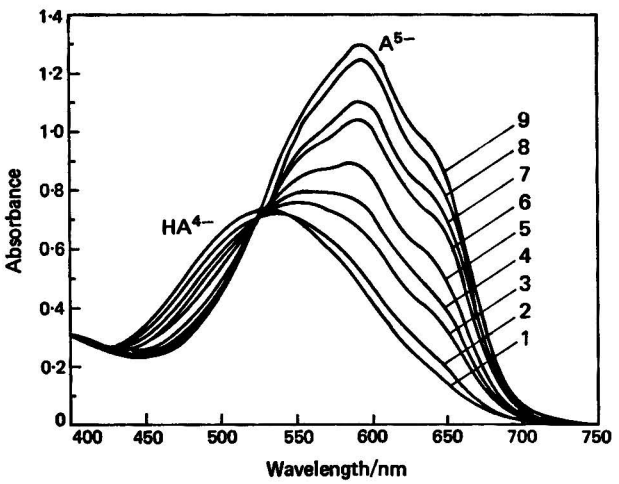


Fig. 4. Change in the absorption spectrum of AFBS with pH in the pH range 11–14. $C_R = 5 \times 10^{-5}$ M, $\mu = 0{\cdot}1$ (KCl) where possible, 2-cm cells, pH fixed with diethylamine or potassium hydroxide as appropriate. 1, pH = 11·08; 2, pH = 11·50; 3, pH = 11·91; 4, pH = 12·09; 5, pH = 12·33; 6, pH = 12·69; 7, pH = 12·89; 8, pH = 13·20; 9, pH = 13·75. 2 M and 5 M KOH gave no further increase.

The pH - absorbance graph at 595 nm between pH values of 7·5 and 14, shown in Fig. 6, clearly shows the distortion due to overlapping ionisations and a little decomposition at about pH 14. By inspection sensible trial values were chosen for $\epsilon_{H_2A^{3-}}$, $\epsilon_{HA^{4-}}$ and $\epsilon_{A^{5-}}$ (λ = 595 nm) and for pK_5 and pK_6, remembering that the onset of pH - absorbance graphs occurs at $pK \pm 1$. Distributions of H_2A^{3-}, HA^{4-} and A^{5-} were calculated at the relevant pH values

by means of equations such as

$$\alpha_2 = \frac{[H_2A^{3-}]}{C_A} = \frac{[H^+]^2}{[H^+]^2 + K_5[H^+] + K_5K_6}$$

and theoretical absorbance values then calculated via the chosen ϵ values.[3] The fifth trial gave a good fit, as is shown by Table I, for the values $\epsilon_{H_2A^{3-}} = 2320$, $\epsilon_{HA^{4-}} = 4000$, $\epsilon_{A^{5-}} = 13\,400$ l mol^{-1} cm^{-1}, p$K_5 = 10{\cdot}0$ and p$K_6 = 12{\cdot}3$.

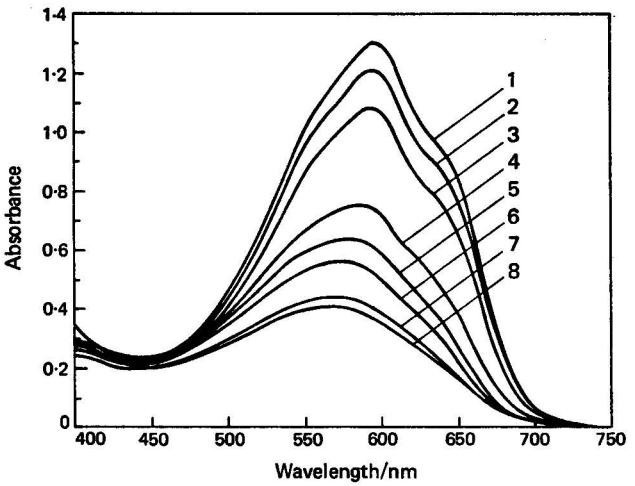


Fig. 5. Stability of AFBS in strong alkali; change of absorption spectrum with time. $C_R = 5 \times 10^{-5}$ M, solvent 1·0 M potassium hydroxide solution, temperature 30 °C, 2-cm cells. Time from start: 1, 2 min; 2, 20 min; 3, 46 min; 4, 140 min; 5, 210 min; 6, 5 h; 7, 10 h; 8, 22 h.

The first three pK values cannot be determined by spectrophotometric means as no colour change is produced by the governing ionisations. Recourse was made to the pH-titration approach now rendered feasible by the suitable solubility and supersaturation properties of AFBS.

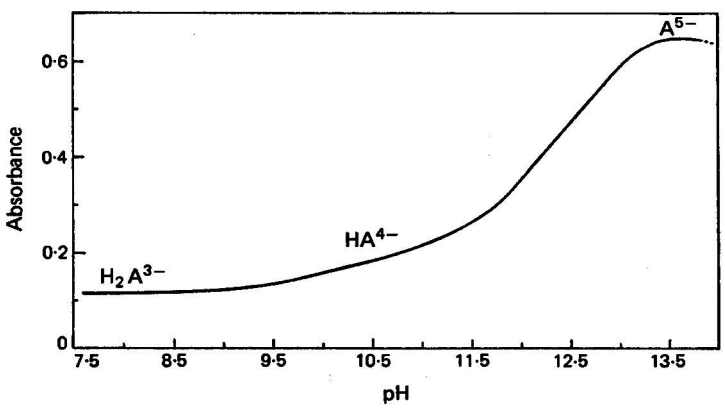


Fig. 6. Variation of absorbance with pH for AFBS in the pH range 7·5–14. $C_R = 5 \times 10^{-5}$ M, $\mu = 0{\cdot}1$ (KCl) at 20 °C, 1-cm cell, wavelength 595 nm. pH fixed with ammonia or diethylamine buffers or potassium hydroxide as appropriate.

An amount (0·1862 g) of AFBS was transferred to the titration cell of a Metrohm E300/364/373 automatic titrator. The cell was gently flushed with argon, then the solid was dissolved by the addition of 20·0 ml of carbonate-free 0·1000 M sodium hydroxide

TABLE I

THEORETICAL AND EXPERIMENTAL ABSORBANCE VALUES FOR THE SYSTEM $H_2A^{3-} \rightleftharpoons HA^{4-} \rightleftharpoons A^{5-}$ UNDER THE CONDITIONS APPERTAINING TO FIG. 6

Assumed ϵ and K values used, as given in the text.

pH	7·5	8·0	8·5	9·0	9·5	10·0	10·5	11·0
A (theory)	0·116	0·117	0·118	0·123	0·136	0·159	0·186	0·213
A (experimental) ..	0·116	0·117	0·118	0·124	0·134	0·156	0·184	0·215
pH	11·5	12·0	12·5	13·0	13·5	14·0	14·5	
A (theory)	0·260	0·354	0·487	0·591	0·642	0·661	0·666	
A (experimental) ..	0·265	0·350	0·471	0·588	0·647	0·640*	—*	

* Decomposition has affected the last two experimental values.

solution (mole ratio of 5). The solution ($C_0 = 0{\cdot}02$ M) was immediately titrated manually with 0·1000 M hydrochloric acid; the titration graph is shown in Fig. 7. Trial values for pK_1, pK_2 and pK_3 were chosen by comparison with compounds, such as benzenesulphonic acid, that possess ionisable hydrogen atoms of a similar nature to those of AFBS. Values

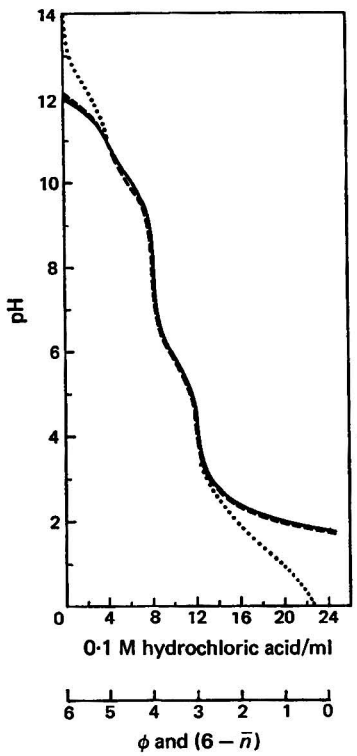


Fig. 7. pH titration curve of AFBS; 0·1862 g of AFBS dissolved in 20 ml of 0·1 M sodium hydroxide solution (5 equiv.), titrated with 0·1 M hydrochloric acid: ——, experimental curve obtained; – – –, theoretical titration curve of ϕ against pH as calculated from the assumed K values; and . . . . ., theoretical dissociation curve, *i.e.*, $(6-\bar{n})$ *versus* pH.

for α,[3] *e.g.*,

$$\alpha_5 = \frac{[H_5A]}{C_A} = \frac{K_1[H^+]^5}{\left([H^+]^6 + K_1[H^+]^5 + K_1K_2[H^+]^4 + K_1K_2K_3[H^+]^3 + K_1K_2K_3K_4[H^+]^2 + K_1K_2K_3K_4K_5[H^+] + K_1K_2K_3K_4K_5K_6\right)}$$

were calculated for various pH values and the theoretical dissociation curve produced from

$$(6 - \bar{n}) = 6\alpha_0 + 5\alpha_1 + 4\alpha_2 + 3\alpha_3 + 2\alpha_4 + \alpha_5$$

where $\bar{n}$ is the average number of acidic hydrogen atoms remaining on the molecule at the particular pH[4]; $(6 - \bar{n})$ is thus the number of hydrogen atoms that have ionised away. The theoretical titration graph can then be obtained from the relationship

$$\phi = (6 - \bar{n}) + \frac{1}{C_0} \times \frac{V_0 + V}{V_0}([OH^-] - [H^+])$$

where $\phi = \frac{CV}{C_0V_0}$, the fraction of acid titrated with respect to one equivalent. Here, C_0 is the initial concentration of compound, V_0 the initial volume, V the volume of titrant added and C the titrant concentration. Values of $pK_1 = 0{\cdot}5$, $pK_2 = 1{\cdot}4$ and $pK_3 = 2{\cdot}5$, together with the pK values found by spectrophotometry, produced a good fit with the experimental titration graph.

To summarise:

$K_1 = 3{\cdot}2 \times 10^{-1}$ mol l^{-1};	$pK_1 = 0{\cdot}5$
$K_2 = 4{\cdot}0 \times 10^{-2}$ mol l^{-1};	$pK_2 = 1{\cdot}4$
$K_3 = 3{\cdot}2 \times 10^{-3}$ mol l^{-1};	$pK_3 = 2{\cdot}5$
$K_4 = 1{\cdot}78 \times 10^{-6}$ mol l^{-1};	$pK_4 = 5{\cdot}8$
$K_5 = 1{\cdot}0 \times 10^{-10}$ mol l^{-1};	$pK_5 = 10{\cdot}0$
$K_6 = 5{\cdot}0 \times 10^{-13}$ mol l^{-1};	$pK_6 = 12{\cdot}3$

K_1 cannot in reality be determined under the above conditions and the quoted value is based on those of model compounds. In fact, it is usually ignored with reagents of this type.

Fig. 8 shows the pH-distribution diagram for AFBS.

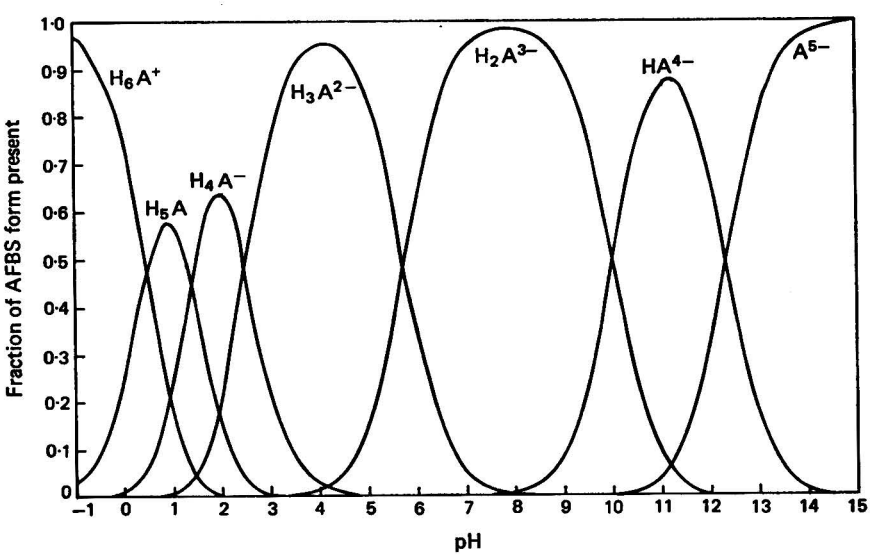
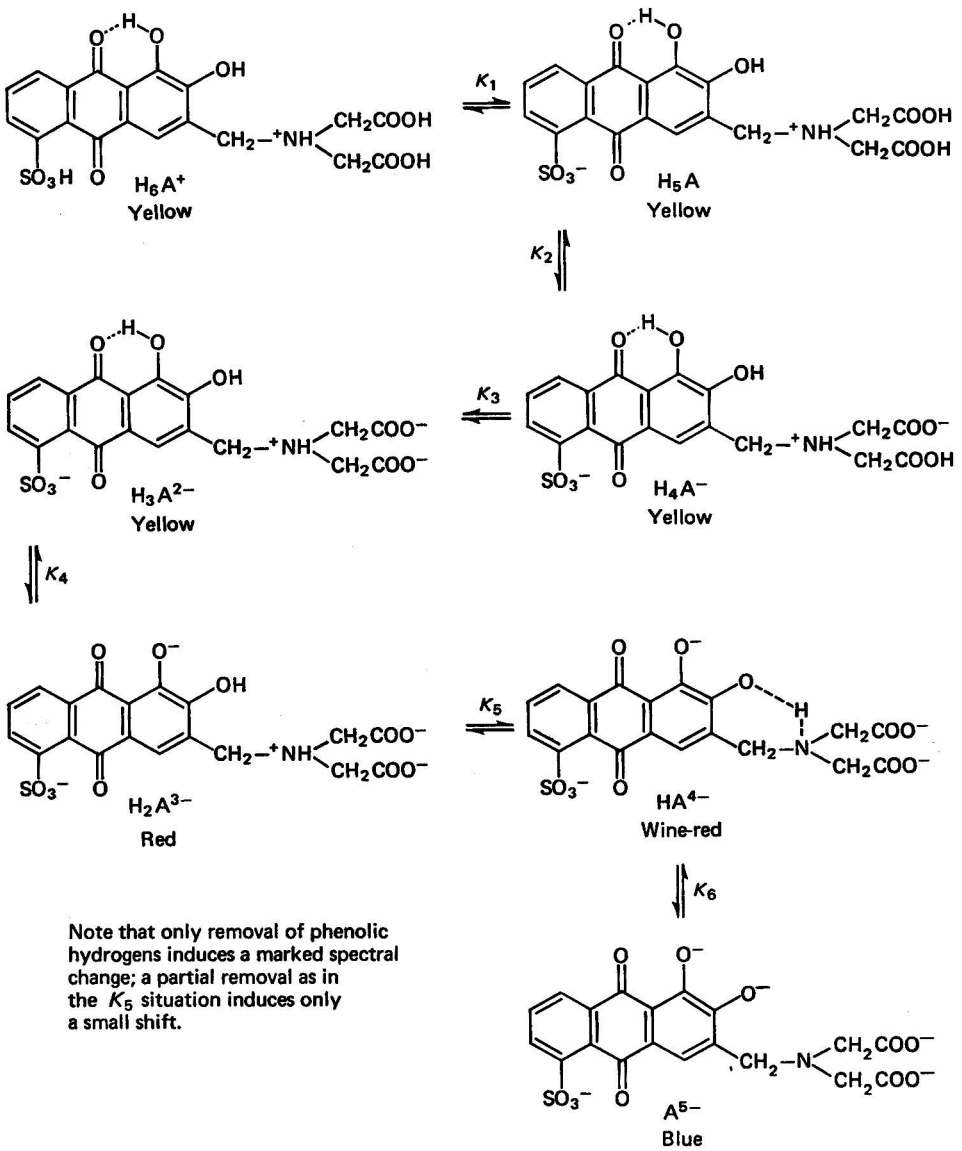


Fig. 8. Distribution diagram showing the fraction of various ionised forms of AFBS present in solution as a function of pH.

From the results obtained the ionisation scheme for sulphonated alizarin fluorine blue appears to be as follows:



That the yellow to red transition is caused by the ionisation of the 1-hydroxy proton is supported by the fact that the spectral transition shown in Fig. 1 is very like the ionisation of sulphonated 1-hydroxyanthraquinone. The ionisation spectral pattern for 2-hydroxyanthraquinone (in 75 per cent. ethanol) is very different.

It is fitting at this stage to draw attention to the excellent investigation of Ingman concerning alizarin fluorine blue.[5] His pK values for this reagent at $\mu = 0{\cdot}1$ are: $pK_1 = 2{\cdot}40$, $pK_2 = 5{\cdot}54$, $pK_3 = 10{\cdot}07$ and $pK_4 = 11{\cdot}98$, which correspond to those for K_3, K_4, K_5 and K_6, respectively, in this paper. In addition, Ingman provides evidence for an H_5L_2 entity.

Solubility of AFBS as a Function of pH

This study was carried out as an aid to the practical use of AFBS.

Procedure

Samples of 0·1164 g or 0·2328 g of AFBS zwitterion ($2·5 \times 10^{-4}$ mol or 5×10^{-4} mol) were weighed into 20-ml plastic-stoppered sample tubes and mixed with 0–10 ml of 0·1 M hydrochloric acid or sodium hydroxide solution (mole ratios of 0–2). The volume was made up to 10 ml with water and sufficient 1 M potassium nitrate solution to render μ equal to 0·1. The tubes were stoppered and the contents shaken at 20 °C intermittently for 24 h. After this time the contents were filtered, the filtrate was adjusted to pH 3 and the absorbance measured at 425 nm in a glass cell of suitable path length. In most instances further dilution was necessary. The molar absorptivity, ϵ, for AFBS at 425 nm had previously been determined by examination of a $5·00 \times 10^{-5}$ M solution at pH 3 in a 2·00-cm cell, with $\mu = 0·1$ (potassium nitrate solution). Here $A = 0·520$ and therefore $\epsilon = 52001\ \text{mol}^{-1}\ \text{cm}^{-1}$ The results are given in Table II.

TABLE II

THE SOLUBILITY OF AFBS AS A FUNCTION OF pH

Temperature 20 °C; $\mu = 0·1$ (KNO_3).

Tube	Amount of AFBS taken/g	Solution added	Mole ratio	pH of filtrate	Concentration of AFBS in filtrate
1	0·1164	2·5 ml of 0·1 M HCl	H^+: AFBS 1:1	1·72	$3·35 \times 10^{-4}$ M, 0·16 g l^{-1}
2	0·1164	1·25 ml of 0·1 M HCl	H^+: AFBS 0·5:1	2·00	$3·80 \times 10^{-4}$ M, 0·18 g l^{-1}
3	0·1164	No acid or base	AFBS only	2·68	$1·31 \times 10^{-3}$ M, 0·61 g l^{-1}
4	0·1164	1·25 ml of 0·1 M NaOH	OH^-: AFBS 0·5:1	3·05	$7·88 \times 10^{-3}$ M, 3·7 g l^{-1}
5	0·1164	2·5 ml of 0·1 M NaOH	OH^-: AFBS 1:1	3·15	$1·50 \times 10^{-2}$ M, 6·9 g l^{-1}
6	0·2328	7·5 ml of 0·1 M NaOH	OH^-: AFBS 1·5:1	3·28	$4·30 \times 10^{-2}$ M, 20·1 g l^{-1}
7	0·2328	8·75 ml of 0·1 M NaOH	OH^-: AFBS 1·75:1	3·38	5×10^{-2} M, 23·3 g l^{-1} complete solubility (just)
8	0·2328	10 ml of 0·1 M NaOH	OH^-: AFBS 2:1	4·98	5×10^{-2} M, 23·3 g l^{-1} complete solubility

Results

Solubility of the various ionised forms

For the addition of sodium hydroxide solution in a mole ratio of 1·5 the pH of the filtrate is 3·28 and the total concentration is 0·043 M. From the distribution graphs (Fig. 8) it is found that at a pH of 3·28 $[H_3A^{2-}] = 0·043 \times 0·85 = 0·036$ M. This is not a limiting solubility as for 1·75 mol of base at pH 3·38, $[H_3A^{2-}] = 0·05 \times 0·875 = 0·044$ M and solubility is complete. Again, at pH 3·28, $[H_4A^-] = 0·043 \times 0·13 = 0·0056$ M, and as a precipitate is present, this must be the solubility of this form (as $K^+.H_4A^-$). This supposition is confirmed by the result for 1·75 mol of base when the pH is 3·38. Here, $[H_4A^-] = 0·05 \times 0·11 = 0·0055$ M and complete solubility is just achieved. For the addition of 1 mol of sodium hydroxide the pH of the filtrate is 3·15 and the total concentration is 0·015 M. Therefore, $[H_3A^{2-}] = 0·015 \times 0·8 = 0·012$ M. This is not a limiting value. $[H_4A^-] = 0·015 \times 0·175 = 0·0026$ M, and this is not a limiting value either. $[H_5A] = 0·015 \times 0·003 = 4·5 \times 10^{-5}$ M; because solid is present this must be the solubility of the zwitterion form. For 0·5 mol of sodium hydroxide, the pH is 3·05 and the total solution concentration is $7·88 \times 10^{-3}$ M. $[H_4A^-] = 7·88 \times 10^{-3} \times 0·205 = 1·61 \times 10^{-3}$ M, which is not a limiting value; $[H_5A] = 7·88 \times 10^{-3} \times 0·005 = 3·94 \times 10^{-5}$ M, which is a limiting value. For a saturated solution of H_5A, the pH is 2·68 and the total solution concentration $1·31 \times 10^{-3}$ M.

Therefore, $[H_4A^-] = 1·31 \times 10^{-3} \times 0·31 = 4·06 \times 10^{-4}$ M, not a limiting value, and $[H_5A] = 1·31 \times 10^{-3} \times 0·018 = 2·6 \times 10^{-5}$ M, which is a limiting value.

To summarise—

Species	Solubility
$K_2^+.H_3A^{2-}$	$> 4{\cdot}4 \times 10^{-2}$ M
$K^+.H_4A^-$	$5{\cdot}6 \times 10^{-3}$ M
H_5A	4×10^{-5} M

A study is in progress on rare-earth metal complexes of this reagent and their ability to carry out the fluoride ternary complexation reaction.

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Received *September 9th*, 1974
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Book Reviews

THERMAL ANALYSIS OF POLYMERS. By W. WRASIDLO. *Advances in Polymer Science, Volume* 13. Pp. 99. Berlin, Heidelberg and New York: Springer Verlag. 1974. Price DM46; $18·90.

One of the areas of most rapid development in polymer science during the last decade has been that of thermal analysis. The reason for this is that not only do the techniques of thermal analysis quickly provide a large amount of data on material properties and transitions, but some have received sophisticated commercial exploitation such that few research and technical laboratories do not now have access to at least one or more of these techniques. Consequently, a review of the complete subject area would necessarily be either voluminous or superficial.

The author has properly selected topics in such a way that within the confines of the size of this publication he has been able to deal with them in some depth. Apart from one chapter he has devoted attention to the principal subject areas of measurement rather than to the techniques themselves. The first chapter is, however, concerned with adiabatic calorimetry, differential thermal analysis and calorimetry, dilatometry and thermo-optical analysis, concluding with an interesting and valuable section on sample preparation and conditioning. The second chapter gives a concise account of glass transitions, with emphasis on models, theories and empirical approaches, and also presents two fairly extensive tables on relevant thermodynamic properties. A different approach is followed in the final two chapters, one on melting and the other on crystallisation. The former pays attention to the behaviour of particular polymer classes, including copolymers and blends, whereas the latter discusses the factors that are associated with crystallisation, with special reference to polyethylene.

In view of the restriction on space, it is not surprising that some sections are brief and in one or two places could be up-dated, but it would be uncharitable to weigh these limitations against the intrinsic value of the publication. The author has produced a concise and yet most readable review, supported by 277 references, of a depth belied by the generality of the title. It is a book that can be highly recommended for reading by graduate students, research workers and, indeed, anyone concerned with the thermal analysis of polymers. R. P. SHELDON

REFRAKTOMETRICHESKIYE METODY V KHIMII (REFRACTOMETRIC METHODS IN CHEMISTRY). By BORIS V. IOFFÉ. Pp. 400. Second Edition. Leningrad: Khimiya. 1974. Price Rble 2·36.

Refractometry, possibly the oldest optical method used in modern chemical analysis, is still central to the work of many physical scientists and industrial chemists. Despite their utility, refractometric methods have somewhat surprisingly not merited a monograph; in the English language literature, they were at best treated as chapters in books concerned with other subjects. Much of the problem derives from the broad dimensions of the field. A monograph on refractometry would require theoretical and technical competence in many widely separated areas of chemistry. We are indeed fortunate that Professor Ioffé of the State University of Leningrad has tackled the task. He has devoted over 30 years to refractometry, having obtained the prestigious Doktor degree for his work.

Professor Ioffé's book is divided into two parts, the first dealing with basic theoretical principles and areas of applicability, the second devoted to technical and instrumental aspects of refractometric measurements. In the first part, the author treats the refractive index as a function of various parameters, such as composition, temperature and pressure, and he demonstrates the peculiar behaviour of a whole series of refractometric variables (specific and molecular refractions, dispersion, intercept, etc.). Ioffé develops refractometry as a relatively simple method of researching binary, ternary and more complex systems, in addition to the usual gas and volumetric analysis. Particularly important are the chapters dealing with the use of refractometric methods for studying chemical composition and kinetics of reactive interaction. The range of these applications of refractometry includes complex formations, phase transformations and tautomeric equilibria, and an extensive number of deviations from the classical additive scheme are all meticulously analysed and interpreted. Characteristic of the author's approach, unusual in such a book, is a survey of the position of refractometry in the contemporary framework of physicochemical analytical instrumentation. Refractometric methods are shown in combination with ultraviolet and infrared spectroscopy, as well as with chromatography and other non-optical methods; the reader will find a stimulating outline of developments in refractometric analysis and a picture of current trends in its applications. One such trend, for example, is the transference of immersion refracto-

metric methods from the field of mineral analysis to microbiology. Noteworthy are pages devoted to continuous-control refractometric methods in petroleum refining, a use that does not seem to be current outside oil refineries in the Soviet Union.

The second part of the book deals with specific techniques and instrumentation of refractometry. Ioffé is well acquainted with the history of the field, so that hardly any techniques of refractometric measurements have escaped his attention. Many kinds of refractometric equipment enjoy just as extensive coverage. Attention paid to instruments manufactured in Russia and Eastern Europe might seem excessive to a Western reader. This peculiarity of the book is not surprising in view of both the limitations of the Soviet market and the availability in the USSR of documentation on modern Western instrumentation. This emphasis could be eliminated in an eventual translation of the book, for which Ioffé could add data on recent instruments made in Europe and the USA.

The book includes convenient reference and calculation tables, which establish an exhaustive compendium for refractometric measurements. For this second edition of the book, a number of improvements have been introduced, including unique specially computed four-digit tables for the Lorentz - Lorenz function derivative. In spite of impressively abundant numerical and graphical data (34 tables and 173 figures) and a 2000-item list of references, the book is carefully produced and surprisingly easy to use.

Ioffé's book represents a work, unique in the field of refractometry, which provides an inside view of the subject and of its perception in Soviet scientific circles (participation of specialists from the USSR Academy of Sciences and the Ministry for Defence Industries of the USSR in the preparation of the book has also contributed to its value). I strongly recommend that Ioffé's book be translated into English so that it might receive wider use in both academic and industrial circles.

YAKOV M. RABKIN

FORENSIC TOXICOLOGY. PROCEEDINGS OF A SYMPOSIUM HELD AT THE CHEMICAL DEFENCE ESTABLISHMENT, PORTON DOWN, 29–30 JUNE 1972. Edited by BRYAN BALLANTYNE. Pp. x + 157. Bristol: John Wright & Sons Ltd. 1974. Price £4·25.

The practice of printing the proceedings of symposia serves the dual purpose of providing participants with a permanent record of the lectures they attended and, at the same time, making the information in the lectures available to all interested workers in the field. In his preface, the editor defines forensic toxicology as "a specialisation dealing with the legal and medical aspects of the detrimental effects of chemicals on humans," a specialisation to which the analytical chemist has made significant contributions in a truly multi-disciplinary sphere. Contributors from a variety of organisations provide a series of papers dealing with carbon monoxide, cyanide, barbiturates, morphine, methadone, hallucinogens, anticholinesterases, paraquat, lead and mercury, and reference to the papers will give a guidance to the practising forensic toxicologist on suitable analytical procedures and the interpretation of the analytical findings. The techniques referred to range from chemical spot tests to the modern instrumental methods, and perhaps serve to illustrate the challenge in this field of work. The 151 pages of text provide a useful survey of the specialisation as defined by the editor, and can be commended to the attention of forensic toxicologists, although the price seems to be rather high.

P. G. W. COBB

FRAME: AN ON-LINE CORRECTION PROCEDURE FOR QUANTITATIVE ELECTRON PROBE MICROANALYSIS. By H. YAKOWITZ, R. L. MYKLEBUST and K. F. J. HEINRICH. *NBS Technical Note* 796. Pp. iv + 46. Washington, D.C.: Institute for Materials Research, National Bureau of Standards. 1973. Price $0·80.

This note introduces, explains and lists, in language intelligible to electron microprobe users, the program FRAME developed by the American leaders in this field, and gives examples of its use. The program is based on the now classical Z-A-F correction procedure of Duncumb *et al.* and exemplified by the well known NBS Special Publication 298. FRAME, however, saves much memory space by calculating internally many of the data required, including mass absorption coefficients.

FRAME will handle up to six elements measured simultaneously and will print results for them and one other, the last obtained by difference or from stoicheiometry, as the composition of each location in a sample is measured.

While of primary interest to those having microprobes with suitable on-line computing facilities, it will be of considerable value to those requiring a program for off-line use on a small computer.

It is an important and up to date contribution to the literature on this subject and is recommended to all microprobe users. P. R. MONK and D. A. PANTONY

CLINICAL BIOCHEMISTRY. PRINCIPLES AND METHODS. Edited by H. CH. CURTIUS and MARC ROTH. Volume 1. Pp. lxix + 1 to 854. Volume 2. Pp. lxix + 855 to 1677. Berlin and New York: Walter de Gruyter. 1974. Price DM390; $156.

One of the expressed aims of this book is to provide the information necessary for a good understanding of the principles governing the analytical procedures used in clinical biochemistry. The magnitude of this task is shown by the size of the book, which covers the subject comprehensively and without redundancies or repetition in 1677 pages.

It consists of contributions by 66 authors, all except nine of whom are from Europe. The first third of the text is concerned with general methodology, including techniques, methods of separation and analysis. Most of this is conventional but well done, and this reviewer found the chapters on GeMSAEC fast clinical analysers and ultramicro techniques particularly interesting. The chapter on automation and data processing is refreshingly iconoclastic, free from jargon or references, but would be of little use to the beginner who wishes to know the current state of the art.

The remainder of the book covers detailed procedures for different groups of substances, and here the treatment of individual topics is uneven. Some authors review the available methods, without always recommending one, whereas others discuss one method in depth and give full practical details. Some contributions tend to catalogue rather than review facts, and large numbers of references to original work, mostly pre-1971, are given throughout the book: 45 are given in the first eight pages and this proportion is not atypical. For some common tests, where there is a choice of well known methods, the treatment is sometimes superficial, whereas some more complex analyses are covered in great detail, so that it is difficult to see the wood for the trees. For example, 27 pages and 140 references are devoted to glycosaminoglycans. When describing analytical methods, principles are not always clearly explained and data on precision are rarely given. In one instance, the techniques of collecting the specimen and preparation of a reagent are not given but indicated only by a reference to the literature.

For a book of this size, there are some important omissions. Laboratory safety and the monitoring of therapeutic drug levels are not mentioned. The section on toxicology deals mainly with forensic applications and does not mention the use of screening tests in clinical toxicology. The emphasis throughout is on manual methods, and although the AutoAnalyzer is not described, a flow diagram is given, which is unintelligible. A more comprehensive account of interference by drugs in chemical analyses would have been useful. The choice of enzyme tests is a strange one and omits CPK, and there is no comprehensive discussion on the selection of enzyme tests in different clinical conditions. There are, however, useful chapters on new topics, including the use of leucocyte preparations. In addition to the purely analytical aspects, the clinical chemist must understand why a test is done and what the results mean. For some substances normal values are not given and in others there is no indication of the clinical relevance of the test.

However, these are minor criticisms, and in comprehensiveness this book has no equal. It is well written and produced and covers not only current analytical procedures, but those which are likely to be important in the future. The breadth of subjects dealt with is a salutary reminder that the horizons of clinical biochemistry are still expanding and that its needs cannot be met by a series of multichannel analysers. Analytical chemists are still essential, and although at this price (about £65) few are likely to buy the book, those who take their work seriously should make sure that their libraries do so. P. M. G. BROUGHTON

AN INTRODUCTION TO LIQUID SCINTILLATION COUNTING. By A. DYER. Pp. xiv + 111. London and Rheine: Heyden & Son. 1974. Price £3; $8·25; DM24·70.

This introduction to liquid scintillation counting manages to include most of the topics of interest and importance in the use of this technique. The general principles, instrumentation, sample preparation, quenching and quench correction, the counting of tritium, carbon-14 and other isotopes, Čerenkov counting and non-radioactive uses of liquid scintillation spectrometers are described. It would be impossible to include much detail on any one aspect in a book of this

size but a good deal of information is given in tables and a useful bibliography includes the various conference reports and reviews, the principal primary papers and the practically useful commercial technical publications on the subject. A selection of "cocktails," solubilisers and gelling agents, etc., with their relevant uses is included in appendices.

In some places the text suffers through brevity. In the section on dual-labelled counting, it is not clear where the tangent is drawn in Fig. 22, and in the description of the use of the Engberg plot for choosing the optimum conditions for counting tritium and carbon-14, only the best conditions for counting the former are apparent. Carbon-14 would be counted under different conditions, *i.e.*, at much lower gain. In measuring low levels of tritium to determine the age of ground waters it would be necessary, for any of the methods quoted, to carry out reconcentration of the tritium first, *e.g.*, by electrolysis. The listing of isotopes that can be determined by liquid scintillation or by Čerenkov counting in the form of a Periodic Table does not enable a distinction to be made between two isotopes that would be counted by different methods, *e.g.*, calcium-45 and calcium-47, strontium-89 and strontium-90. The information would have been better included in the existing table of beta emitters measured by liquid scintillation counting.

This book can be recommended for a preliminary reading before going to the more extensive and more expensive reports and reviews given in the bibliography. D. I. COOMBER

Errata

DECEMBER (1974) ISSUE. Page 994, line 17 of main text: *for* "200 nm" *read* "2 nm." Page 995, line 16 of text: *for* "50 to 400 nm, compared with the 200 000 nm" *read* "0·5 to 4 nm, compared with the 2000 nm." Page 995, line 27: *for* "10^{-10} mtorr" *read* "10^{-10} torr."

APPOINTMENTS VACANT

A Comparison of the Extraction of Mercury from Sediments by Using Hydrochloric - Nitric Acid, Sulphuric - Nitric Acid and Hydrofluoric Acid - Aqua Regia Mixtures

A study of the extraction of mercury from various sediments with hydrochloric - nitric acid, sulphuric - nitric acid and hydrofluoric acid - aqua regia mixtures is reported. The mercury in the extract is detected by using an automated cold-vapour atomic-absorption technique. Although greater extraction of mercury from sediments is possible with a hydrofluoric acid - aqua regia mixture than is possible with hydrochloric - nitric acid and sulphuric - nitric acid mixtures, the results, for the purposes of environmental surveys, are equivalent. The lower detection limit, better precision and simpler application of the methods involving the last two mixtures make them preferable. The hydrofluoric acid - aqua regia extractions were performed in a PTFE bomb.

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Analyst, 1975, **100**, 253–258.

Determination of Trace Amounts of Phosphorus in Trichloro- and Tetrachlorosilane

A method is described for the determination of phosphorus below the parts per million level in halosilanes. The sample is hydrolysed in an enclosed vessel and the silicic acid formed is volatilised off. Phosphorus is extracted as molybdophosphate into chloroform - butan-1-ol mixture. The molybdenum associated with the extracted phosphorus is determined by the thiocyanate method.

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Analyst, 1975, **100**, 259–262.

A Method for Determining 2-Aminoethanephosphonic Acid in Rumen Contents

The work described is an attempt to develop a reliable method for determining AEP when mixed with related substances from micro-organisms in the rumen. Hydrochloric acid hydrolysates of rumen contents, rumen bacteria, rumen ciliate protozoa and clarified rumen contents were applied to a column (10 × 1 cm) of Dowex 50–X8, eluted with 0·6 N hydrochloric acid and 2·4-ml fractions collected. Inorganic phosphorus was separated in column fractions 2–10 and AEP appeared in fractions 11–23.

When fractions containing AEP were spotted on to Whatman No. 1 filter-paper strips the developed chromatograms showed six ninhydrin-positive spots in addition to that of AEP, which was the slowest acid to migrate (R_F = 0·3). The substance giving spot No. 4, another phosphonic acid (R_F = 0·59), was present in ciliate protozoa and ciliate-free fractions of rumen contents, whereas AEP was confined to protozoa. A highly significant correlation was found between the AEP concentrations and protozoal counts in samples of rumen contents collected at different intervals. It is therefore suggested that the concentration of AEP can be used as a marker of the protozoal growth in the rumen.

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Department of Animal Production, Faculty of Agriculture, Alexandria University, Alexandria, Egypt.

Analyst, 1975, **100**, 263–268.

Determination of the "Potential Surface-active Hydroxyl Content" of Fatty Alcohols and Their Ethoxylates

A quantitative sulphation procedure for hydroxyl groups has been developed, which when used in conjunction with an established method for determining anionic surfactants, gives a rapid analytical method for the determination of the "potential surface-active content" of alcohols or their ethylene oxide derivatives. With this procedure it is possible to discriminate between potentially surface-active and other hydroxyl groups.

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Analyst, 1975, **100**, 269–274.

Sulphonated Alizarin Fluorine Blue Part II. Fundamental Solution Chemistry and an Improved Isolation Procedure

Sulphonated alizarin fluorine blue {3-[*NN*-di(carboxymethyl)aminomethyl]-1,2-dihydroxyanthraquinone-5-sulphonic acid}, or its monopotassium salt, shows promise as a general reagent for metals and as a positive absorptiometric reagent for fluoride. An improved purification procedure for the reagent is described. The acid-dissociation constants are $pK_1 = 0{\cdot}5$; $pK_2 = 1{\cdot}4$; $pK_3 = 2{\cdot}5$; $pK_4 = 5{\cdot}8$; $pK_5 = 10{\cdot}0$; and $pK_6 = 12{\cdot}3$. Detailed pH - absorption spectrum graphs and pH - absorbance graphs are given, together with the distribution diagram and the likely ionisation scheme. The following solubilities at 20 °C have been deduced: H_5A, 4×10^{-5} M; $K^+.H_4A^-$, $5{\cdot}6 \times 10^{-3}$ M; and $K_2^+.H_3A^{2-}$, $>4{\cdot}4 \times 10^{-2}$ M.

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Analyst, 1975, **100**, 275–284.

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ORIGINAL PAPERS

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